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Abstract

Testing Hypotheses about Diffuse Idiopathic Skeletal Hyperostosis (DISH) using Stable Isotope and aDNA Analysis of Late Medieval British Populations

Rosa Kelly Spencer

This thesis explores two of the major hypotheses associated with diffuse idiopathic skeletal hyperostosis (DISH) – that DISH is related to diet, and that DISH is genetically linked. Using rib and tooth samples of individuals with and without DISH from both monastic and non-monastic sites, this study attempts to investigate these theories by using carbon and nitrogen stable isotope analysis to assess diet, and mtDNA analysis to assess maternal relatedness.

The data from these analyses demonstrate the complexity of attempting to explore the links between DISH and diet and DISH and genetics. The stable isotope analysis shows a statistically significant difference in $\delta^{15}N$ between those with DISH and without DISH when examining all data together, and between monastic samples with and without DISH, but not when data is separated into individual sites, males and/or females, or non-monastic categories. Although not statistically significant, a pattern exists for DISH samples to plot higher in terms of $\delta^{15}N$ values than non-DISH samples in all comparisons, which suggests that there is an isotopic difference between DISH and non-DISH and that this is related to animal protein. However, it is also possible that the differences observed are reflecting physiological rather than dietary processes. The aDNA analysis, on the basis of the data obtained, provides no evidence that the individuals with DISH are maternally related. This aspect of the research explores only one way in which DISH could be genetically conferred; it is still possible that DISH has a genetic aetiology.

It is cautioned that small sample sizes for both the stable isotope and aDNA analysis may be affecting interpretation of the data. Alternative theories are put forth for the aetiology of DISH and, ultimately, it is concluded that more data is needed in order to explore these questions thoroughly.

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Chapter 1: Introduction

Diffuse idiopathic skeletal hypertostosis (DISH) is a condition about which very little is known. Also known as Forestier's disease, or ankylosing vertebral hyperostosis, DISH is a condition that exists amongst both living and archaeologically derived populations, causing ossification of the anterior longitudinal ligament of the vertebrae and ossification of entheses at other non-vertebral sites. This condition has been reported as not causing any significant pain to some individuals, whilst causing secondary problems such as dysphagia (difficulty in swallowing), Achilles tendonitis, or reduced range of movement in others (Cammissa *et al.* 1998, Childs 2004, Sarzi-Puttini and Atzeni 2004).

Although there is a wide knowledge base, both clinical and archaeological, about the signs, symptoms and diagnosis of DISH, little is known about its aetiology. Yet, despite this lack of knowledge, there are many accepted theories in the bioarchaeological literature about the causes of DISH. Various theories have been postulated, for example, that it may be a 'state' rather than a disease (Hutton 1989), or that it may be an occupationally related disease (Macones *et al.* 1989, Pappone *et al.* 1996, Waldron 1985). Others suggest that DISH may be present only in 'bone-formers' and that it has a genetic component (Crubezy *et al.* 1996, Havelka *et al.* 2001, Havelka *et al.* 2002, Kim *et al.* 2004, Macones *et al.* 1989, Pappone *et al.* 1996, Pappone *et al.* 2005, Rogers *et al.* 1997, Sarzi-Puttini and Atzeni 2004). The most prevalent theories seem to link DISH to obesity and diabetes (although recent clinical data suggests no definable link between diabetes and DISH exists; see Sencan *et al.* 2004, Daragon *et al.* 1995) and, in particular, to past monastic communities in England and elsewhere, proposing high animal protein, high calorie diets are to blame (Rogers and Waldron 2001, Stroud and Kemp 1993:212-213, Waldron 1985). It is this latter theory that pervades the bioarchaeological literature and has spawned a whole generation of papers focused on diagnosing or reporting DISH in medieval communities. Some studies have even used the presence of DISH as an indicator of high status or obesity because of the suggested links with monastic communities overeating

and bone fusion. It would appear from the literature that a link between monks, diet, obesity, and DISH is accepted in the bioarchaeological community but this theory has never been tested scientifically. This study attempts to do just that.

The purpose of this research is to test two of the major hypotheses surrounding the aetiology of DISH:

1. That DISH may be related to diet
2. That DISH may be genetically linked;

by using carbon and nitrogen stable isotope analysis and ancient mtDNA analysis to look at individuals with and without DISH buried in both monastic and non-monastic cemeteries from the late medieval period in England. This time period is most frequently associated with monastic studies (since this was a period of monastic development and expansion) and with reports of DISH (for reasons discussed earlier - see above paragraph).

1.1 Stable Isotopes and DISH

There are currently very few studies using stable isotope analysis to investigate disease and no studies that use stable isotopes specifically to study DISH. One of the purposes of this thesis is to use stable isotope analysis to explore the aetiology of DISH by using carbon and nitrogen to help illuminate diet amongst late medieval monks from selected sites in England. In order to make the data more meaningful, stable isotope analysis will also be carried out on non-monastic late medieval populations and the two data sets compared. Essentially, the objectives in terms of dietary analysis are three-fold:

1. To compare the diets of monastic and non-monastic, DISH and non-DISH populations
2. To identify the components of these diets and proportions, if possible
3. To establish, if any, links between animal protein and DISH.

Although no such studies have been carried out on DISH before, previous studies of medieval diet and other periods can be used as a guide. Using the methods of

analysis discussed in Chapter 5 it should be possible to achieve the objectives set out. Any existing differences between monastic and non-monastic, DISH and non-DISH groups should be easily determined by looking at the stable isotope values of ^{13}C and ^{15}N obtained for each group, as should any differences between male and female diet. Similar work comparing different sites has been carried out with success by Müldner and Richards (2005), Mays (1997) and others. Studies comparing groups within sites, including men and women, has also seen success, for example the work of Polet and Katzenberg (2003), Richards *et al.* (1998), Privat *et al.* (2002), Ambrose *et al.* (2003) and others.

Identifying the components of these diets, and their proportions will be more difficult. As will be discussed in Chapter 5, it is not very easy to determine the exact foods being eaten by a population, nor the exact percentages or proportions with which these foods contribute to the diet. Even with the use of linear mixing models, such clarity in terms of dietary components is not always possible. However, documentary evidence from the late medieval period can help to determine what types of foods were being eaten during this time.

The very notion that diet may be a major contributing factor to DISH is due to studies of medieval monasteries that report monks were consuming as much as 6200 calories a day consisting of foods such as deer, geese, chicken, partridge, mutton, eggs, cheese, wine and ale (Waldron 1985, Rogers and Waldron 2001). This diet, combined with little exercise, is thought to have "induced corpulence and invited coronaries" (Waldron 1985:1763) something which is difficult to ascertain from osteological data. This is also the type of lifestyle that can lead to type II (adult-onset) diabetes, a disease that is thought to have a causal relationship with DISH. It is hoped that the stable isotope analysis used in this research can be used to determine if monastic diets really did contain such a high proportion of animal protein and animal fats, and whether the monks were consuming the type of diet that could have led to type II diabetes and/or be related to the existence of DISH.

Although carbon and nitrogen stable isotopes are being utilised, this study is not, however, meant to be an in-depth analysis of medieval diet. Rather, it is an

attempt to elucidate any potential dietary links with DISH through the comparison of dietary isotopic signatures of those individuals with and without DISH and of monastic and non-monastic communities in England during the late medieval period. In order to do this, and in order to help interpret the data, a review of the literature on medieval and monastic diet was necessary and therefore some in-depth discussion on diet is presented.

1.2 Ancient DNA (aDNA) and DISH

Little research has been carried out on genetically inherited diseases in the past, so this study presents a unique opportunity to explore the potential that ancient DNA (aDNA) has to contribute to palaeopathology. As mentioned above, DISH has been suggested to have a genetic component (Crubezy *et al.* 1996, Havelka *et al.* 2001, Havelka *et al.* 2002, Kim et al 2004, Macones *et al.* 1989, Pappone *et al.* 1996, Pappone *et al.* 2005, Rogers *et al.* 1997, Sarzi-Puttini and Atzeni 2004) and reports of the condition manifesting in family groups exist (Gorman *et al.* 2006). Therefore, one of the objectives of this research is to test for genetic links between individuals with DISH.

There are several different methods of genetic analysis available for establishing family relationships. However, these are limited by the problems associated with using ancient DNA (aDNA). Ancient DNA degrades quite rapidly which can mean short fragments, loss of bases, and chemical modification of bases, all of which make amplification of the material difficult and can lead to errors in replication (Jobling 2004:111). Initially, the idea was to perform a kinship analysis of the skeletal remains using short tandem repeats or microsatellites but this technique was deemed unlikely to succeed as to do so thoroughly would take years of laboratory work and analysis. This aDNA analysis, therefore, tests only one aspect of the many potential ways in which DISH could be genetically conferred – whether it exists in families.

Maternal relatedness was chosen as a way of testing for a genetic link between those individuals with DISH – in other words, are those individuals with DISH from the same maternal lineage? Using mitochondrial DNA (mtDNA) to investigate this question is a well-established tool within genetics. Although it does not give a full genetic profile (we cannot determine the paternal lineage or sex of the individual), it has advantages in that mtDNA exists in larger numbers within the cell and is more likely to be detectable within ancient samples than nuclear DNA (see Chapter 6).

The ancient DNA analysis is primarily undertaken to determine if there is any possibility of a genetic link between individuals with DISH amongst the skeletal material analysed. However, it is also intended as a preliminary study to see if late medieval material, that has been previously studied and handled by many different researchers, is a viable resource from which aDNA can be extracted and sequenced successfully.

1.3 Chapter Summaries

The next chapter defines DISH, its aetiology, diagnosis, and differential diagnosis. It discusses the prevalence rates for DISH, in both modern and archaeological populations, with examples from the literature. It also explores the links between DISH and diabetes, and DISH and obesity.

The third chapter explores the archaeological and documentary evidence for monastic diet and lifestyle. It first gives a general overview of monastic orders that existed during the late medieval period, and then a more specific discussion about the structure and day to day activities of the three monastic orders used in this study – Augustinians, Cistercians, and Dominicans. After presenting the archaeological and documentary evidence for diet, it attempts to assess the nutrition of the diets of these monks and similarities and differences between the Benedictines, Augustinians, Cistercians, and Dominicans.

Chapter 4 functions as a comparison to monastic diet by exploring the archaeological and documentary evidence for medieval lay diet. It discusses the basic components of diet and their nutritional quality for the different socio-economic groups in existence during the late medieval period and then uses these descriptions as a template for the non-monastic sites used in this study.

Chapter 5 is an overview of stable isotope analysis, how it relates to diet, and how it can be used to explore the question of whether there is a relationship between DISH and diet and DISH and protein.

Chapter 6 is an overview of aDNA analysis, how it has been applied to archaeological studies, the limitations of such analysis, and how it can be used to look at relatedness amongst the DISH samples used in this study.

Chapter 7 is the materials and methods chapter. It describes the sites used for sampling and the methods of data collection for the DISH and non-DISH samples. It also describes the laboratory protocols for the aDNA and stable isotope analysis and how these samples were analysed.

Chapter 8 presents the results of the scientific analyses. It presents a summary of the skeletal data, and then the data for the stable isotope and aDNA analysis. A discussion of the problems encountered with the stable isotope analysis is included here.

Chapter 9 discusses the results. Incorporating the data from the dietary chapters, it attempts to interpret the findings of the stable isotope analysis and the links between DISH and diet. The aDNA analysis discusses the issues of contamination, presents evidence in support of the authenticity of the samples, and then discusses the results with regards to genetic links between individuals with DISH.

Chapter 10 draws the final conclusions of this research into the aetiology of DISH. It discusses how the objectives were met, how they could be improved upon with further research, and what the final conclusions are about DISH, diet,

and genetics. Consideration is also given to alternative theories that could explain the patterns observed in the data.

Chapter 2: DISH, Diabetes, and Obesity

2.1 DISH

Diffuse idiopathic skeletal hyperostosis is a disorder characterised by hyperostosis and ankylosis of the spinal column on the antero-lateral side, and also ossification of extra-spinal entheses and ligaments (Forestier and Rotes-Querol 1950, Pappone *et al.* 1996, Rogers and Waldron 2001). In laymen's terms, this means that there is excessive bone growth and fusion on the front and sides of the vertebrae and that some of the (soft-tissue) tendons and ligaments that connect the muscles to bone, and bone to bone, at joints throughout the rest of the skeleton have 'hardened' and turned into bone (see Figure 1). The spinal fusion, usually most prominent in the thoracic region, is often described as a 'flowing' ossification that resembles dripping candle-wax and is usually located on the right hand side of the vertebrae, the presence of the aorta on the left preventing ossification on the opposite side (Mader 2002, Reale *et al.* 1999, Sarzi-Puttini and Atzeni 2004). Although there is new bone formation on the vertebral bodies, it should be noted that the intervertebral disc spaces and apophyseal surfaces of the vertebrae are unaffected (Forestier and Rotes-Querol 1950, Reale *et al.* 1999, Rogers and Waldron 2001). This is an important pathognomonic feature of DISH and is key for differential diagnosis, especially when differentiating between DISH and ankylosing spondylitis (AS). The most commonly affected peripheral (non-vertebral) joints are the insertion sites of the Achilles tendon, patellar tendon (see Figure 2), triceps tendon (heel, knee, and elbow, respectively), and the pelvis (Mader 2002, Rogers and Waldron 2001).

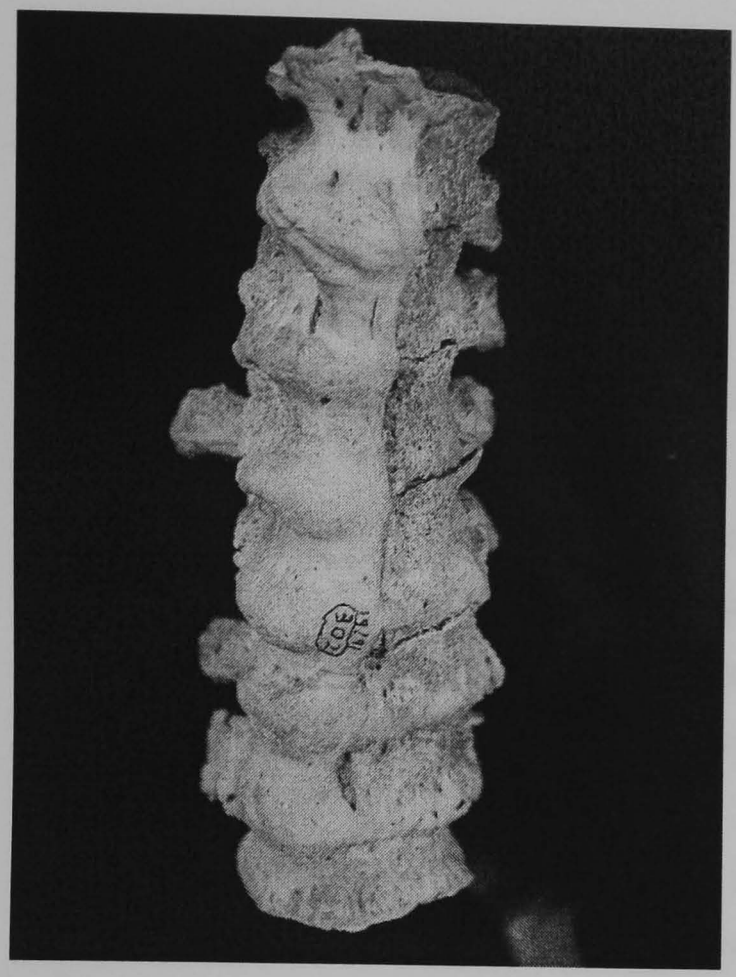


Figure 1. Example of a vertebral column affected by DISH. Skeleton 302 from Blackfriars Friary, Gloucester

Figure 2. Example enthesophytes on the patellae of a skeleton affected by DISH. Skeleton 2905 from Merton Priory, Surrey.

2.1.1 Diagnosis

Different suggestions have been made for the diagnosis of DISH, in both living and bioarchaeological situations, but the most commonly accepted clinical

criteria are based on those of Resnick and Niwayama (1976). Their criteria, based on radiographic studies, are four-fold:

- flowing calcification and ossification of at least four contiguous vertebrae
- intervertebral disc height should be maintained with no evidence of disc degeneration
- no apophyseal joint ankylosis, sacroiliac joint erosion, sclerosis, or fusion
- ossification of the anterior longitudinal ligament (ALL).

Rogers and Waldron (2001) modified Resnick's and Niwayama's criteria suggesting that the following three criteria should be met before diagnosis of DISH can be made in an archaeological skeleton:

- hyperostosis of the spine, affecting at least three vertebrae, with or without ankylosis
- vertebral changes affecting only the right-hand side of the thoracic vertebrae, except in rare cases of *situs inversus* (where organs are located on the opposite side of the body to normal)
- evidence of calcification or ossification in extra-spinal ligaments and/or at entheses.

It is important to be clear on the criteria for diagnosis of DISH as there are several other disorders which result in similar manifestations of spinal fusion such as ankylosing spondylitis, retinol-related hyperostosis, fluorosis, and severe osteophytosis (Childs 2004, Nesher and Zuckner 1995, Rogers *et al.* 1985). These disorders are all considered in a differential diagnosis of DISH but, with careful analysis, can usually be ruled out as they all present slightly different patterns of bone fusion and extra-spinal changes.

2.1.2 Differential Diagnosis

2.1.2.1 Ankylosing spondylitis

Ankylosing spondylitis predominantly affects males in late adolescence or early adulthood, with an age of onset between 15 and 35 years of age, although late-onset of ankylosing spondylitis can also occur (Olivieri *et al.* 2001, Roberts and

Manchester 2005:158). Primarily, it is the spine that is affected, with thin vertical syndesmophytes (bone growth) uniting contiguous vertebrae, beginning in the sacroiliac joints and moving upwards through the lumbar spine (Rogers and Waldron 1995:65, Littleton 1999, Van der Linden *et al.* 1984). The spine is ultimately fused into a 'block' of bone that moves as a single unit, the vertebrae taking on a squared appearance which is sometimes referred to as 'bamboo spine' (Rogers and Waldron 1995:64). The vertebral joint surfaces become eroded and their disc spaces obliterated. Peripheral joints that are affected, mainly by joint erosion, are predominantly in the lower limbs including the knee, ankle, wrist, hand and foot, as well as the hip and shoulder (Littleton 1999, Roberts and Manchester 2005:158, Van der Linden *et al.* 1984). Those affected by ankylosing spondylitis often have the HLA-B27 antigen in their blood and most commonly are Caucasian or Native American (Olivieri *et al.* 2001, Roberts and Manchester 2005:158). Apart from the presence of HLA-B27, this disease differs from DISH in that the sacroiliac joint is affected, the disc spaces are obliterated, there are not usually any peripheral joints affected; osteophytes found in DISH are also generally larger and more irregular and confined to the right side in the thoracic region (Rogers and Waldron 1995:68).

2.1.2.2 Retinol-related hyperostosis

Retinol-related hyperostosis is caused by excess vitamin A. Vitamin A toxicity can cause damage to the liver and central nervous system, problems with child-bearing, hyperlipidemia, and anaemia, as well as mimicking DISH (Nesher and Zuchner 1995). The hyperostoses that develop as a result of excess vitamin A are often located in the axial skeleton but are especially prevalent on the cervical spine, often involving several contiguous vertebrae. Extraspinal hyperostoses are also seen, the most common site for these being the knee. Those with the condition may be asymptomatic or may experience pain, stiffness and a decreased range of motion, similar to those patients with DISH (*ibid.*). Unlike DISH, however, these bone changes are usually seen in younger patients and the bone spurs that develop are usually smaller.

2.1.2.3 Fluorosis

Skeletal fluorosis is a result of an excessive intake of fluoride, though this level of 'excess' appears to depend on the period of exposure, climate, nutritional status of the person, water storage methods, work patterns, tea-drinking habits, and other trace elements in the water, and not just the amount of fluoride (Littleton 1999, Wang et al. 2007). In fact, there does not appear to be a clear linear relationship between the level of fluoride in the water and development of fluorosis, although the minimum level ever reported as causing bone changes is 0.7ppm (*ibid.*). Once fluoride is ingested, it is absorbed into the bone where it replaces the hydroxyl portion of the hydroxyapatite, forming fluoroapatite which increases the density of the bone. This fluoride stimulates irregular bone formation at tendon and ligament insertion sites, causing ossification; increased bone production; thickening of the bone trabeculae; and ossification of spinal ligaments, usually accompanied by enlarged vertebrae completely fused by thick osteophytes (Littleton 1999, Resnick and Niwayama 1983).

The development of fluorosis is influenced by the temperature because, in hot, dry climates, water intake is higher and also evaporation rates are greater, which leaves the water's mineral content, including fluoride, much more concentrated. It is also influenced by nutrition. Malnourished individuals seem to be more prone to developing fluorosis, as do those who drink a lot of tea and consume a lot of fish from the sea, both of which contain high levels of fluoride (Littleton 1999, Whyte *et al.* 2005). The disease is usually seen in older adults, with men more frequently affected than women. The bone lesions that are seen in fluorosis differ from DISH in that the spinal osteophytes form initially in the lumbar vertebrae, as opposed to the thoracic vertebrae as in DISH, the new bone growth is usually centrally placed on the vertebrae and not restricted to the right hand side in the thoracic region, there is usually accompanying tooth discolouration/staining, and both lower and upper peripheral joints are affected (Littleton 1999, Wang *et al.* 2007).

2.1.2.4 Osteophytosis

Osteophytosis is a normal component of an ageing skeleton (van Schoor *et al.* 2005). The stresses of everyday activity such as bending and lifting cause the development of bone or osteophytes at the margins of the vertebral bodies (Roberts and Manchester 2005:135). In extreme cases these osteophytes may fuse together contiguous vertebrae, resulting in ankylosis of the spine. This severe osteophytosis differs from DISH in that the osteophytes are horizontal in nature, rather than vertical as is seen in DISH, and it does not cause the general ossification of tendons and ligaments seen in DISH; therefore, only the spine is usually affected and none of the peripheral joints (Littleton 1999, Prescher *et al.* 1998, Rogers *et al.* 1985). The osteophytes seen in the vertebrae can be accompanied by Schmorl's nodes, small depressions in the vertebral body caused by the nucleus pulposus of the vertebra invading the annulus fibrosus of the vertebra below (Prescher *et al.* 1998, Roberts and Manchester 1995:106).

2.1.3 Symptoms and Aetiology of DISH

Despite the severe appearance of DISH and the extensive spinal fusion often involved, the clinical symptoms of the disorder are usually mild or non-existent. The most common finding is stiffness and a decreased range of spinal movement, especially of the neck and trunk (Rogers and Waldron 2001, Sarzi-Puttini and Atzeni 2004). Studies have found that this spinal stiffness is usually most noticeable in the morning and late evening, and often exacerbated by wet weather, cold, and inactivity (Coaccioli *et al.* 2000). The extra-spinal new bone formation may result in pain; limited movement in joints such as those of the fingers, elbow and shoulder; Achilles tendonitis; and, if spinal fusion has occurred, the individual may also experience some mild back pain (Childs 2004, Sarzi-Puttini and Atzeni 2004). Occasionally, DISH may cause more serious problems such as irritation of vertebral nerve roots or spinal cord compression which can result in paraplegia, and also dysphagia (difficulty in swallowing), if cervical vertebral fusion is involved (Cammisa *et al.* 1998, Rogers and Waldron 2001). However, such conditions are quite rare. The majority of people with

DISH do not experience any discomfort, and some are not even aware that they have the condition.

The aetiology of DISH is as yet unknown, but several theories exist. As discussed above, environmental agents such as excessive fluoride in the air and drinking water can cause spinal fusion through the process of fluorosis. This has led to speculation that a similar mechanism may be responsible for DISH (Childs 2004). Trauma may also be a causal factor in DISH. Clinical studies have shown that those people who exhibit hyperostosis have often been exposed to repeated microtrauma, usually in the form of repetitive heavy lifting or hard physical labour, but this is also true of people who have undergone joint-replacement surgery (Childs 2004, Greenfield and Goldberg 1997, Macones *et al.*. 1989, Pappone *et al.* 1996). In yet another theory, Rogers *et al.* (1997) postulated that it was not the stress itself that caused the bone formation, but that the genetic makeup of the individual was such that they were "bone formers" and would respond to musculoskeletal stress with exaggerated bone deposition. Genetic links have been investigated (Crubezy 1996, Havelka *et al.* 2001, Havelka *et al.* 2002, Gorman *et al.* 2006) and many papers cite or allude to genetic factors as playing a role in the aetiology of DISH (Kim *et al.* 2004, Pappone *et al.* 1996, Pappone *et al.* 2005, Sarzi-Puttini and Atzeni 2004). A more detailed discussion on genetics and DISH can be found in Chapter 6.

In addition to studies of DISH in humans, there have been several studies on DISH in animals but none have revealed any definitive aetiology for the condition. DISH appears to be more common in dogs than other mammals (Wright *et al.* 1982, Woodard *et al.* 1985) but it has also been reported in whales, horses, Rhesus macaques and even prehistoric saber-tooth cats (Bjorkengen *et al.* 1987, Lagier 1989, Sokoloff 1968). Unfortunately, whilst all of these studies clearly describe the bone formation present, they are unable to offer any more insight into the cause of DISH than studies on humans have. Most papers suggest mechanical stress and nutritional disorders as potential links, which is similar to the hypotheses put forth by researchers studying DISH in humans.

In human studies, one of the most common theories on the aetiology of DISH, however, is that it is related to obesity and type II diabetes. Studies on diabetes and DISH have found that there seems to be an association between the two, with DISH being more prevalent in people with type II diabetes but, as yet, there has been no statistically significant evidence found to support these findings (Daragon *et al.* 1995, Sencan *et al.* 2004). This concept has been particularly prevalent in the archaeological literature, most likely perpetuated by the image of the medieval fat monk, especially as the documents and account books from medieval monasteries seem to show us that they were consuming many different types of dishes on a daily basis including several types of game, fish, beef, suckling pigs, cheeses, sauces, ales and wines (Harvey 1993:44-52, Rogers and Waldron 2001, Waldron 1985). The average daily allowance in terms of calories has been calculated to be over 6000 (see Harvey 1993:64-65), which is more than double the 2500 calorie allowance for a man today (NHS Direct, accessed August 2008). If this dietary picture is accurate, then the suggestion that obesity and type II diabetes existed amongst the inhabitants of medieval monasteries must certainly be true (see Chapter 3 for discussion).

2.1.4 Prevalence of DISH in Modern and Archaeological Populations

The reported modern day prevalence rate for DISH varies depending on the study. However, there is a general consensus that it exists in greater frequency in older people (it appears to be absent in people under 40 years of age), increasing in both frequency and severity with increasing age and weight, and is more prevalent in men than women, many studies reporting an approximate 2:1 prevalence rate (Cammisa *et al.* 1998, El Miedany *et al.* 2000, Sarzi-Puttini and Atzeni 2004). The modern prevalence rate for those over 40 years of age has been reported as 3.8% in men and 2.6% in women, and for those over 65 years of age as being 12-22% in men and 12-13% in women (El Miedany *et al.* 2000, Rogers and Waldron 2001). It seems to be more predominant in Caucasians, Japanese, Pima Indians, African Blacks and Jews in Jerusalem and less

predominant in American Blacks, Asians, and Native Americans (Childs 2004, Rogers and Waldron 2001).

2.1.4.1 Modern Studies of DISH

Julkunen *et al.*'s paper (1971) was one of the first population based studies of DISH. They took chest radiographs of 12, 858 people over the age of 15 from six different communities in Finland and examined them for the presence of a minimum of three contiguously fused vertebrae. In total, they found 164 people with DISH; the youngest person being 43 years of age. In those over 40 years of age, there was a prevalence rate of 3.5% in men (92/2691) and 2.2% in women (70/3238). Amongst women, there was also a geographic difference in prevalence rates with numbers of DISH being higher in East Finland than West Finland.

A clinical study of 247 radiographs from a hospital in Campania, Italy, resulted in the identification of 69 individuals with DISH (22 males, 47 females), a prevalence rate of 28% using Resnick and Niwayama's (1976) criteria (Pappone *et al.* 1996).

Kiss *et al.* (2002) carried out a population based study of DISH in Budapest, Hungary. The authors randomly selected 635 people from a population of 120,000 to take part in the study. Using criteria of three contiguously fused vertebrae, they found a prevalence of 27.3% (84/307) in men and 12.8% (42/328) in women; using four contiguously fused vertebrae, they found a prevalence of 6.1% (19/307) in men and 1.2% (4/328) in women. They calculated the male to female ratio as being 2.1:1.

Kim *et al.* (2004) carried out a large-scale study on hospital outpatients treated for non spinal-related conditions in two cities in Korea. Using both Resnick and Niwayama's criteria and Julkunen *et al.*'s (1971) they examined 3595 chest radiographs for evidence of DISH. The found 2.9% (104 of 3595) of their study group had DISH using Resnick's criteria, and 4.1% using Julkunen's. There was

a significant increase in DISH with age and a sex bias with 5.4% of men being affected as opposed to 0.8% of women.

Despite reports of a male bias amongst DISH subjects, an Italian study of DISH reported a 15.1% overall prevalence in the female participants, with a 7.5% prevalence in those aged 50-59, and a 40% prevalence in those over 70 years of age (Pappone *et al.* 2005).

Westerveld *et al.* (2008.) also looked at chest radiographs from hospital outpatients. The 501 Dutch subjects were admitted for non spine-related conditions and were aged over 50 years of age. Using Resnick and Niwayma's criteria, they found a 17% prevalence rate for DISH with significant increases in prevalence amongst older individuals and men.

2.1.4.2 Archaeological Studies of DISH

In archaeological populations prevalence rates also indicate that DISH is a common occurrence, particularly in medieval monastic communities in England, an idea that has been perpetuated by Waldron (1985) and Rogers and Waldron (2001). They have reported rates of DISH in late medieval sites as being 8.6% at Merton Priory, 13.4% amongst those over 40 years of age at Blackfriars Friary, Ipswich, and 9.1% at Wells Cathedral, all in England, and 100% at the Basilica of Saint Servaas, Maastricht, in the Netherlands. Waldron (1985) and Rogers and Waldron (2001) believe that this high prevalence rate may be attributable to a 'high status' lifestyle and, in particular, to diet and consumption of lots of meat, but these are only theories and, as yet, they have not been investigated thoroughly.

2.1.4.2.1 Archaeological Examples from the Medieval Period

Merton Priory in Surrey, England, was one of the first Augustinian houses founded in 1140. Excavations in the late 1970s recovered 35 almost complete

skeletons and the bones from an additional seven individuals from the chapter house and the canon's cemetery that lay to the east (Waldron 1985). Of these 42 skeletons, only one was female, assumed to be a benefactress of the priory, and the rest were male and thought to be priors. Three skeletons were found to have changes consistent with DISH, that is, spinal and extraspinal lesions, and an additional eight had extraspinal manifestations of the disorder. Of these eight, four had sufficient vertebrae to make a diagnosis but three did not have any changes to the vertebrae that would suggest DISH. The remaining skeleton had extensive fusion all the way around the anterior bodies of the vertebrae suggesting a seronegative arthropathy, rather than DISH, but no apophyseal or sacroiliac joints survived in order to confirm or reject this diagnosis. Four of the skeletons with extraspinal hyperostoses did not have any preserved vertebrae and so an assessment could not be made regarding DISH. The prevalence of DISH at Merton Priory was calculated to be 8.6% (Waldron 1985).

Blackfriars Friary, Ipswich, England, was founded in 1263. It was excavated between 1983 and 1985 with 250 inhumations found, mostly from the church nave, although a number of burials were also uncovered from the church choir, the cloister, the chapter house, and to the south of the nave (Mays 1991). Twenty-one individuals were found showing bone changes thought to be associated with DISH but only ten of those met the criteria for diagnosis – i.e. had sufficient vertebrae to examine that were fused. Of these 21 skeletons, only two were female, one of which was included in the ten that met the criteria for a diagnosis of DISH. However, it must be kept in mind that there were only 58 females with vertebrae to study in contrast to 120 males. The prevalence of DISH in those over 40 years of age who had three or more vertebrae available to study was calculated to be 13.4%.

The Saint Servaas Basilica in Maastricht was in use between 1070 and 1521 and excavated between 1981 and 1989 (Janssen and Maat 1999). Twenty-seven burials were excavated from the chapel (Siftskapel) that was reserved for burial of the canons. Even though only 22 of the skeletons could be sexed and determined to be male, all 27 burials were believed to be males since all canons in the late medieval period were males and only canons would have been buried

beneath the Siftskapel. All of these skeletons were older adults, aged between 50 and 79 years old, with an average age at death of 56.

Reale *et al.* (1999), published the case of a single male adult (aged 23-35 years) with DISH from the cemetery of S. Angelo Abbey in Montescaglioso, Southern Italy. The skeleton, whose burial dated from 1100-1400AD, had flowing ossifications on the right side of the anterior ligament of T6 to T12 and also new bone formation on the extra-spinal elements consistent with a diagnosis of DISH. Although the identity of the individual could not be given with any certainty, it was suggested that he might have been a monk.

Mays (2000b) explored DISH in skeletons of peasants from the rural village of Wharram Percy, Yorkshire, comparing them to lay benefactors from Blackfriars Friary, Ipswich, and then comparing both medieval populations to a modern study. Using the criteria of three contiguously fused vertebrae, Mays found five cases of DISH from Wharram Percy (all males) and 11 from Blackfriars Friary (10 males, 1 female). These represented prevalence rates of 3.4% and 15.9%, respectively.

Vidal (2000) examined 99 skeletons with preserved vertebrae from four cemeteries dating to the 6^{th} to 8^{th} century in Eastern France. Two of the four cemeteries contained individuals with DISH with varying degrees of vertebral ossification. Vidal identified 13 potential cases of DISH, of which nine were males.

Wells Cathedral, in Somerset, England, was excavated between 1978 and 1982, during which time 337 skeletons were recovered (Rogers and Waldron 2001). These included lay individuals buried in the lay cemetery and also religious inhabitants of the Cathedral buried in the 13^{th} century lady chapel and 16^{th} century Stillington's chapel. In total, six males from the lay cemetery (6.5%), two from the lady chapel (13.3%), and three from Stillington's chapel (23.1%) were found to have DISH. Although this difference in prevalence rates between the lay cemetery and the chapels was not statistically significant, the authors found them highly suggestive of a difference in prevalence of DISH between lay and religious burials, especially as the Bishop of Wells, Giso, exhibited all the

classic manifestations of DISH. The overall prevalence rate for males at this site was calculated at being 9% (11 of 121 males affected).

Verlaan *et al.* (2007) examined 51 individuals from the Onze Lieve Vrouwe Church in Maastricht (Netherlands), which was founded in the 11th century. The authors reported 17 of 42 adults had DISH (40.4%) – ten males, five females, two adults of indeterminate sex. The adults ranged from 23 to 69 years in age, with a mean age of 49.5 +/- 13 years. The authors believe that the males from this site were clergymen.

2.1.4.2.2 Archaeological examples of DISH from outside the medieval period

DISH also existed outside the medieval period, both in Europe and the rest of the world. Examples from Africa, Asia, South America, and Eastern Europe have been found in archaeological populations.

Crubezy and Trinkaus published their diagnosis of DISH in the Shanidar 1 Neanderthal skeleton (from Shanidar Cave in Iraq) in 1992. Although not a complete skeleton, this individual was sexed as male and aged at 35-40 years of age. The evidence for DISH, which is not particularly convincing, consists of a large osteophyte present on L3, a smaller osteophyte on L5, and enthesophytes on the ulna, patella, calcanei, femur, and coracoid process of the scapula. Although not conclusive, Crubezy and Trinkaus found this pattern of lesions to be highly suggestive of DISH making this the oldest documented case in humans. More recently, another Neanderthal skeleton (from Kiik-Koba in the Ukraine) underwent a re-assessment of its palaeopathological lesions. The skeleton, thought to be male and approximately 40 years of age, was found to have large patella and calcaneal osteophytes and smaller osteophytes on the distal hallucal phalanx, talus, and intermediate cuneiform and, on the basis of these bone growths, which, again, are not very convincing, was also suggested to have DISH (Trinkaus *et al.* 2008).

Arriaza has studied DISH in populations from both prehistoric South America and Africa. He found a 1.4% prevalence rate (5 of 340 skeletons) amongst adults from Arica and Iquique, Chile, which increased to 4% if only those over 40 years of age were considered (Arriaza 1993a). In Sudan, Arriaza found a 13.4% prevalence rate (18 of 134 skeletons) amongst Meroitic Nubians dating from 350BC to AD350 (Arriaza 1993b).

Crubezy (1996) published a study of DISH in two European populations – one from Vredovic, Moravia, dating to 5700BC and the other from Nitra-Horne Krskany, Slovakia, a few centuries younger. Of those skeletons aged over 30 years, four of 36 (11.1%) from Vredovic and two of 27 (7.4%) from Nitra-Horne Krskany had DISH, a total of six of 63 skeletons (9.5%).

Jankauskas (2003) studied 458 skeletons from two Lithuanian populations – 142 skeletons from the first millennium AD and 316 from the second millennium AD – aged between 20 and 50 plus years old. The incidence of DISH was similar in both groups, with the earliest appearance in the 30-40 year age group, resulting in a combined prevalence of 18.01% in males and 2.61% in females. A significant increase in DISH was found with those over 40 years of age.

Oxenham *et al.* (2006) published the first two probable cases of DISH from prehistoric Japan. Two adults representing the late Jomon culture from Funadomari, on an island just off the northern tip of Hokkaido, exhibited signs of DISH. The first adult skeleton was fragmentary but showed fusion of a minimum of four contiguous thoracic vertebrae. The second skeleton, an adult male aged 40-45 years, had fusion of only two vertebrae but also hyperostosis in the appendicular skeleton, most markedly in the sternum, ulna, and patella.

As can be seen from this brief literature review, there have been many reports of DISH amongst medieval religious communities. However, as the numerous other reports from non-medieval populations suggest, DISH is not confined to any one time period or archaeological context (see Table 1). It has been seen in ancient hominids through to modern day humans and in as diverse populations as rural peasants, subarctic sea mammal hunters, and prehistoric Indians, as well as

medieval monastic males. It is hard to believe that such diverse groups of people, many of whom lived in marginal environments, were all obese and partaking in great feasts as Rogers and Waldron (2001) suggest is the aetiology of DISH. However, as religious communities have so often been cited as having high prevalence rates of DISH and suggestions of obesity, type II diabetes, and high intakes of meat put forth as aetiological reasons for this, the relationship between medieval monks and obesity/diabetes/meat must be explored.

Table 1. Modern and Archaeological Prevalence of DISH

Site/Population	Period	Prevalence	%	M	F	Criteria	Authors
Italy	Modern	69/247	28%	22	47	Resnick	Pappone *et al.* 1996
Netherlands	Modern	85[1]/501	17%			Resnick, >50years	Westerveld et al. 2008 [1] actual value not reported, estimated from percentage value
Korea	Modern	104/3595	2.9%			Resnick	Kim et al. 2004
			4.1%	5.4%	0.8%	Julkunen	Kim et al. 2004
Hungary	Modern	126/635	19.8%	84/307 27.3%	42/328 12.8%	4 fused verts	Kiss et al. 2002
		23/635	3.6%	19/307 6.1%	4/328 1.2%	3 fused verts	Kiss et al. 2002
Finland	Modern	164/12,858	1.3%	[2]92/269 1 3.5%	[2]70/32 38 2.2%		Julkunen et al 1971 [2]over 40 years
Italy (females only)	Modern		15.1%		15.1%		Pappone et al. 2005
Merton Priory, Surrey, UK	Archaeological Late Medieval		8.6%				Waldron 1985
Blackfriars, Ipswich	Archaeological Late Medieval		13.4%				Mays 1991 (monks)
Blackfriars, Ipswich, UK	Archaeological Late Medieval		3.4%				Mays 2000b (lay benefactors)
Wells Cathedral, Somerset, UK	Archaeological Late Medieval		9%	11/121 9%			Rogers and Waldron 2001
Saint Servaas Basilica, Maastricht, Netherlands	Archaeological Late Medieval	17/17	100%	17/17 100%			Janssen and Maat 1999

Onze Lieve Vrouwe Church, Maastricht, Netherlands	Archaeological Late Medieval	17/42	40.4%				Verlaan et al. 2007
S Angelo Abbey, Italy	Archaeological	Case study					Reale et al. 1999
Wharram Percy, Yorkshire, UK	Archaeological Late Medieval		15.9%			3 verts	Mays 2000b
France	Archaeological 6^{th}-8^{th} century	13/99	13.1%	9			Vidal 2000
Eastern Europe	Archaeological ~6000BC	6/63	9.5%				Crubezy 1996
Lithuania	Archaeological 1^{st} and 2^{nd} millennium AD			18.0%	2.6%		Jankauskas 2003
Japan	Archaeological Late Jomon period						Oxenham et al. 2006
Shanidar Cave, Iraq	Archaeological ~50,000 years BP	Case study					Crubezy and Trinkaus 1992
Kiik-Koba, Ukraine	Archaeological ~50,000 years BP	Case study					Trinkaus et al. 2008
Chile	Archaeological Prehistoric	5/340	1.4%				Arriaza 1993a
Sudan	Archaeological 350BC-AD350	18/134	13.4%				Arriaza 1993b

2.2 Diabetes

Diabetes develops either when there is a shortage of insulin, the hormone produced by the pancreas that stimulates the movement of glucose into the cells of the body so that it can be converted into energy, or when the insulin that does exist doesn't work properly – both of which result in hyperglycaemia, an excess of glucose in the bloodstream (Kelleher 1988:1-3). Two types of diabetes exist – Type I diabetes, also called Juvenile Onset Diabetes or Insulin Dependent Diabetes; and Type II diabetes, also called Adult Onset Diabetes. The terms Juvenile Onset and Adult Onset are being used less frequently though because, as obesity increases rapidly amongst young children, it leads to a rise in the occurrence of Type II diabetes, previously thought to only exist in adults.

In Type I diabetes, the body attacks its own insulin-producing cells in the pancreas resulting in an insulin deficiency which must be corrected with insulin injections (Watkins 2003:3). In Type II diabetes, it is thought that insulin production is working properly but that the receptor sites that allow insulin to deliver glucose to the cell are not, resulting in an accumulation of glucose and insulin in the bloodstream (*ibid.*). The actual mechanisms of type II diabetes are unclear but this type of diabetes is usually prompted by a poor diet, obesity, environment, and a genetic predisposition (Kelleher 1988:6-13, Watkins 2003:3), and is the form thought to be associated with DISH. There are, in fact, many aetiological similarities with DISH, including that the typical age of onset is over 40 years of age and that it may be related to obesity (Watkins 2003:3). The symptoms of Type II diabetes include extreme fatigue, weight changes, blurred vision, drowsiness, tingling or numbness in the hands and feet, slow wound healing, unwarranted hunger, frequent urination and excessive thirst (Kelleher 1988:4-6). Treatment of the disease is through oral medication, diet and exercise; if left untreated, the end result can be neurological disorders, cardiovascular disease, organ failure and even foot amputation if ulceration occurs (Kelleher 1988:5,13). Unless otherwise stated, the use of the term diabetes throughout this chapter, and the remainder of the thesis, refers to Type II diabetes, the type that has links with obesity.

Diabetes Mellitus is a disease that is rapidly increasing in modern societies. In India, cases of Type II diabetes are escalating at phenomenal rates, having quadrupled in less than 30 years (Yajnik 2004). It is estimated that by 2025, India will have over 60 million diabetic patients, representing one patient out of every five in the world (Yajnik 2004). In Great Britain more than two million people have been diagnosed with diabetes, the equivalent of three in every 100 people, and there are thought to be a further one million undiagnosed cases throughout the country (Diabetes UK 2000, accessed February 2006). The World Health Organisation reported in 2005 that cases of obesity are due to rise in the UK in the next ten years by 25% and, because obesity and diabetes are related conditions, this will result in an increase in diabetes in the British population. Those most at risk of developing the disease are minorities; Afro-caribbeans and Asians in the UK have a prevalence rate that is five times higher than the rest of the population (Diabetes UK 2004, accessed February 2006)

2.2.1 Aetiology of Diabetes

Some common theories as to the aetiology of diabetes that are emerging in the literature include 'thrifty genes' and the 'fetal origins hypothesis'. The idea of thrifty genes, first proposed by Neel (1962), is that the genes that favour the economical use and storage of energy may have developed as a survival advantage during times of prolonged famine in early human history (Lazar 2005). There would have been an evolutionary pressure for glucose to be preserved for use by the brain during times of starvation and also a propensity to increase adipose tissue for fat (energy) storage. This means that in present day settings, where lifestyles are more sedentary and food higher in calories, people will have an increased tendency to accumulate fat thus, according to the theory, leading to the epidemics of obesity and diabetes. The fetal origins hypothesis or 'thrifty phenotype' hypothesis, as proposed by Hales and Barker (2001), is based on clinical observations that fetal malnutrition alters metabolic pathways in such a way as to favour the 'thrifty' use of nutrients in utero and postnatal life. This means that once adequate nutrition is finally introduced, the end result is

increased insulin resistance and an accumulation of fat (Lazar 2005, Yajnik 2004). However, the association between increased risk of diabetes and low birth weight may be more complex than this, and recent studies have suggested that fetal diabetes and low birth weight may actually be genetically determined but also influenced by the intrauterine environment (Yajnik 2004).

There are several factors that influence the development of diabetes, many of them dietary. The consumption of just an extra 100 calories a day can result in 5kg of weight gain over a year (Levine *et al.* 2005); if more carbohydrates are consumed than necessary for energy requirements, the excess is stored as fat, which can lead to obesity. However, not all obese individuals are insulin resistant, and approximately half of hyperinsulinemic people are of normal weight (Kelleher 1988:3, Levine *et al.* 2005). Complex carbohydrates (polysaccharides) are the most beneficial to metabolism e.g. cellulose (plant cell walls), hemicellulose (cereal fibres), pectin (found in fruit and vegetables), gums and mucilages (plant secretions). In terms of food sources, these equate to foods like wheat, oat bran, stalks and leaves of vegetables, seeds and fruits (Department of Health 1991:62-63). Consumption of complex carbohydrates results in a steady blood sugar, which reduces the chance of flooding the bloodstream with a sudden burst of insulin. There are mixed opinions on the influence of protein on insulin, but the majority of researchers say that excess protein results in increased insulin levels; which also creates demands for vitamin B6 and calcium and stresses the kidneys (Barzel and Massey 1998, Johnston *et al.* 2004, McAuley *et al.* 2004).

It has been suggested that, in order to combat this insulin resistance, a diet consisting of 45% of calories from carbohydrate, 40% from 'good' fats, and 15% from protein would be beneficial (Department of Health 1991:54). However, this was considered to put diabetics at risk of developing other diseases related to the higher blood cholesterol that a high fat diet would create, such as cardiovascular disease. More recently, it has been recommended that diabetics consume a diet high in carbohydrates, and low in fat and saturated fatty acids, in order to reduce such risks and also combat diabetes. However, diets very high in

carbohydrate (60% plus) may be detrimental as they raise blood triglyceride concentrations and lower HDL cholesterol (Department of Health 1991:54).

2.2.2 Diabetes and DISH

Several studies looking at the relationship between diabetes and DISH have been published in the medical literature, though none seem to have conclusive results. Kiss *et al.* (2002) compared the lifestyles of those with DISH and with spondylosis, looking at factors such as weight gain from the age of 25yrs, illnesses, alcohol, calcium intake, and smoking habits. One hundred and thirty one patients - 69 males and 62 females – with a mean age of 65yrs were examined. They found that occurrences of hypertension and cardiovascular disease were similar in both groups, but non-insulin dependent (type II) diabetes was more frequent in those with DISH. DISH was also found to be associated with high BMI (body mass index), but not diabetes, in medical history records. They concluded that, as those with DISH were more obese at age 25 than the control group and weighed more, it was highly suggestive that obesity was a risk factor for DISH.

Julkunen *et al.*'s (1971) study of DISH in a Finnish population, discussed above, also looked at hyperglycaemia (a potential indicator of diabetes) in association with DISH. They found a higher prevalence of hyperglycaemia amongst people with DISH than in control subjects. However, geographical differences had also been found amongst prevalence rates of DISH and the glucose tolerance did not correlate with this.

Daragon *et al.* (1995) looked solely at diabetes and hyperostosis. They observed 50 people aged 60 yrs+ with vertebral hyperostosis (DISH) and matched them for age, sex, height and weight with 50 people without vertebral hyperostosis. These were examined radiographically and a glucose tolerance test was performed. They found that six in the test group and six in the control group had diabetes, whilst 12 in the test group and 16 in the control group had an impaired glucose tolerance. There was no statistically significant difference in terms of

weight/height index between the vertebral hyperostosis and control groups and, although it was slightly greater in diabetics than non-diabetics, this difference was not statistically significant either. As no difference in glucoregulation was found between the test and control groups, no relationship between vertebral hyperostosis and diabetes was concluded but, like Kiss *et al.* (2002), they suggested that weight may be an aetiological factor for vertebral hyperostosis.

Littlejohn (1985) postulated that DISH was related to increased levels of insulin. He reviewed studies of vitamin A toxicity, obesity, and type II diabetes and their relationships to DISH, finding that higher frequencies of the disease occurred in those patients with adult onset diabetes and, vice versa, that those with type II diabetes had high incidences of DISH. Littlejohn noted that there was a relationship between hyperglycaemia (impaired glucose tolerance due to high levels of insulin), as occurred in type II diabetes, and bone growth, and that no such bone growth occurred in cases of type I diabetes where insulin was deficient. He supports these observations with studies of the Pima Indians, a population who have a very high prevalence of type II diabetes and associated hyperinsulinaemia; they also have a high prevalence of DISH, the highest of any population, with over 50% of males over the age of 55 having the condition. Additionally, he points out that other bone growth stimulating disorders such as acromegaly and hyperostosis frontalis interna are also accompanied by hyperinsulinaemia.

Coaccioli *et al.* (2000) re-investigated the links between hyperinsulinemia and DISH with a three-fold objective: to examine the relationship between DISH, diabetes, glucose tolerance and obesity; to examine the relationship between hyperinsulinemia and DISH; and to determine the proportion of symptomatic patients. They looked at 170 subjects which they divided into six groups: type I diabetes, type II diabetes, non-obese type II diabetes, impaired glucose tolerance, obese, and normal. They found 40% of the obese type II and 37.5% of the obese subjects had DISH, versus 30% of the non-obese, and that a very small proportion of those with DISH were symptomatic. However, despite a higher prevalence of DISH in diabetic patients, there were no statistically significant

correlations between blood glucose and DISH so the authors concluded that the aetiology was still unknown.

Sencan *et al.* (2004) took a slightly different approach and, rather than examining the prevalence of diabetes in those patients with DISH, examined the prevalence of DISH in patients with diabetes. They used 133 endocrinology outpatients with type II diabetes as a test group and 133 age/sex/weight comparable non-diabetics as controls and performed various laboratory tests such as glucose levels, cholesterol, blood insulin levels etc. They diagnosed DISH (radiographically) in 16 patients (12%) in the diabetes group and nine (6.8%) in the control group but these differences in prevalence rates were not statistically significant. The mean age of those with DISH was, however, statistically significantly higher than that of the 241 remaining subjects, but this is in line with what we already know about the late onset of DISH. The authors concluded that those factors thought to be responsible for the aetiopathogenesis of DISH such as diabetes, high insulin levels, and growth peptides, need further evaluation.

The studies on DISH and diabetes examine different aspects of the disease. Whilst links between diabetes, insulin levels, and weight are continually being investigated it is evident from these studies that there are no clear links between DISH and diabetes. There do, however, appear to be some possible links with hyperinsulinaemia and some strong correlations with increased weight.

2.3 Obesity

Obesity, currently the world's most common metabolic disease, is a chronic disease defined as the increase in body fat stores (Formiguera and Canton 2004). Studies report frightening statistics such as a 50% increase in the disease worldwide in the past ten years and that one third of all Americans born in the year 2000 will develop diabetes, mostly as a result of increasing obesity (Formiguera and Canton 2004, Lazar 2005). More alarming is that the UK, not

the USA, leads the developed world in the fastest growing obesity rates (Diabetes UK 2004, accessed February 2006). Obesity amongst adults in Britain increased from 14% to 22% in the years 1994 to 2004, and a quarter of all children are currently overweight or obese (Diabetes UK 2004, accessed February 2006). 'Central-type obesity', accumulation of fat around the waist and hips, carries the highest risk for developing type II diabetes, as well as high blood pressure, heart disease, stroke, dyslipidemia, joint disease, and respiratory disorders (Formiguera and Canton 2004). This is because those individuals with central fat distribution are more prone to insulin resistance, something which only improves with weight loss, best achieved through a reduced calorie diet and regular exercise (Klein *et al.* 2004).

2.3.1 Obesity and Diabetes

Obesity is deemed to be the most important predictive factor when it comes to type II diabetes. Studies have shown that the disease is three to seven times more prevalent in obese adults than normal-weight adults, and that the only way to regain glycemic control is through weight loss (Klein *et al.* 2004). Diet is as important as exercise for those with diabetes as an increase in carbohydrates exacerbates dyslipidemia (elevated triglyceride levels and low HDL cholesterol concentrations), a disorder which is associated with insulin resistance and type II diabetes. In order to reduce the risk of developing diabetes, or reduce the mortality rate in those who already have type II diabetes, a controlled diet, regular exercise and aerobic fitness are essential (Klein *et al.* 2004).

According to Lazar (2005), the link between obesity and diabetes is thought to be due to the hormone leptin. This hormone acts as a signal of energy stores; when energy is sufficient, leptin will inhibit food intake and accelerate metabolism. Studies on rodents and in humans have shown that a fall in leptin will result in increased appetite and decreased metabolic rate, whilst an absence of leptin will result in obesity and insulin resistance. Those with only one functional copy of the gene will have increased body fat, hence elevated leptin levels are found in obese individuals.

There are also other theories with respect to obesity and diabetes, one of which proclaims that obesity and diabetes are both inflammatory conditions, so the presence of inflammation can predict the development of type II diabetes (Dandona *et al.* 2004). There is a significant correlation between body mass index and concentration levels of a proinflammatory cytokine called tumour necrosis factor-alpha (TNF-α). Expression of this cytokine is found to be increased in obese animals, whereas neutralisation of it leads to a decrease in insulin resistance. It is also known that stress can induce an inflammatory state which can result in the decline of glucose homeostasis in those individuals with diabetes. However, it is not known whether stress alone can cause the development of diabetes.

Wannamethee *et al.* (2005) studied a group of 7176 British men aged 40-59 who had no diagnosis of either cardiovascular disease or diabetes in order to find out whether any weight changes they experienced over the next 20 years influenced the risk of developing these diseases. Their weight, height, and body mass index (BMI) were calculated at the initial screening, five years later, and at 20 years when the study ended. On the basis of their BMI, the men were divided into four groups – normal, moderately overweight, considerably overweight, and obese – and, across all these groups, weight loss was associated with a decrease in the risk of developing diabetes and weight gain was associated with an increase in the risk of developing diabetes.

Schwartz and Porte Jr (2005) proposed that the brain plays an important role in glucose levels in the body, an idea that was first hypothesised in the 1850s. The brain is able to sense when the body's energy content and nutrient availability is sufficient and resist any further release of fat from the body's energy stores and circulating nutrients such as glucose. The neuropeptides in the hypothalamus of the brain are inhibited by leptin and insulin, and therefore reduced signalling from these hormones increases the output of these neuropeptides, which will stimulate food intake and reduce energy expenditure, thereby promoting weight gain. These neuropeptides have also been found to cause insulin resistance and glucose intolerance even when their effects on food intake have been blocked.

Therefore, any defects in the brain's sensing of, or response to, insulin and leptin will put into effect a chain of events that leads ultimately to obesity and diabetes.

2.3.2 Obesity and DISH

Few clinical studies focused solely on obesity and DISH exist. Whilst several papers cite obesity as a likely contributing factor to DISH (Coaccioli *et al.* 2000, Julkunen *et al.* 1971, Kiss *et al.* 2002, Sarzi-Puttini and Atzeni 2004), it is usually in relation to the link between obesity and diabetes, whereby obesity is a major contributor to type II diabetes and the insulin resistance that causes diabetes has links with bone formation (although these relationships are still unclear and undergoing further investigation). The majority of bioarchaeological papers, however, still point to obesity as being a likely cause of DISH in medieval monastic communities, citing examples of eating practices or, rather, *overeating* practices, but could these links between obesity and monastic communities really exist?

A study conducted in the late 1960s by Strong *et al.* (1967) overfed both normal weight and obese subjects by amounts ranging between 740 and 1970 kcal per day for four days, the average being 1530kcal. The differences in calorie excess depended upon how much exercise the subject took, which varied between 30 minutes and an hour per day. Their weight gain in those four days ranged from 370g to 5460g, with, surprisingly, some of the obese subjects gaining the least weight. The metabolic rate of the patients increased slightly, to compensate for the extra energy, but it amounted to burning off only about 20% of the dietary excess. Overall, the body was unable to adapt to the surplus food supply and, hence, increased fat stores. If we use this study as a model for monastic communities, we can draw comparisons between the two groups. It appears from the dietary records that the monks were consuming excess calories on a daily basis, at least as much as 600 kcal on an average day. As they appear to have done little exercise, with manual labour reduced to a bare minimum in most monastic communities (see Chapter 3), it was probably comparable to the 30 minutes to an hour exercise undertaken by the subjects in the overfeeding study.

Therefore, we could postulate that if the monks were eating to excess then they were certainly gaining weight, as the subjects in the overfeeding study did, even if we cannot calculate precise amounts.

The amount of exercise undertaken on a daily basis certainly impacts on the energy expenditure of an individual. However, it has been shown by Levine *et al.* (2005) that non-exercise activity, specifically posture, can also impact on energy expenditure. They studied the movements of lean and obese subjects over ten days and calculated their NEAT (non-exercise activity thermogenesis) rates associated with standing, sitting and lying, to see whether posture impacted upon weight gain. They found that the lean participants were upright for 152 minutes longer than the obese participants, and seated for 164 minutes less, which resulted in expenditure of an average of 352 kcal per day. In order to investigate whether these differences were a cause or consequence of being obese, Levine *et al.* asked the obese subjects to undergo a period of weight loss, and the lean subjects to undergo a period of overfeeding, and then re-measured their NEAT values. They found that both groups maintained their original values, thereby indicating that it was the posture behaviour, and not the weight of the individual, that influenced energy expenditure. The authors calculated that if the obese subjects adopted the posture behaviour of the lean subjects they could expend an extra 350 kcal per day, resulting in weight loss of 15kg over the course of a year. Applying this to monastic life, it is likely that posture and body movements changed little on a day to day basis as the daily round was so prescribed. Since the monastic Rules tended to focus on praying, singing, and copying books, a good portion of a monks' day would likely be spent sitting or kneeling. One could postulate that this might be imitative of the 'obese' NEAT postures and, if so, then the monks would have easily put on weight, especially as regular aerobic exercise was not part of their routine.

Another potential source of weight gain in monastic communities is a lack of sleep. According to a study conducted by Gangswisch *et al.* (2005), which used data from a 20 year cross-sectional and longitudinal study of Americans about their health, a lack of sleep is strongly associated with obesity. They report that there were significant differences between sleep duration and obesity for subjects

between the ages of 32 and 49. The highest percentage of obese people were in the category reporting less than seven hours sleep a night, with those getting two to four, five, and six hours sleep being 235%, 60%, and 27%, more likely to be obese, respectively. The average increase in BMI (body mass index) was highest in those people getting two to four hours sleep a night and lowest for those reporting ten or more hours sleep a night. We know that monastic orders had a strict routine of services and activities that they had to follow, and, although the monks got eight hours of sleep in the winter, in summer this was reduced to five hours on account of the earlier sunrise and later sunset. Five hours sleep per night would, according to Gangswisch *et al.*'s study, put them in the "60% more likely to be obese" category. Even if they were only put at risk of being obese for half the year, when combined with other factors such as little exercise and a high calorie diet, it seems likely that obesity could be an issue in medieval monasteries. Sleep loss has also been linked to decreased glucose tolerance, insulin resistance and a prediabetic metabolic state with those people on four hours sleep a night experiencing a 30% reduction in insulin response to glucose. This would suggest another link to DISH, that a lack of sleep could contribute to the development of diabetes and also insulin resistance.

One PhD thesis examined the opposite relationship between obesity and DISH. Using DISH as a marker for obesity, Patrick (2005) found a greater association of obesity-related osteoarthritis with monastic skeletons than secular ones. However, she did not find any significant differences in BMI (body mass index, calculated as weight in kilograms divided by height in meters squared) between monastic and secular assemblages, with an average BMI of 24.47 for monastic individuals and 24.64 for secular, placing them in the "acceptable" weight range and not "obese" categories as defined by the World Health Organisation (see Patrick 2005:318). Despite 11 of 12 calculations for BMI and weight falling in the normal range for adults and not the obese one, Patrick concludes that monks have higher rates of obesity-related pathology than secular counterparts and also skeletons that were "built to cope" with the increased body weight and thus proposes that obesity in monks is a real phenomenon (Patrick 2005:376).

It would seem from the above studies, therefore, that the potential for obesity amongst monastic communities is there. However, much of this 'potential' depends upon whether the dietary accounts, records, and assumptions that exist about many monastic communities are accurate. Although the posture, activity levels, and calorie intake of monastic groups could create a predisposition towards obesity, studies such as Patrick's show that obesity may not actually be an issue as, even though Patrick concludes that obesity was a reality faced by monastic communities, her data shows that monks were of a similar weight and BMI to secular communities. In order to explore this issue of obesity, we must first assess whether the documentary evidence for monastic diet is accurate.

Chapter 3: Monastic Orders, Lifestyle and Diet

Individuals from three different religious orders have been used in this research – Cistercians, Augustinian Canons, and Dominicans. In the bioarchaeological literature, the prevalence of DISH in monastic communities has been strongly linked to their diet. In order to explore what their diet entailed, we must first gain an understanding of how these orders were formed, what their daily activities were and what the influences from their daily lives were that may have impacted upon their health.

3.1 Monastic Orders

Several different types of monastic orders existed during the medieval period but the most prominent groups were the Benedictines, the Augustinians and the Cistercians who, by the end of the 13th century, represented about half of the monastic population in Britain. The Benedictines, who were founded by St. Benedict, were the original template for new monastic orders such as the Cistercians, the majority of which arose during the eleventh and twelfth centuries. These new orders sprang from debates regarding how a monastic life should be lived – whether it should be a life of prayer or a life of preaching and evangelism (Brooke 2003:154). Some groups created their own interpretations of the Rule of St. Benedict, often creating stricter and more literal interpretations than those of the Benedictines, whilst others chose to break away from St. Benedict's Rule altogether and follow a new set of religious writing, that of St. Augustine. However, even those groups committed to following the Rule of St. Augustine often borrowed traditions and observances from the Rule of St. Benedict because St. Augustine's Rule was quite brief and lacked the detail necessary to lead a proscribed religious life. Since the orders share common roots it means that the differences between the different groups are not clear cut, making it difficult to categorise them. A basic distinction can be made between monks and canons; a monk being someone who follows the Rule of St. Benedict and a canon being

someone who follows the Rule of St. Augustine (Brooke 2003:156), although, as with any generalisation, there are exceptions. As many publications within bioarchaeology do not make a distinction between monks, canons, and friars, for the purposes of this research, the religious orders will be referred to collectively as 'monks' in the later chapters, except where indicated.

3.1.1 The Rule of St. Benedict

The Rule of St. Benedict, written in approximately 526 AD, consists of a prologue and 73 chapters which detail the layout and organisation of a monastic community, including the aims and virtues of a monastic life; the daily round of prayers, reading and psalms; the political structure of the monastery; and much more (The British Library 1980:14, Lawrence 1990:22). Essentially, it was a manual for the monks, which Benedict designed to show how they could live in a community and lead a cenobitic life, rather than the hermitic, solitary existence that had previously been the norm for those seeking religious vocations (Lawrence 1990:22). Several different orders followed the Rule of St. Benedict including the Carthusians, Grandmontines, Cistercians, the Knights Templar and, of course, the Benedictines.

3.1.1.1 Benedictines

The Benedictines are one of the oldest Christian orders, originally just called 'monks', in the 12th century they became known as 'black monks' because of the colour of their habits, distinguishing them from the 'white monks' – the Cistercians. Prior to the 10th century, there was no centralised or uniform order as such; each house existed independently although they all observed one rule (The British Library 1980:15). Although it is known that St Gregory the Great sent his prior, Augustine (who later became the first archbishop of Canterbury), to evangelise England in 597, the earliest record of the use of St. Benedict's rule in Britain is not until the seventh century and is associated with St. Wilfrid (The British Library 1980:18). This early movement died with the Viking raids of the

ninth century but was revived in the year 970 with the creation of the *Regularis Concordia* in Winchester. The *Regularis Concordia* was a set of edicts based on the Rule of St. Benedict and after its inception the monks were referred to as the Order of St. Benedict (The British Library 1980:18, Derwich 2000:137). They followed St. Benedict's Rule and its characteristic features of intellectual pursuits and liturgical duties, using the motto 'pray and work' as their guide (*ibid.*). After the Norman invasion in 1066, English churches changed and the cathedral monastery appeared. Cathedral monasteries are an almost entirely British phenomenon and are some of the most well known religious monuments in England – Durham Cathedral, Canterbury Cathedral, and Westminster Abbey (*ibid.*). The monks inhabiting these monasteries wore a black tunic with a leather belt underneath a black scapular and cowl. Fully professed monks wore a black hood for the choir whilst temporarily professed monks and novices wore a cope (Derwich 2000:136). Benedictine abbeys and monasteries were meant to be self-sufficient fraternal communities for unordained monks, but they also housed the sick, excommunicated persons, oblates and novices as well as guests and resident benefactors (Derwich 2000:138). The Benedictines vowed to serve God by imitating Christ, and thus their days revolved around daily liturgy and *opus Dei* (God's Work) as well as non-religious "labour" such as gardening, working in the monastic workshops, and copying books (Burton 2000:164-5).

3.1.1.2 Carthusians

The Carthusians were part of a movement of new monasticism that embraced a desire to live in solitude and poverty by retreating into remote areas of southern France and northern Italy. These groups appealed to both the educated man and layman alike and it was not uncommon for wealthy landowners to leave their comfortable existence to join one of these remote communities (Wickstrom 2000:244). Carthusians wore a traditional monastic tunic under a cowl and scapular which was joined at the sides by a large band of cloth, and, like many of the new monastic orders it was white rather than the traditional black. The colour of the robes was not the only thing that changed, the liturgy was vastly reduced so that there were only two daily services of Matins and Vespers, unlike

the eight services of the Benedictines. They also changed the physical structure of the monastery, creating private cells for each monk to live in rather than dormitories, and limited each monastery to twelve monks plus a prior imitating the twelve apostles led by Christ (Wickstrom 2000:244). The Carthusians became famous for their book production, which was probably aided by easy access to parchment due to the fact that they raised sheep – the only form of agriculture that was usually possible due to the remote locations that they lived in. The tending of these flocks was carried out by laymen, illiterate peasants who lived in separate dormitories from the monks and wore simple brown habits.

3.1.1.3 Grandmontines

The Grandmontines were founded by St. Stephen of Muret at Muret, near Limoges, France in 1076 (Aston 2000:72). An austere order, even more so than the Carthusians, they lived as hermits in cloistered communities in abject poverty. Only three houses were ever founded in Britain, the first of which was founded at Grosmont, Yorkshire in 1204 (ibid).

3.1.1.4 Cistercians

The Cistercians became the most successful of the new monastic orders. They sought a simpler interpretation of the Rule of St. Benedict, reducing the liturgy as the Carthusians did, and emphasising a return to manual labour (Berman 2000:297). They also sought to recruit adult converts rather than child oblates. The Cistercians were the puritans of the Catholic church, dedicated to following the Rule of St. Benedict quite literally. Believing that prayer and spiritual reading should take precedence in the daily activities, they were very austere, pursuing perfection, seclusion and self-sufficiency (Brooke 2003:169-171). Cistercian documents dictated that the community should be remote from 'the habitation of men' with simple liturgy and plain undecorated churches (Burton 2000:65). In some cases, their desire to be remote and isolated was so strong that it caused them to destroy villages and evict tenants from land endowed to them

so that they could maintain adherence to their principles (Butler and Given-Wilson 1979:37)! They also shunned the forms of revenue that supported the Benedictine houses – rents, labour services, tithes etc from peasant workers– in favour of manual labour carried out by themselves. This enabled them to be self-sufficient and in some parts of the country, such as Yorkshire and Wales, they were able to develop sheep farming and a wool trade which rendered them, ironically, quite rich and powerful (Burton 2000:65-72). Although the Cistercians prided themselves in their undertaking of manual labour, this was actually carried out by a separate class of monks known as *conversi* (the remainder of the order being choir monks). *Conversi* were essentially laymen turned monks who, in theory, were illiterate and therefore performed a greater proportion of manual work in return for a lesser proportion of the literary work and *opus Dei*. The lay brothers were in reality farmers, shepherds, millers, masons, and weavers (Brooke 2003:173-174). They grew their own corn and wove their own clothes, eventually abandoning the black habit in favour of undyed (but bleached) wool in order to follow Benedict's recommendation to wear the "basest garments possible" which meant that the Cistercians became known as the 'white monks' (Tobin 1995:38). One of the monastic sites used in this study is a Cistercian foundation – the Abbey of St. Mary Graces, London.

3.1.1.5 The Knights Templar

The Knights Templar was a group of fighting monks (or a group of monastic knights) who were formed in Jerusalem in 1118 to protect pilgrims journeying from Europe to religious places in Palestine (Aston 2000:82). They followed the rule of St. Benedict and were influenced by the Cistercians, living in communal poverty and following the religious offices except for when in military duty (Burton 2000:82). The Knights Templar made their headquarters at the Old Temple in London in 1128 and, by the end of the twelfth century, had established 34 houses in England and Wales (Aston 2000:82, Burton 2000:82).

3.1.2 The Rule of St. Augustine

The Rule of St. Augustine was followed by, or influential to, numerous orders including the Knights Hospitaller, Augustinian Canons, Premonstratensians, Gilbertines, Franciscans, Dominican Friars, Augustinian Friars, and Carmelites because it was quite broadly defined and easily adapted (Burton 2000:43). Its main emphasis was on poverty, simplicity, and practical good works and Augustinian houses, founded by kings, prince, nobles and bishops, were often formed in close association with hospitals so that they could serve the needs of the poor, sick and aged (Brooke 2003:161). The Rule itself was not a major work like St. Benedict's but rather a collection of documents, in particular a letter that St. Augustine (Augustine of Hippo) wrote to his sister regarding the practice of religious life (Burton 2000:43, Hughes 2000:106). Some scholars have suggested due to its brief nature that it is perhaps meant as a summary or reminder of oral teachings rather than a detailed work (Hughes 2000:106). There are two parts that form the Rule of St. Augustine, the first is known as the *Regula Tertia*, which outlines the nature of a monastic community and monastery, the second is known as the *Regula Secunda*, which lists the daily services, and regulations on discipline and manual labour (Burton 2000:43). The Rule asks its followers to "live together in harmony and be of one mind and heart in God" and recommends the typical monastic observances of communal praying, common property, manual labour, silence, obedience, regular reading, fasting, and chastity (Hughes 2000:106).

3.1.2.1 The Knights Hospitaller

The Knights Hospitaller were very similar to the Knights Templar, only their constitution was more similar to the Rule of St. Augustine than to St Benedict's (Burton 2000:82). They began with the establishment of a hospital in Jerusalem in the early 1100s but later provided armed escorts to pilgrims journeying to the Holy Land (Aston 2000:82). Like the Knights Templar, they too, made London their headquarters and in 1144 they were granted land in Clerkenwell to create the Hospital of St John of Jerusalem (Burton 2000:82). The Knights Hospitaller

established a further 34 houses in England and Wales during the 12th century, and another 50 in the century following (ibid). When the Knights Templar was suppressed by the pope in 1312, the Knights Hospitaller inherited their lands.

3.1.2.2 Augustinian Canons

The Augustinian Canons, or Austin Canons as they were also known, had a pastoral role in the community and so were often founded close to, or in, towns and cities. They were the largest group of canons in Britain and they first settled in England at St. Botolph's Priory in Colchester in 1103 (Butler and Given-Wilson 1979:44). Henry I founded five of the houses of the Order in England and was also involved in the foundations of seven others during his reign in the early 12th century, including the Priory of St. Mary Merton – Merton Priory – which is used in this study (Malden 1905:94, Postles 2000:102). St James Abbey, in Northampton, also used in this study, was also an Augustinian House. Those that were "regular" canons were celibate and were cloistered, whilst those who were "secular" canons were often married and lived in the towns (Butler and Given-Wilson 1979:44). This order was also called the Black Canons on account of their black robes.

3.1.2.3 Premonstratensian Canons

The Premonstratensian Canons, also known as the White Canons due to their white habit, were named after their mother house at La Prémontré. They followed the Rule of St. Augustine but a more austere, stricter version than that of the Austin Canons, founding their houses in more isolated areas (Burton 2000:57, Postles 2000:101).

3.1.2.4 Gilbertines

The Gilbertines are the only order to be founded in England. Gilbert of Sempringham's order started in 1131 with the building of a cell adjoining his

Lincolnshire church for a group of women who wished to live an enclosed life (Burton 2000:98). This soon developed into an independent priory and lay sisters were added to serve the nuns, and at a later date, lay brothers. The nuns followed the Rule of St. Benedict, whilst the lay brothers and sisters followed the Cistercian customs (Burton 2000:99). In 1147, after a visit to Cîteaux (the Cistercian foundation), Gilbert introduced regular canons into the houses who followed the Rule of St. Augustine. It was thought that the canons would serve the spiritual needs of the nuns and the lay brothers and sisters the domestic and manual needs allowing the nuns to follow their religious vocation within the strict confines of the cloister. The Gilbertine Order became quite popular and several "double houses" were founded, most of these in Lincolnshire.

3.1.3 Mendicant Orders

Mendicant orders appeared in Britain during the 12th and 13th centuries (LaCorte and MacMillan 2004:11). These were groups that mostly followed the Rule of St. Augustine and represented yet another attempt to return to the austere lifestyle of earlier religious groups. They renounced all possessions so wandered freely from place to place begging for a living and were frequently given buildings to live in on the outskirts of towns where they offered spiritual guidance to the population (Aston 2000:84). The four main mendicant orders were the Franciscans (Friars Minor or Grey Friars), Dominicans (Friars Preacher or Black Friars), Augustinian (Austin) Friars, and Carmelites (White Friars).

3.1.3.1 Franciscans

The Franciscans, who have been immortalised in the paintings and frescoes of Giotto, were inspired by the life and work of St. Francis of Assisi, embracing poverty and preaching to inhabitants of towns and cities about issues such as urban violence and poverty (Powell 2000:507). They arrived in England in 1224 and within a few weeks were settled at Canterbury, London, Oxford, Cambridge, and Northampton. By 1230 there were Franciscans in most of the major

medieval towns (Aston 2000:85). Strictly speaking, they followed the Rule of St. Francis who created a way of life based on how Christ lived – without possessions, teaching and preaching to the masses (Burton 2000:109).

3.1.3.2 Dominicans

The Dominicans, whose most notable member was Thomas Aquinas, were founded by Dominic of Castile and followed the Rule of St. Augustine (Burton 2000:110). They scattered themselves throughout Western Europe and, although they embraced poverty like the Franciscans did, they were even more focused on preaching to the communities they resided in. This led to tensions between the Friars and the parish priests because the mendicants often preached at the same time as mass, drawing the local parishioners away from their churches (Izbicki 2000:413). The Dominicans first arrived in Britain in 1221, settling in major towns such as York, Shrewsbury, Northampton, London and Exeter (Aston 2000:85). They became known as the Preaching Friars and the Black Friars, on account of their emphasis on preaching and the colour of the robes that they wore. Unusually, the Dominicans had a "third order" of lay persons who followed a "third rule" that allowed them to combine secular life with religious life, thus enabling them to be married, live at home, and follow secular occupations (LaCorte and MacMillan 2004:11). Two of the sites used in this study, Ipswich Blackfriars and Gloucester Blackfriars were, as their names suggest, Dominican foundations.

3.1.3.3 Augustinian Friars

The Augustinian Friars or Hermits of St. Augustine were founded by Pope Alexander VI by the union of three very similar orders in 13th century Italy – the Hermits of Tuscany, the Hermits of Blessed John the Good, and the Hermits of Brettino (McWilliams 2000:103). This Order was famed for its charity, spirituality, and theological study as well as its emphasis on intellectual pursuits; Gregor Mendel, the founder of genetics, was an Augustinian Friar. The

Augustinian Friars were decimated by the Black Death, losing 5000 of their 8000 members, and were completely destroyed in England by Henry VIII's Reformation (McWilliams 2000:104). They first arrived in England in 1248, and their earliest foundation was in Suffolk (Aston 2000:87). The Augustinian Friars' constitution was very similar to the Dominicans (and hence based on the Rule of St. Augustine) but as their spiritual tradition was rooted in a hermitic lifestyle, many of their earlier friaries were isolated places. By the end of the 13th century, however, most medieval towns had an Austin Friary (Aston 2000:87).

3.1.3.4 Carmelites

The Carmelites, whose founder is unknown, were originally an eremitic order from Israel whose primary focus centred on solitude and prayer in cells around a chapel dedicated to Mary (Egan 2000:242). When they first arrived in Britain in the early 1240s they selected isolated sites such as Hulne in Alnwick as their homes but they were later reformed by St. Simon Stock, who, influenced by the Dominicans and St. Augustine's rule, relaxed their severe hermit lifestyle and moved them towards communal life in close proximity to the cities where they became preachers and spiritual counsellors (Aston 2000:87, Egan 2000:242). The Carmelites became known as White Friars due to the white cloak that they adopted in 1287.

Table 2: Monastic Orders and their Key Features

Rule	Order	Other Names	Date (UK)	Example Sites	Key Features
St. Benedict's	Benedictines	Black Monks	c.600s	Durham Cathedral, Westminster Abbey	-Cathedral monasteries -one of the oldest orders -wealthy order
St. Benedict's	Cistercians	White Monks	1128	Fountains Abbey and Rievaulx (North Yorkshire), **Abbey of St. Mary Graces (Royal Mint Site, London)**[1]	-austere, self-sufficiency -manual labour (through laypersons) -rejected forms of income that supported Benedictines
St. Benedict's	Carthusians	Poor Brothers of God of The Charterhouse	1170s	Witham (Somerset)	-remote, isolated sites -cloister surrounded by cells
St. Benedict's	Grandmontines		1204	Grosmont (Yorkshire)	-lived as hermits -lived in abject poverty -only founded 3 houses in Britain
St. Benedict's	Knights Templar		1128	Old Temple (London)	-rule similar to Cistercians -fighting monks; protected pilgrims to Palestine
St. Augustine's	Knights Hospitaller		1144	Clerkenwell (London)	-gained lands from Knights Templar when they were suppressed by Pope 1312 -established hospital in Jerusalem, later provided armed escort

St. Augustine's	Augustinians	Austin Canons/ Black Canons	1106	**Merton Priory (Sussex), St. James Abbey (Northampton)**	-largest group of canons in Britain -founded close to towns -pastoral role in community
St. Augustine's	Dominicans	Order of Friars Preachers/ Blackfriars	1221	**Gloucester Blackfriars, Ipswich Blackfriars**	-emphasised missionary work and preaching -had a "third order" of secular friars
St. Augustine's	Augustinian Friars	Austin Friars/ Order of Hermit Friars of St. Augustine	1248	Clare (Suffolk)	-constitution like Dominicans but spiritual tradition of hermits
St. Augustine's	Premonstratensians	White Canons	1143	Newsham (Lincolnshire)	-closely related to Cistercians -concentration of house in Lincolnshire
St. Augustine's	Gilbertines		1130s	Tunstall (Lincolnshire), Sempringham (Lincolnshire)	-only English order, founded by Gilbert of Sempringham -some double houses (men and women)
St. Augustine's	Carmelites	White Friars	1240s	Hulne Priory (Alnwick)	-began as hermits, sought isolated places -reformed (influenced by Dominicans) and became communal/pastoral
St. Francis'?	Franciscans	Order of Friars Minors/ Conventual Friars / Greyfriars	1224		-pastoral role in community -renounced possessions

1. Sites in **bold** are those used in the research

3.2 Monastic Lifestyle

Monastic orders had strict rules governing their daily life. How the monks should dress, what they should eat, even what hours they should sleep and awaken were prescribed to them by St. Benedict's Rule. This discussion will focus upon the Benedictine Order as this is the group upon which most of the other major religious groups are based, all with their own modifications and deviations. It is also the group for which most evidence about lifestyle, including daily activities and diet, exists. A discussion with respect to the differences between the Benedictine order and the three religious orders used in this research – the Cistercians, the Austin Canons, and the Dominicans – will follow.

3.2.1 Recruitment

Up until the twelfth century, children were a major source of recruitment for the monasteries. Boys from the age of seven were given to the monasteries by their parents as child-oblates in order to be educated and eventually become monks themselves (Lawrence 1990:37). By and large, these children came from landed families as it was a way of ensuring their children were provided for without having to divide up the family land into smaller plots for all to live off (*ibid.*). Once accepted, they were committed for life, but this came to an end in the twelfth century when it was decided that only adults could be permanently committed (Lawrence 1990:37). As monasteries were often located on the edges of towns, adult monks were recruited from the local area. Due to this local recruitment strategy, many of the monks living in the monastery had relatives amongst the local town's community, sometimes brothers or sisters, and sometimes, if they joined later in life, wives, sons or daughters (*ibid.*). Although many of the other European monasteries, such as those in Germany, required noble birth for entry into the monastery, recruitment in England occurred regardless of social standing. However, as some form of endowment was requested it meant that most recruits were from the middle or upper classes, or as Lawrence (1990:126) describes them, the 'middling landowners' or 'better-off

townspeople'. Recruitment was restricted further still by the need to be educated as those living a monastic lifestyle were required to be able to read books and sometimes even to produce them (Lawrence 1990:142).

3.2.2 Daily Routine

The daily routine varied according to the seasons, with eight hours sleep in the winter and five hours sleep plus an afternoon 'siesta' in the summer (Lawrence 1990:31). Every minute of the day was accounted for by St. Benedict's routine of work, prayer, study, and sleep (Burton 2000:159). The liturgy, or communal prayers, was the focus of the daily routine, with manual work being so much reduced that it was only a gesture towards fulfilment of the Rule. In winter, approximately nine and a half hours were spent in church, one and a half hours were spent reading, three and three quarters of an hour working, one and a quarter hours eating, and eight hours sleeping. In summer, the same proportion of the day was spent praying, but there was more reading time, less work time, an extra hour eating (supper) and only five hours sleeping (Burton 2000:161). The brethren would sleep fully clothed, minus their cowl and scapula, in communal dormitories, and they were prohibited from having any more than their feet or arms uncovered at any one time, resulting in an uncomfortable night's sleep in summer (Lawrence 1990:113).

The day began at 2.30am in winter (1.30am in summer) with the preparation for the night office, which was the singing of *Nocturns* (later known as *Matins*) (see Table 2). The dormitories, located on the first floor in the east section of the cloister, had 'night stairs' at the north end which led directly into the south transept so that easy access was provided for the night office (Burton 2000:141). In summer this was followed by *Matins* (*Lauds*) at daybreak and then a wash and change of clothes; in winter, it was preceded by reading until daybreak and followed by *Prime*, another service (Burton 2000:160). The day continued with short offices of *Terce*, *Sext*, and *None*, interspersed with work and reading. In the evening there were services of *Vespers* and *Compline*, the final service at sundown. The major difference between the seasons was that in winter there was

only one meal, dinner, which was eaten at 2pm and bedtime was at 6.30pm following *Compline*.

In summer, the timetable was somewhat extended to fill the increase in daylight hours; dinner took place at noon and was followed by a siesta and later a supper at 5.30pm with bedtime at 8.15pm, following *Compline* (Burton 2000:161). This busy schedule of services left little time for much else but there were a few hours in the day when the monks could read or perform tasks about the house. Manual labour, as had been prescribed by the Rule, became ritualised so that it amounted to nothing more than baking a few loaves of bread in the bakery or performing a short spell of weeding (Lawrence 1990:115). Instead, servants were employed to carry out the menial work around the abbeys, reflecting the social thinking of the time that working in the fields was a job for peasants. Peter the Venerable declared that as his monks came from a 'social milieu unfamiliar with toil, [they] were more suitably employed furrowing parchment with pens than ploughing furrows in fields' (Lawrence 1990:115). Any time not spent in church was mostly used for reading, copying or composing books, or artistic work, although any monks with useful skills, such as goldsmithing or wood-carving, were put to good use. This literary work took place in the cloisters, which were built deliberately south of the church so that the monks could take advantage of the light to work, study, and write. The entire day was conducted in silence except for two short periods after the morning chapter and after dinner when talking was permitted (Lawrence 1990:118).

Table 3: Benedictine Daily Routine

Summer		**Winter**	
1.30am	Rise – preparation for night office (Nocturns)		
2am	Nocturns (later known as Matins)		
		2.30am	Rise – preparation for night office (Nocturns)
		3am	Nocturns (Matins)
3.30am	Matins (Lauds) at sunrise		

4am	Wash (face and hands) and change		
5am	Reading	5am	Reading
6am	Prime – mass	6am	Matins (lauds) at sunrise
7.30am	Work	7.30am	Reading
8am	Terce – sung mass	8am	Wash and change Terce - mass
9.30am	Reading		
		9.45am	Work
11.30am	Sext		
12pm	Dinner	12pm	Sext – sung mass
1pm	Siesta		
		1.30pm	None – service
		2pm	Dinner
2.30pm	None; drink		
		2.45pm	Work
3pm	Work		
		4.15pm	Vespers
5.30pm	Supper	5.30pm	Change into night shoes; drink
6pm	Vespers	6pm	Collatio
		6.15pm	Compline
		6.30pm	Bed – 8hrs sleep
7.30pm	Change into night shoes; Collatio		
8pm	Compline		
8.15pm	Bed - ~ 5hrs sleep		

Adapted from: Burton 2000:160-1

3.2.2.1 Cistercian Variations

The Cistercians had a very different outlook to the Benedictines, seeking remoteness, simplicity, and a literal interpretation of Benedict's writing. They renounced all wealth and luxury, refusing to accept gifts of cultivated manors; the monopoly rights over local fisheries, bakeries, mills; or rights to the tithes and burial fees of parish churches like the Benedictines (Butler and Given-Wilson 1979:35). They also had very simple, plain churches with none of the stained glass, gold or jewels that decorated the Benedictine houses and also simple services (*ibid.*).

One of the key changes that the Cistercians made to the Benedictine model was to remove the child oblate. They raised the minimum age for a novice to 15 years of age, not necessarily because they were concerned about the wellbeing of the children in the monastery or his or her freedom of choice, but because they felt that children were a disruptive influence within the monastic community (Burton 2000:174). The Benedictines, influenced by the Cistercian amendments, later increased the age for a novice to adult.

The Cistercians altered the emphasis of the liturgy so that they could re-incorporate periods of manual work and also simplified it, removing the decorative altar vessels, the processions and many of the ceremonial features of the Benedictine horarium so that there was more time for individual prayer and spiritual reading (Burton 2000:163, Knowles 1941:211). The schedule of the liturgy, however, was somewhat similar to the Benedictine model (see Table 2).

Table 4: Cistercian Daily Routine

Summer		**Winter**	
1.30am	Rise Matins – service 1hr	1.30am	Rise Matins – service 1hr
	Short interval then Lauds – service		4 ½ hrs in bed
4am	Prime – private mass at sunrise		
4.30am	Chapter – readings, discussion of business		
Work	Individual tasks – gardening etc	7am	Lauds – service
		8am	Prime
		9.30	Chapter – readings, discussion of business
		Work	Individual tasks – scriptorium etc
11am	Sext – service	11am	Sext – service
	Lunch – 30 mins		Nones – Service
	Siesta 1-2 hrs		Lunch – only meal of the day

			Work –few hrs
2pm	Nones – service		
	Work – few hrs	3.30pm	Vespers
		4pm	Compline
		4.30pm	Bed – 9hrs sleep
6pm	Vespers		
	Supper		
7.30pm	Compline – service		
8pm	Bed – 5 ½ hrs sleep		

Adapted from Tobin 1995:133-138

Manual labour was very important to the Cistercians, even though the heavy agricultural labour was in actuality carried out by servants and lay brethren - *conversi*. They felt, as written in one of their statutes, that the "food for the monks of [their] order ought to come from manual labour (de labore manuum), agriculture and the raising of animals" (Burton 2000:164). It was their desire that everything consumed by the monks should be produced by the abbey itself.

3.2.2.2 Augustinian Canon Variations

The houses of the Austin Canons usually contained much smaller numbers than those of the Benedictines, averaging between 30 and 50 people, but they still represented about 3000 of the 20,000 religious in England (Butler and Given-Wilson 1979:73-74). Since their rule, the Rule of St. Augustine, was based on a collection of letters and hence not very detailed, some of their traditions were strengthened by borrowing from St. Benedict's Rule. Due to this, they were said to be less strictly governed and food, drink and conversation were less tightly restricted than the Benedictines (Butler and Given-Wilson 1979:46, White 1993).

The church services of the Austin Canons were said to be of a "moderate length", that is, shorter than the Benedictines (Burton 2000:163), but unfortunately there is little detail of their daily horarium in the literature. These shorter church services left more time for manual tasks which included "preparing parchment, writing, illuminating and correcting books, making and repairing clothes, making implements, such as wooden spoons and candlesticks, and weaving

mats…digging the garden, laying out beds and sowing seeds, pruning and grafting, weeding, ploughing, reaping and mowing" (Burton 2000:165). Although the Rule of St. Augustine had no absolute rule about silence in the cloister/house, silence was still usually observed in the church, refectory, and dormitory (*ibid.*).

3.2.2.3 Dominican Variations

It was the Dominicans' duty to "master sacred truth" consequently they replaced manual labour with mental labour; attending lectures, studying and writing books instead of sowing seeds and weeding (Hinnebusch 1965:339). They spent their time in the church, chapter house, refectory, library and classroom, rather than the kitchen, workroom or storerooms. Unlike the Benedictine houses, the dormitory that the friars slept in often contained individual cells that functioned as places to study and sleep. Those that didn't require study space slept in the open section of the dormitory on a hard matting of straw, wool or sacking (Hinnebusch 1965:353).

The sacred liturgy was central to Dominican life and all other activities were fitted around it. The timetable (see Table 4) varied according to the seasons, with winter beginning on the Feast of the Holy Cross on September 14th and ending at Easter, and summer beginning at Easter and lasting until the Feast of the Holy Cross.

Table 5: Dominican Daily Routine

Summer		**Winter**	
Midnight	Matins and Lauds		
	Private prayer		
	(study or sleep)	2am	Matins and Lauds
			Private prayer
	Chapter (may be omitted if Prior felt more time was needed for study)		(study or sleep)
4am?	Prime		Chapter (may be omitted if Prior

			felt more time was needed for study)
		6.30am	Prime
	Terce Sext		Terce Sext
	Dinner		None
	Siesta		Dinner
2pm	None		
		4.15pm	Vespers
			Short interval
		5pm	Compline
		5.30pm?	Bed-8 ½ hrs sleep
6.30pm	Vespers		
	Supper		
7.45pm	Compline		
8.15pm?	Bed-<4hrs sleep		

Adapted from Hinnebusch 1965:351

Compline was considered by the friars to be one of the most important offices of the day as that is when the lay people from the community they were preaching in could, and would, attend (Burton 2000:164).

Silence was observed at all times, and was especially enforced in the cloister, dormitory, cells, refectory, infirmary, and church. The friars were permitted to speak elsewhere, provided that they obtained special permission (Hinnebusch 1965:361).

3.3 Analysis of Monastic Diet

3.3.1 Archaeological Evidence

One of the most obvious sources of evidence for monastic diet from archaeological sites is animal remains, but excavating animal remains at monastic sites is made difficult by the fact that religious houses had good drainage and water systems that carried kitchen and other waste away from the main site and into streams and rivers (Jones 1989:174). Sieving soil from the more obvious sites such as the kitchen, refectory, drains, and cesspits would

likely recover some remains but this is not always done on site (ibid.). Most likely, one would expect to find high concentrations of fish and marine foods at monastic sites but this is not always the case, not only because recovery of such small finds is unlikely without employing the use of sieves, but because the survival rate of such remains is less than that of animal and bird remains (Jones 1989:174, O'Connor 1993:108, Bond 2001:73, Serjeantson and Woolgar 2006:106).

The lands owned by the monks/canons themselves were a great source of food. Crops were sown, livestock reared and fishponds built on the vast estates that many groups had at their disposal. However, considering the size of many monastic sites, little bone debris has been recovered (O'Connor 1993:108). Within the few large bone assemblages that exist, cattle bones generally predominate but the importance of fish, especially herring, in the monastic diet is also apparent where deposits have been sieved, as is freshwater fish (O'Connor 1993:109). Unfortunately, any foods produced on, or harvested from, these lands (e.g. cattle, freshwater fish) were often not recorded in the account ledgers as they were not purchased. Therefore, in the absence of bone assemblages, such foods are essentially 'invisible' to the researcher and may be inadvertently excluded from a dietary analysis.

The little archaeological evidence for food that exists from monastic sites has been reviewed by Bond (2001), along with its potential uses and limitations, and the sections below predominantly discuss his findings. He recognises that written sources will provide a more complete picture than the archaeological evidence, mostly due to the nature of monastic sites – that major stratified concentrations of domestic refuse are rarely found and that efficient waste removal systems exist – but highlights the knowledge gained from excavation. As there is little archaeological food evidence from the sites used in this research, the archaeological data below present a general picture of monastic diet and, therefore, will be considered in terms of the basic food groupings of cereals, fruits and vegetables, meat, fish, poultry and dairy, and beer and wine.

3.3.1.1 Cereals

As religious houses often had large estates used for farming, much of the archaeobotanical evidence gained from them is similar to that of other late medieval rural sites (see section 4.2.1) with the presence of seeds and cereal crop remains and, of course, the same rules apply; only those plant remains that are charred or in waterlogged conditions are likely to remain (Bond 2001:56). Much of the evidence for cereal production points to wheat and barley as the main staples, with both used in making bread and ale, but that oats and rye were also important. Oats were more important in the north as they grew better in the cooler wetter areas of the north and west of the country (Bond 2001:63). In addition to the cereal crop remains, there is also archaeological evidence of buildings used to store the produce, clearly indicating that a large quantity of cereals were stored, if not also produced. The size of the barns could also give an indication of the amount of land that was being farmed and the amount of crop produced on the estates, but these ideas have not been thoroughly explored in the archaeological literature (Bond 2001:60).

3.3.1.2 Fruit and Vegetables

An absence of evidence does not equal proof that a particular food was not consumed. We know from the accounts of various religious houses that beans and peas were important foods, used mainly in pottage and in soup, but there are very few found in the archaeological record because they do not survive well (Bond 2001:64). The same is true of produce from the vegetable gardens of monastic estates. Many evaluations of monastic diet assume that vegetables were not eaten in any large quantity because they are absent from the kitchen accounts of foods purchased but, as discussed above, any foods that were produced on the estate were not recorded so absence from the documentary record does not mean an absence from the diet. There is little archaeological evidence for monastic gardens but a few sites have yielded preserved remains. At the Abingdon grange of Dean Court (Benedictine) peas, beans, fennel seeds and lentils were recovered and lentils were also recovered at Hyde Abbey

(Benedictine) (Bond 2001:66). The excavation of waterlogged drains at Oxford Blackfriars (Dominican) resulted in the recovery of various fruits (apple, pear, plum, cherry, raspberry, strawberry, grape, fig) and nuts (hazel and walnut) as well as mustard and marigold (*ibid.*). Orchards in the monastic estates, particularly in the south and west of England, most likely provided some of this fruit and as there are records of the purchase of cider-making equipment, there must have been considerable amounts of apples and pears at some sites (Bond 2001:67).

3.3.1.3 Meat

The archaeological evidence for meat consumption is based upon the animal bones found. At Kirkstall Abbey (Cistercian) a large collection of animal remains were found immediately west and south of the misericord kitchen. Approximately 5000 animals were represented, of which 90% were ox or cow, 5% were sheep, and 3% were pig (Bond 2001:80). The majority of these animals were old; it is likely that only those cattle that were no longer used for ploughing or milk were slaughtered. The cut marks on the bone also support this idea as most bones had been chopped, suggesting that they were stewed rather than roasted, a common way to cook older tougher meat (*ibid*). The animal bones found at other religious sites paint a similar picture. For the majority, cattle are the dominant species but pigs and sheep vary in their significance. Cooking equipment specifically used for meat has also been found, for example, dripping pans that were used beneath a spit to collect juices from the meat (Bond 2001:81).

3.3.1.4 Fish

The archaeological evidence for fish consumption at monastic sites is limited due to poor survival rates of fish bones and lack of adequate recovery techniques (i.e. sieving) upon excavation. Of those remains that do exist, the majority seem to be marine fish rather than freshwater, even at the inland sites (Bond 2001:73). Cod

and herring are the most commonly found fish at medieval sites (Serjeantson and Woolgar 2006:107). Cod is found most abundantly at sites in Eastern England – York, Norwich, London - and it is certainly predominant at St Mary Graces (the Royal Mint Site) in the 13th and 14th centuries (Serjeantson and Woolgar 2006:115). Herring could be caught in the Ouse, Humber and the Thames Rivers until intense fishing in the tenth and eleventh centuries reduced their number (Serjeantson and Woolgar 2006:116). After that, herring were caught at coastal sites and preserved so that they could be transported to various locations inland (*ibid.*). It is difficult to distinguish between fresh and preserved fish from their remains but, generally, if only vertebrae of a species of fish are found consistently then it is likely that the fish was prepared (and the head removed) and preserved at another location and then transported (Serjeantson and Woolgar 2006:105, 117).

Freshwater fish were also eaten and many monastic houses had the right to fish in local lakes, fens and marshlands (Bond 1988). They also leased or owned river-fisheries – either a section of river that was fished, a series of fish traps, or a dam/barrier that was built to catch fish (Bond 1988). From the 12th century onwards, monastic houses invested in fishponds on their estates, which they used for storing and breeding bream, pike, perch, tench and roach (Bond 2001:73). In the late 15th century, after being introduced from the Danube region, carp became a favourite fish to stock as it needed little artificial feeding and would thrive in still-water ponds (Bond 1988). These fish ponds could be a single structure or several linked together. Few of the monastic fishponds have actually yielded substantial quantities of fish remains, possibly because they were emptied, cleaned, and refilled at regular intervals when they were in use (Bond 2001:74) but there is little doubt of their importance. Many authors have speculated that the monastic fishponds were sources of income for the monastic communities that held them. Currie (1989:147) refutes this by demonstrating that, in addition to a lack of evidence of sales of fish in the monastic account books, there would be an insufficient quantity of fish contained in the fishpond to sustain a community, which is why the monastic diet was supplemented by the purchase of marine fish. In fact, due to the prohibition of meat in the monastic diet, fish were important and it is likely that the cheaper marine fish were the everyday

staples of the monastic community and the freshwater fish reserved for feasts and other special celebrations (Bond 2001:74).

3.3.1.5 Poultry and Dairy

Poultry was not forbidden in the diet according to St Benedict's Rule, so it featured prominently in monastic diets (Serjeantson 2006:131). As with fish remains, the recovery of bird bones increases with the use of sieving at excavation sites (*ibid.*). Unfortunately, even with good preservation, it can be difficult to identify species of bird found as some members of the same family have very similar skeletons (Serjeantson 2006:133). The majority of bird bones found at religious sites are domestic chicken and goose and such remains, for example, have been found at Norton Priory (Augustinian), Oxford Blackfriars and Leicester Austin Friary (Bond 2001:75). Wildfowl have also been found – mallard, teal and woodcock at Norton Priory, and duck, moorhen and woodcock at Oxford Blackfriars (Bond 2001:76) – although in smaller quantities. Other birds recovered from archaeological sites include peacocks, pigeons, swans, partridges, and quail (Serjeantson 2006:140-5).

Eggs were consumed in great quantities by religious houses, sometimes as many as five a day (Harvey 1993:61). In the documentary records there are recipes for various egg-based dishes, but the archaeological evidence is sparse and restricted to a few eggshells recorded from Oxford Blackfriars (Bond 2001:77). Milk was obtained from the cattle kept on the manors, but it seems to have been limited in supply throughout the winter months so was often turned into cheese during this period. Butter was also used, mostly in cooking, but had to be heavily salted in order to preserve it. Although most of the evidence for the above dairy produce comes from the accounts and documentary sources, there is archaeological evidence in the form of dairy equipment such as strainers, presses, buckets, and butter-crocks used in making and storing cheese and butter (Bond 2001:81-82).

3.3.1.6 Beer and Wine

Large amounts of ale were consumed in religious houses, mostly because there was little else to drink. The daily allowance of ale varied depending on the order, the persons rank within the community, and at the discretion of the prior (Bond 2001:63). Some houses produced their own ale, whilst others bought it in because they could not produce enough to satisfy the needs of the community. Archaeological evidence of ale production is restricted to the buildings associated with the brewing process. Malting kilns have been identified, and also brewhouses, but few have been examined (*ibid.*).

Vine cultivation existed, mostly in southern England, and certainly expanded after the Norman Conquest, although only a handful of vineyards appear to have been on monastic property (Bond 2001:68). Much of the wine that was drunk by the monks on special feast days would just as likely have come from northern France or the Rhineland as England (*ibid.*). Identification of monastic vineyards is possible from some maps but archaeologically the evidence is limited to a few grape pips; no wine-making equipment has as yet been recovered from an English monastic site (Bond 2001:69).

Although we can gain a basic understanding of the types of foods that were eaten through the archaeological evidence we cannot, unfortunately, translate this into quantities of food eaten. Admittedly, the animal remains do yield quantifiable amounts of bone, but unless the exact numbers of people being fed, the parts of the animal that were being eaten, and the amounts of food eaten in one sitting are known, one cannot assess the nutrition adequately. For this type of analysis, documentary evidence is much more informative.

3.3.2 Documentary Evidence

Much of our evidence for monastic diet comes from the account ledgers of the larger and more prominent monasteries in Britain, mostly Benedictine monasteries. Harvey (1993) provides a very detailed description of diet and

dietary analysis based on the Benedictine house of Westminster Abbey which will provide the basis for the discussion which follows. Although Westminster Abbey was perhaps more wealthy than some of the other Benedictine houses in late medieval Britain at the time, its meal time practices have been assumed to be similar to all other monastic communities. Many researchers have used the Westminster Abbey dietary analysis as a template for all monastic diet in late medieval Britain. Unfortunately, this may not be a good model to use for all religious houses as they each varied in terms of access to resources and money to purchase food. The accounts themselves only span a short period of time, approximately 12 years between the years 1495 and 1525 (Bond 2001:56) so they may not even be typical of the Benedictine diet at Westminster, especially as adherence to the Rule during these years was not as strict as in the earlier part of the late medieval period. However, as this is one of very few sources of information about monastic diet it is heavily relied upon in the archaeological literature and, as such, is often used to support the idea that monks were eating large amounts of food. For this reason, and also because it is one of the best sources of documentary evidence for monastic diet we have, the evidence gleaned from Harvey's (1993) detailed descriptions (as discussed in the following section, 3.3.2.1) will be used in the dietary analysis as it is key to understanding how the idea of the fat monk and its associations with DISH developed. Dietary information from the other orders will be discussed where it exists.

3.3.2.1 Benedictine Diet

As with activity, St. Benedict also prescribed strict rules for diet in the monastic houses. Meat was forbidden unless you were ill, though fish was permitted, and, at Easter and Christmas, fowl could be eaten (Gasquet 1922:52; Lawrence 1990:31,79). According to Lawrence (1990:31), a typical meal for the Benedictines might have consisted of two or three dishes of cooked vegetables with bread and wine, if they lived in a wine-growing region. Usual foods appearing on the dinner table, in addition to bread and vegetables, were cheese, beans and cereals with extra dishes (known as pittances) of fish and eggs on

special occasions (Burton 2000:166). However, logs in the accounts book of the Abbot of Westminster show that fish, both fresh and dried and usually salted, were often eaten instead of meat as well as a large variety of game including chickens, ducks, geese, herons, pheasants, partridge, pigeons, quail, teal and swans; fruit and vegetables were seldom mentioned (Gasquet 1922:52,65). According to the Rule, the monks were supposed to eat only a single meal per day from September 14th to Easter, with two meals per day – dinner and supper - taken during the remainder of the year though there was some variation with some houses keeping the double meal until November (Knowles 1948:18). In the 13th century, the Benedictines tried to introduce the practice of eating two meals per day during the winter months (Burton 2000:166).

However, despite the strict rules governing meals, the rules were often adapted and sometimes even broken. In reality, the Benedictine monastic diet was a form of upper-class diet (Harvey 1993:34). At Durham, during the fifteenth century, the monks were invited in rotation to dine at the Prior's table on rare foods such as oysters, salmon, and dates (Lawrence 1990:278). The monasteries also provided extravagant feasts for their guests, which they too would partake in. One visiting scholar to Canterbury apparently experienced the service of 16 different dishes in their sauces, together with beer, ale, claret, wine, mead and mulberry wine (Waldron 1985). Abstinence from meat was also breached, and in several different ways. As the consumption of game was permitted, monasteries would sometimes send out hunting expeditions led by dogs to capture deer or wild boar on the estate which would then be suitable for eating because it had been 'transformed' into game (Gasquet 1922:58-59). There is also evidence that the larger monasteries owned country houses where it was customary to eat meat and to which the monks retreated in rotation (Knowles 1948:20). In fact, there were three circumstances under the Rule in which the monks were permitted to consume meat. Firstly, as already mentioned, the monks were allowed to consume meat in the infirmary when they were ill; secondly, meat was allowed at the Abbot's table when guests were present and he would invite the monks to join him on occasion; and finally, those monks who, on occasion, had their blood let were permitted to eat meat afterwards in order to restore their strength (Burton 2000:167). However, these leniencies soon became

abused and the brethren would often claim that they were in need of a "fortifying diet" and hence retreat to the infirmary where meat was allowed to be consumed (Gasquet 1922:58). The blood-letting became a regular event by the 12th century and, even though it was not supposed to be enjoyed, it was somewhat anticipated, not only because the monks were permitted to eat meat for one or two days afterwards, but because they were also permitted to miss the night office, take early meals, and talk to the other monks present (Burton 2000:167). It also evolved that the Abbot would invite the monks to dine at his table in rotation, and eat meat, whether guests were present or not (Knowles 1969:118).

The largest breach of St. Benedict's Rule on meat consumption came in the twelfth century. It was then that the Benedictine monks made a distinction between different cuts and preparations of meat, with the muscle ('butchers meat') and fresh meat cut from the joint seen as 'prohibited' or 'irregular' meat; offal or entrails and salted, pre-cooked and chopped meat seen as 'permitted' or 'regular' meat (Harvey 1993:40). Therefore, eating fresh roast beef would be in breach of St Benedict's Rule prohibiting meat, but eating a pork fritter would not. The orders decided that they could eat both the permitted and prohibited types of meat so long as the Rule was still upheld in the refectory and so the monasteries built special rooms called misericords where irregular eating practices could take place. At Westminster Abbey (a Benedictine abbey) there were in fact three rooms for eating – the refectory, misericord, and a two storey chamber called the *cawagium* of which the lower storey was used for meals (Harvey 1993:40). The monks would justify this consumption of prohibited food by ensuring that a small number of people ate in the refectory so that, to some extent, normal practice was still observed and Benedict's Rule upheld. Of course, when Benedict wrote his Rule, the refectory was the only dining room in existence so everything he wrote about mealtimes applied only to that room. In 1334, Pope Benedict XII, dissatisfied by these recent meal-time practices, insisted that at least half the monastic community must eat regular meals in the refectory on any given day and that no irregular (i.e. prohibited) food was allowed on a Wednesday, Friday, Saturday or during Advent or Lent (Harvey 1993:40). Despite these new boundaries, there was still less than half the community eating in the refectory because the monks excluded from the calculations anyone who was away from

the monastery, who was sick, or who was dining at the abbot's table. With both the refectory and misericord in use, the brethren were able to eat both dinner and supper five or six days a week (outside Advent and Lent) and to eat meat four days a week (Harvey 1993:42). Therefore, in spite of the regulations against eating meat, it would seem that meat was in actuality eaten not infrequently and was even consumed at regular intervals.

It is these documented accounts of monastic feasts and rule-breaking at meal-times that has led the osteoarchaeological community to speculate about the true nature of the monastic diet. Should these accounts be typical of a monastic community then it would be no surprise to find the inhabitants of them to be overweight and plagued by disorders and diseases associated with obesity. According to Rogers and Waldron (2001) calculations have shown that late medieval monks consumed an average of between 4870 and 6207 calories per day, the differences depending on the time of year and whether they were eating two meals a day or just one. The monks often left part of their food allowance to the poor of the local community so they didn't always consume this many calories. However, even if they only consumed 60% (an arbitrary figure calculated by Harvey that assumes they give 40% away to the poor), Harvey (1993:70) has calculated from dietary records that the monks would still be taking in 3723 calories a day, one and a half times as much as the recommended daily intake for a male today, although perhaps not a lot more than would be required for a heavy male (3160 calories) (Harvey 1993:70).

3.3.2.2 Cistercian Diet

In general, the Cistercians followed a much more rigorous diet than the Benedictines. Their meals were quite sparse consisting of coarse brown bread, vegetables flavoured by herbs from the garden, the occasional piece of cheese, 750mL of beer (or wine if in warmer southern climes), and eggs and fish on feast days (Tobin 1995:136). Eggs and cheese were banned during Advent and Lent and of course meat was not allowed to be consumed at any time, the exception being if a monk was taken ill and in the infirmary (Burton 2000:167). The

Cistercians were quite strict when it came to meat eating (including lard), and its consumption was not allowed outside of the infirmary. As written by one Cistercian monk: "I abstain from meat because if I feed my body too much, I nourish carnal desire thereby; I eat bread in moderation, because a heavy stomach prevents me from standing up straight at my prayers" (St. Bernard quoted in Tobin 1995:136). In addition, the monks were subjected to blood-letting four times a year to "help guard against the temptations of the flesh" (Tobin 1995:136).

It should be noted that although the early groups of Cistercians were quite strict when it came to prohibition of meat eating, later groups were not. Snape (1926:155) explains that the Cistercian Rule on the question of meat-eating was relaxed sometime in the 14th century to allow the monks to eat meat in the infirmary and by invitation of the abbot at his table. By the mid-fifteenth century further relaxations had taken place permitting the consumption of meat in special rooms outside of the refectory which meant that, other than during periods of fasting, the Cistercians were consuming meat as much as three times a week, mirroring those changes made by the Benedictines (*ibid.*).

3.3.2.3 Augustinian Diet

According to the accounts at Bolton Priory (an Augustinian foundation), 19lb (8.6kg) of grain, over 3lb (1.4kg) meat, ½ lb (0.2kg) butter and cheese per person was consumed each week (White 1993). Fruit and vegetables were not recorded, presumably because they came from the manor and so were not entered into the accounts (*ibid.*) The canons used oatmeal for pottage and wheat for bread; the remainder of the oats being used for brewing beer and also feeding horses, dogs etc (White 1993). All religious houses supported the impoverished. Bolton Priory gave corn and beans to paupers; in some houses monks were given very generous daily diets so they could support one man each (White 1993). Despite the regulated appearance of their mealtimes, the Bolton Priory canons were disciplined after a visit by Archbishop Wickwane in 1280 for "eating too sumptuous meals and drinking after Compline" (White 1993:14). According to

one observer, 'Gerald of Wales', (late 12th to early 13th centuries) who stayed as a guest in an Augustinian house, the Black Canons ate meat three times a week (Burton 2000:167)! In fact the canons at Bolton Priory were eating meat by the end of the 13th century with two thirds of their expenditure on fish and one third on meat. By 1320 this ratio was equal (White 1993). During the years 1304 - 1315, Bolton Priory slaughtered 98 cattle, 122 sheep, and 93 pigs a year (White 1993). Augustinian Canons were said to have the most periods of blood letting of any religious community at eight per year (Butler and Given-Wilson 1979:72), perhaps this quantity of meat was deemed necessary to fortify themselves after such events.

3.3.2.4 Dominican Diet

Dominican meals have been described as meagre by Hinnebusch (1965:358). The same food was served to everyone in the community, including the prior, except for those who were ill and required special foods. Like all the other religious communities, in summer there were two meals, in winter only one. There were usually two cooked dishes per meal though the prior could order a third if he thought it was necessary and affordable (*ibid.*). Additional food, called pittances, might also have been provided if it was a feast day or a benefactor had made a donation. These usually consisted of pastry or fruit. Meat was of course abstained from except by the infirm who were permitted to eat meat if necessary, but only in a special room, and was also permitted when dining outside the priory so as to not inconvenience the host (Hinnebusch 1965:359). Special days such as Good Friday allowed for a diet of only bread and water for the entire day, whilst fasting (eating only one meal a day, like in winter) was observed on Fridays, and other significant religious days (*ibid.*).

3.4 Dietary Analysis

3.4.1 Components of a Healthy Diet

According to the Department of Health, the recommended components (dietary reference values) of a healthy diet if alcohol is included, is made up of 50% carbohydrate, 35% fat, and 15% protein (Department of Health 1991:xx). Alcohol should account for no more than 7% of the total dietary energy (Department of Health 1991:48). The estimated average energy requirements for adult men and women with a low level of activity are 2550 kcal and 1900 kcal, respectively (Department of Health 1991:xx). Those who are obese, however, will have greater energy requirements on account of having greater "fat free mass", the major determinant of resting metabolic rate. Following the recommended government intakes will help to maintain a healthy body and below is a brief discussion of the major components, vitamins, and minerals of such a diet (see Table 5 for a summary of the key components).

It should be noted, however, that energy intake values are based on the basal metabolic rate (BMR), the rate at which we metabolise foods, which will differ from person to person depending on physical activity level and weight. The total energy expenditure gives an accurate basis for which to estimate energy requirements and this is calculated by adding BMR plus the thermic effect of the food eaten, plus the energy expended in physical activity (Department of Health 1991:21). The basal metabolic rate is estimated from body weight and composition, which will vary with sex and age. The energy expended in physical activity examines the length of time spent engaged in any one activity plus the type of activity and then comes up with an energy cost which is expressed as a multiple of BMR. For example, for sitting, the energy expended is 1.2 xBMR, for walking 4xBMR, and for standing activities at work 1.7xBMR (Department of Health 1991:22). Total energy expenditure is then calculated by combining these data with the energy expenditure during non-working hours and during sleeping. Therefore, an overweight person who does little exercise may have completely different energy requirements to that of a lean very active person. Hence, if monks, canons, and friars truly were overweight, they may have had to

consume more calories than an average person in order to sustain their energy levels.

3.4.1.1 Carbohydrates

Dietary carbohydrate should consist of sugars, oligosaccharides, starches, non-starch polysaccharides (NSP), and complex carbohydrates (Department of Health 1991:12). The term "non-starch polysaccharides" replaced "dietary fibre" as a more precise, measurable and chemically identifiable component of diet, especially as NSPs still make up the bulk of dietary fibre no matter what its definition is (Department of Health 1991:61). The main sources of NSP are cereals, and in particular, whole grain foods. This includes barley, oats and rye, but also wheat, maize and rice which are mostly insoluble sources. Most fruits and vegetables also provide soluble sources of NSP although some vegetables such as peas, beans and leafy vegetables are insoluble. Foods that are rich in NSP are on the whole less energy dense, have more bulk to them, and induce a greater feeling of satiety than foods low in NSP. This is beneficial to the body because it can prevent obesity as well as lower glucose, cholesterol and insulin levels (Department of Health 1991:63). The recommended daily average intake for non-starch polysaccharides has been set at 18g/day although this can range anywhere between 12g/day and 24g/day for individuals.

3.4.1.2 Sugars

Sugars are soluble carbohydrate sources and important sources of energy. They consist of glucose, lactose, sucrose, and fructose, to name but a few, and they are divided into two main groups – intrinsic and extrinsic sugars. Intrinsic sugars are those that are contained within the cellular structure of foods such as fruit, whilst extrinsic sugars are those that are not, for example honey (Department of Health 1991:72). Milk products were deemed to be a special class of extrinsic sugars and so a subgroup exists known as non-milk extrinsic sugars (or NMES). It is thought that a high intake of extrinsic sugars (with the exception of milk sugars)

will deplete the amount of fibre in the diet as well as contribute to the development of caries. Whilst there is no recommended daily intake for sugars, the Department of Health has deemed more than 200g/day or 30% of dietary energy as being unhealthy, causing elevated blood glucose, insulin and lipids levels as well as possibly compromising the intake of other nutrients (Department of Health 1991:74).

3.4.1.3 Starches

Starches are polysaccharides and can be present in the diet in a number of different forms. The form or source of this starch influences how much of an increase in blood glucose and insulin will occur (Department of Health:75). Carbohydrate is seen as an important part of the diet, as a source of energy that can prevent ketoacidosis (where muscle stores of energy are used because of insufficient energy in the blood stream), and as one that can be safely increased in place of fat, protein and alcohol all of which should not be present in too high quantities. Therefore, it was decided by the British government's Panel on Dietary Reference Values that starches, together with intrinsic and milk sugars should provide 37% of the total dietary energy (Department of Health:77).

3.4.1.4 Fats

Dietary fats include unsaturated fat, saturated fat, and trans fats. Of these unsaturated fats, some are considered essential fatty acids because they play an important role in the breakdown and excretion of cholesterol as well as being precursors for a number of other important molecules (Department of Health:39). These are more commonly found in plants and marine animals with the main sources being vegetables, fruit and nuts, cereal products, and meat or meat products. Trans-fatty acids are found in industrially hydrogenated products such as margarine but they also naturally occur in the stomachs of cows and sheep through the action of ruminal bacteria and can therefore be transferred into milk and also beef and lamb. These trans fats have negative effects on health and

have been linked to increases in low density lipid (LDL) cholesterol and cancer which prompted the Department of Health to restrict their intake to 5g/day or 2% of dietary energy (Department of Health:44). Saturated fat intake should also be restricted on account of its association with cardiovascular disease and increased cholesterol and its limit has been set at 10% of total dietary intake. Total fat intake in general has been linked to cancer, particularly breast, bowel, pancreatic and prostate cancers as well as obesity and diabetes mellitus. For these reasons, total fat intake is recommended as being 30% of total dietary energy (Department of Health:55).

3.4.1.5 Protein

Protein is essential for maintaining a healthy body. In fact, it is the amino acids found in proteins that are essential to body maintenance – these are known as the essential amino acids (Carpenter 2000:885). As long as the minimum amount of protein required by the body is consumed, the amino acids will be supplied in adequate amounts (*ibid.*). This protein can be consumed from either animal products or plant products, although animal products are usually higher in protein. The recommended intake of protein is 0.75g per kilogram of body weight per day. It is advised that people should not consume more than twice this amount as excessive levels of protein may not be safe. High intakes of protein have been found to have an adverse effect on the body, increasing blood pressure, and causing a deterioration of renal function and also calcium loss through urine which can lead to bone loss (Carpenter 2000:888). For this reason, the upper safe limit for protein intake has been set at 1.5g/kg/day (Department of Health:82).

3.4.1.6 Vitamin A

Vitamin A, also known as retinol, is a fat soluble vitamin that is essential to the health, survival and reproduction of all vertebrates (Wolf 2000:741). It is predominantly found in green leaves and yellow and red vegetables (in the form

of beta-carotene, which can be converted into vitamin A) such as sweet potato, broccoli, lettuce, tomato, and cabbage and in lesser amounts in legumes such as chickpeas. The preformed vitamin A is found only in animal foods, the highest levels being found in animal livers, then kidneys and fatty fish like salmon, and then lesser amounts in milk, butter and eggs, if the animals are able to consume sufficient quantities of the vitamin for it to pass into their milk and egg yolk (Drummond and Wilbraham 1958:78, Wolf 2000:749). However, according to Drummond and Wilbraham (1958:78), if vegetables are the main source of food then three times as much vitamin A must be consumed to meet daily requirements than if dairy is the primary source. Foods lacking in vitamin A or beta-carotene include wheat, oats, rice, potatoes, onions, pork and lard. Deficiencies in vitamin A can manifest themselves in the form of 'night blindness', a condition whereby the affected person can see properly during daylight hours but their vision deteriorates at night; other conditions that appear are skin lesions and loss of the sense of taste and smell (Wolf 2000:742,746). An excess in vitamin A however can lead to skin rashes, liver damage, weakness and fatigue, and also calcification of spinal ligaments (Wolf 2000:746).

3.4.1.7 Vitamin B

The vitamin B complex is made up of several vitamins that were originally thought to be one – thiamine (also known as B1), niacin, riboflavin, biotin, folic acid, pyroxidine (B6), and cobalamin (B12) (Roe 2000:751). A sufficient intake of thiamine prevents the development of the disease beriberi which can induce paralysis. People subsisting on polished rice (rice with the husk or outer layer removed; i.e. white rice) have been found to develop the disease as have alcoholics if they drink heavily without eating, although this is an acute form resulting in confusion and paralysis of the eye muscles (Roe 2000:751). Niacin deficiency is associated with the disease pellagra which manifests itself as dermatitis on areas of the body exposed to strong sunlight, as diarrhoea, and a confused state. It can be synthesised from the amino acid tryptophan which is found in animal proteins, therefore meat and milk are needed in the diet to sustain an acceptable level of niacin. This vitamin is necessary for the synthesis

and breakdown of fatty acids, carbohydrates, and amino acids, and in high levels can reduce cholesterol levels in the blood (Roe 2000:752). Riboflavin is necessary for energy use and so is important during physical activity. It is found most abundantly in dairy products and green vegetables, and without it produces a form of dermatitis, cracks at the corners of the mouth, a sore tongue and eyes that are light sensitive. Vitamin B6, pyridoxine and its closely related compounds pyridoxal and pyridoxamine, is important for proper amino acid metabolism, synthesis of haemoglobin, and neurotransmittance so without an adequate supply of it dermatitis, neurological problems and anaemia appear (Roe 2000:753). Pantothenic acid is readily abundant in foods and so only humans who are very severely malnourished have been found to suffer from a deficiency of it. Biotin assists with biosynthesis of fatty acids in the body, and, again, has rarely been reported as a deficiency. Folic acid deficiency can result in anaemia due to lack of cell maturation, especially amongst pregnant women. Green vegetables and fruit are needed in order to gain sufficient quantities of the vitamin. Vitamin B12 deficiency is also associated with anaemia and sometimes neurological disorders that lead to sensory loss, loss of balance, and confusion. This vitamin is only present in animal foods so vegans are at risk of a deficiency, although it may take several years to develop. The rest of the population should not be at risk of low intake.

3.4.1.8 Vitamin C

Vitamin C (ascorbic acid) was discovered as a cure for scurvy first and a vitamin second. It is critical in the biosynthesis of collagen thereby preventing the breakdown of the gums and aiding the healing of wounds that would be caused by scurvy (Hughes 2000:758). Whilst vitamin C is readily available in potatoes, citrus fruits and cabbage, the way that these foods are cooked/prepared can have a valuable impact on the amount of vitamin C that is actually consumed. However, most people today are able to consume enough vitamin C that scurvy is not an issue and according to Hughes (2000:759) only if someone is subsisting on "a diet devoid of fruit and vegetables (such as one based on nuts, grain, and/or cooked meat/fish) is scurvy likely to emerge".

3.4.1.9 Vitamin D

Vitamin D is not actually a vitamin since it is synthesized in the skin using ultraviolet light (i.e. exposure to sunlight). It is an important substance used to keep blood calcium and phosphate levels normal and also maintain the skeleton and cellular function (Jones 2000:763). Without it, the most commonly presented ailment is rickets (in children), which results in bending or bowing of the long bones, along with flaring of the epiphyses and enlargement of the rib cage. In the early 20th century it was discovered that vitamin D2 could be synthesized by exposing plant sterols to UV light and hence there is now food fortification. The only significant sources of vitamin D in foods are animal liver, egg yolks, and fish oils whilst most grains, meat, vegetables, and fruit contain virtually none. Fish itself, especially the fatty saltwater varieties of herring, mackerel, tuna, halibut and salmon, are also good sources as they have fat stores in the muscle where fat-soluble vitamin D can be found (Jones 2000:765). Jones (2000:765) comments that some social practices may have prevented the development of rickets in the past, for example, the Christian tradition of serving fish on Fridays, and also June weddings, which would have resulted in babies being born in the spring and hence spending the first six months of life in the sunshine.

3.4.1.10 Vitamin E

Vitamin E is an important anti-oxidant that helps prevent damage done by free radicals and maintains cell membrane integrity (Jones 2000:769). The adverse effects of a deficiency are difficult to pinpoint but it has been reported that red blood cells are adversely affected, either shortening their lifespan or causing them to hemolyze, and that there may be some associated muscle and neurological problems (Jones 2000:771). Foods that contain vitamin E include plant oils, and to a lesser extent animal fats and fish oils. Wheat germ bread is also a rich source of this vitamin.

3.4.1.11 Vitamin K

Vitamin K is responsible for blood coagulation, preventing hemorrhaging. The best sources of this vitamin are found in green leafy vegetables and oils such as soybean, rapeseed and olive oil. Few documented cases of vitamin K deficiency have been found in modern life suggesting that gaining adequate amounts to meet daily requirements is not difficult. However, should a deficiency exist then one could potentially bleed to death (Thierry-Palmer 2000:774-6).

3.4.1.12 Calcium

Calcium is an important mineral but its absorption is dependent on vitamin D availability and therefore the quantities consumed make little difference if vitamin D intake/synthesis is low (Crawford 1993:92). It is also adversely affected by high intakes of protein and excessive alcohol consumption, both of which have been said to cause calcium loss (Spencer 2000:792-3). A minimum of 800mg of calcium per day is required for maintaining homeostasis; any less and there is a risk of a decrease in adequate bone maintenance and the development of osteoporosis (Spencer 2000:788). Calcium is most often consumed in the form of dairy products, mostly milk, but milk was rarely drunk fresh in the late middle ages as it was seen as something to cook with or to give to children (Dyer 1998b:63). The nobility refused to drink it as they believed it would curdle in the stomach (Spencer 2000:1220). In the late middle ages milk, which could be goat's, ewe's or cow's, was usually made into cheese, either separately or sometimes mixed together but, even though this was the preferred way to consume dairy, it was not very prominently featured in secular homes. Lesser amounts of calcium can also be gained from green leafy vegetables such as spinach and cabbage leaves, which were certainly eaten during the medieval period (Greico 1999:309).

3.4.1.13 Iron

Iron is an important mineral for oxygen transport in the blood, collagen synthesis, antibody production, and the conversion of β-carotene to vitamin A, to name but a few (Kent and Stuart-Macadam 2000:811). It is found in red meat, liver, kidneys, egg yolks and also oatmeal. Whilst the eggs and red meat are foods most widely consumed by the upper classes, oatmeal was certainly consumed by the lower classes and thus both sections of society should have been sufficiently supplied with iron. However, just because its presence existed in the foods eaten doesn't mean that it was adequately absorbed. Vitamin C, meat, and alcohol are said to aid the absorption of iron, whilst phytic acids like those found in bran, tannins, coffee, calcium phosphate and some forms of dietary fiber inhibit it (Crawford 1993:92, Kent and Stuart-Macadam 2000:813). This means that fruit and vegetable consumption would be important factors affecting iron levels in the body. Wholewheat and brown bread also contain high iron levels, particularly when compared to white bread (Nelson 1993:114); therefore the lower working classes would be expected to have been privy to higher levels of iron in their food than the upper classes. Approximately 1mg of iron a day is needed to maintain levels in adult men, and 1.4mg a day in adult women (Kent and Stuart-Macadam 2000:813). An absence of sufficient iron can result in iron-deficiency anaemia

3.4.1.14 Iodine

Iodine deficiency has probably one of the best known consequences – goiter. Goiter, an enlarged thyroid gland, is most frequently associated with mountainous areas where iodine has been leached from the soil and therefore does not become incorporated into the crops grown in that soil nor is it present in the drinking water (Hetzel 2000:797). One of the earliest artistic depictions of goiter, and its associated cretinism, is from a Cistercian Abbey in Reun, Austria in the late middle ages. Here there is a picture showing "a figure with three large goiters and a stupid facial expression, brandishing a fool's staff in one hand while reaching up with the other toward a toad. This was doubtless a goitrous

cretin" (Hetzel 2000:798). Apparently, live or dismembered frogs were commonly used in the treatment of goiter. Iodine deficiency causes goiter because a deficiency in iodine means that thyroid iodine stores are depleted. This results in a reduction in the production of the thyroid hormone which stimulates release of the pituitary gland's thyroid stimulating hormone, thereby increasing thyroid activity and resulting in an enlarged thyroid (Hetzel 2000:800). Today, iodine deficiency can be prevented through ingestion of iodising salt and water but, in the past, it would have been difficult in iodine-depleted areas, and treatments would have been restricted.

Table 6. Summary of Dietary Components

Dietary Component (alternative name)	Major Food Source(s)	Responsible For	Negatives	Associated Conditions/ Diseases	RDA
Carbohydrate	Cereals, wholegrain foods	Preventing obesity, lowering glucose / cholesterol / insulin levels			18g/day (12-24g/day)
Sugars	Fruit, honey, milk	Energy	Excess: depletes fibre and can cause caries; elevates blood glucose / insulin / lipids; compromises other nutrients		<200g/day <30% of dietary energy
Starches	Carbohydrates	Prevents ketoacidosis			37% dietary energy (incl. intrinsic & milk sugars)
Fats	Plants, marine animals, meat (unsaturated fat) Cow/sheep milk (trans-fats)		Trans fats: increase LDL cholesterol, increase cancer Saturated fats: increase cholesterol, cardiovascular disease	Cancer, obesity, diabetes	Trans fats 5g/day; saturated fat 10% dietary energy; Total fat 30% dietary energy
Protein	Animal or plant products		Increases blood pressure, calcium loss, decreases renal function		0.75g/kg body weight/day; safe limit 1.5g/kg/day
Vitamin A (retinol)	Animal foods, green leaves, red/yellow vegetables		Excess causes: skin rashes, liver damage, fatigue, calcification of spinal ligaments	Night blindness	

Vitamin B (B1, B6, B12, niacin, riboflavin, biotin, folic acid)	Meat, dairy, green veg, fruit (*B12 only in animal foods)	Breaks down fatty acids, carbs, amino acids; synthesis of haemoglobin	Deficiencies cause: dermatitis, anaemia, neurological problems	Beriberi, pellagra	
Vitamin C (ascorbic acid)	Potatoes, citrus fruits, cabbage	Biosynthesis of collagen		Scurvy	
Vitamin D	Liver, egg yolks, fish oils, (UV light)	Maintains skeleton		Rickets	
Vitamin E	Plant oils, wheat germ	Maintains cell membrane			
Vitamin K	Green leafy vegetables, soyabean /rapeseed / olive oils	Blood coagulation	Deficiency leads to haemorrhaging		
Calcium	Dairy products	Bone maintenance		Osteoporosis	>800mg/day
Iron	Red meat, eggs, oats	Oxygen transport in blood, collagen synthesis, antibody production		Iron-deficiency anaemia	~1mg/day
Iodine	Through food/water in iodine-rich environment		Deficiency stimulates thyroid activity; enlarges thyroid	Goiter	

3.4.2 Dietary Assessment

Determining whether a monastic diet was well balanced depends upon which religious order is examined (see Table 6). In terms of dietary components, some of the diets seem to be partially deficient, and some certainly appear to be prone to excess.

The diets of the Benedictines, Austin Canons, and Cistercians (after the 15th century) appear to be quite similar in terms of dietary intake, differing only in the quantity of meat eaten each week. Carbohydrates would have been gained from coarse brown bread or wheat bread, and pottage, as well as fruit and vegetables. Sugars would have been obtained from fruit, and possibly milk; fats from meat,

Table 7: Nutritional Assessment of Monastic Diets

	Benedictines	**Cistercians pre-15th century**	**Cistercians post-15th century**	**Austin Canons**	**Dominicans**
Carbohydrates	Wheat bread, breadcrumbs, pottage	Coarse brown bread, pottage	Coarse brown bread, pottage	Wheat bread, gruel bread, pottage	Brown(?) bread, pottage
	Fruit and vegetables	Fruit and vegetables	Fruit and vegetables	Fruit and vegetables	Fruit and vegetables
Sugars	Fruit and milk	Fruit and milk	Fruit and milk	Fruit and milk	Fruit and milk
Fats	Meat,		Meat	Meat	
	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs
	Nuts	Nuts	Nuts	Nuts	Nuts
Protein	Meat and fish 2-3x per week (5-6lbs)	Some fish (feast days)	Meat and fish 3x per week	Meat and fish 3lb per week	Some fish (feast days)
	Cheese, eggs	Cheese, eggs	Cheese, eggs	Cheese, eggs	Cheese, eggs
	Oats, pottage	Oats, pottage	Oats, pottage	Oats, pottage	Oats, pottage
Vitamin A	Green/red vegetables	Green/red vegetables	Green/red vegetables	Green/red vegetables	Green/red vegetables
	Animal livers/kidneys	Animal livers/kidneys	Animal livers/kidneys	Animal livers/kidney	
	Salmon, fatty fish	Salmon, fatty fish (feast days)	Salmon, fatty fish	Salmon, fatty fish	Salmon, fatty fish (feast days)
	(Milk, butter, eggs)	(Milk, butter, eggs)	(Milk, butter, eggs)	(Milk, butter, eggs)	(Milk, butter, eggs)
Vitamin B	Meat		Meat	Meat	
	Dairy, green vegetables	Dairy, green vegetables	Dairy, green vegetables	Dairy, green vegetables	Dairy, green vegetables
Vitamin C	Berries, cabbage	Berries, cabbage	Berries, cabbage	Berries, cabbage	Berries, cabbage
Vitamin D	Herring, salmon	Herring, salmon	Herring, salmon	Herring, salmon	Herring, salmon (feast days)
	Animal livers, egg yolks	Egg yolks (feast days)	Animal livers, egg yolks	Animal livers, egg yolks	Egg yolks (feast days)
Vitamin E	Wheat germ (bread)	Wheat germ (bread)	Wheat germ (bread)	Wheat germ (bread)	Wheat germ (bread)
Vitamin K	Leafy green vegetables, oils	Leafy green vegetables, oils	Leafy green vegetables, oils	Leafy green vegetables, oils	Leafy green vegetables, oils
Calcium	Milk, butter, cheese	Milk, butter, cheese	Milk, butter, cheese	Milk, butter, cheese	Milk, butter, cheese – minimal
Iron	Red meat		Red meat	Red meat	
	Oatmeal, bread	Oatmeal, bread	Oatmeal, bread	Oatmeal, bread	Oatmeal, bread
	Eggs	Eggs	Eggs	Eggs	Eggs (feast days only)

fish, milk, cheese and eggs; protein would also have been obtained from meat, fish, milk, cheese, and eggs, with the additional component of oats and pottage. Vitamin A would have been gained from any green or red vegetables eaten, as well as animal livers and kidneys, salmon (or other fatty fish), and milk, butter, and eggs if the animals consumed feed with enough vitamin A. Vitamin B would have come from meat, dairy and green vegetables; vitamin C from citrus fruits (although these were imported and not necessarily available to monastic communities), berries, and vegetables such as cabbage, although the vitamin content is dependent on the amount of cooking time as vitamins break down when heated. Vitamin D could have been gained from fatty fish such as herring or salmon or from animal livers and egg yolks. It is unlikely that the monks' daily activities would have exposed them to enough sunlight (especially considering their long clothing) to synthesise their own vitamin D so this vitamin would have to have been obtained from food. Vitamin E would have been supplied by the wheat germ in the bread, vitamin K by any leafy green vegetables consumed and calcium from milk, butter, and cheese. Iron would, of course, have been supplied by any red meat consumed but also through oatmeal, eggs, and wholewheat/brown bread.

The only potential deficiencies in nutrients would be vitamin C, if the vegetables were cooked for long periods of time thus depleting them of nutrients and, in calcium, as milk and butter were mostly used as cooking ingredients and cheese was not consumed regularly. There is, however, an obvious excess and that is in protein consumption. If we assume that the average late medieval male weighed 70kg (see Schweich and Knusel 2003) then the recommended intake of protein, based on the World Health Organisation calculation of 0.75g of protein per kilogram of body weight, should be 52.5g per day or 367.5g per week. The recommended safe level of protein intake would be twice this amount – 105g per day or 735g per week. Harvey (1993:56) estimates that the Benedictine monks were consuming 129.5kg (285lbs) of meat per year, the equivalent of 2.49kg or 5.48lbs per week; nearly seven times the value of the protein consumption calculated above. This calculation is only based on the protein consumed from red meat, and does not include that gained from fish, cheese, eggs, or oats, so the

real total is likely to be even higher. However, these data are misleading as the estimates for meat consumption provided by Harvey do not take into account that meat is not 100% protein. Calculating the protein intake using lean beef, which has a high protein value (on average) of 28g per 100g of meat (Food Standards Agency accessed September 2007), gives a total of 697.20g of protein per week, less than twice the recommended intake and therefore within the safe intake level of protein. The actual intake of protein might be even less than this as fattier beef, and other meats, such as lamb/mutton and pork, contain even lower amounts of protein per 100g of meat.

The diet of the Dominicans and the pre-fifteenth century Cistercians was quite different to that described above. The basic elements of brown bread, pottage, vegetables and fruit were the same, and provided the same basic nutrients, but their diet differed in their consumption of meat, fish and dairy products. From the documentary evidence, it would appear that the Dominicans and pre-fifteenth century Cistercians did not eat any meat at all and only consumed fish on special feast days. This would impact upon their fat, protein, calcium, vitamin A, vitamin B, vitamin D, and iron intake. They could still have obtained protein from the oats and pottage they were consuming, and iron also from the oats and wholewheat or brown bread, but vitamins A, B and D, as well as calcium, would have been more difficult to obtain. Most of the B vitamins are only available from animal products and, although the Dominicans and pre-fifteenth century Cistercians ate some eggs, the consumption of these was generally restricted to feast days. The requirements for vitamin A could have been met by fatty fish consumption, but, again, fish was restricted to feast days. It can also be supplied by green or red vegetables, but three times the amount of vitamin A needs to be eaten if vegetables are the only source (Drummond and Wilbraham 1958:78). Vitamin D can also be gained from fatty fish and eggs but these were only consumed on feast days and their intake may not have been regular enough to satisfy the body's needs. It is unlikely, although certainly not impossible, that the Dominicans and pre-fifteenth century Cistercians would have been exposed to enough sunlight to synthesise their own vitamin D. Calcium could really only have been supplied by butter, milk, or cheese, the latter being again restricted to feast days which may have limited the availability of calcium. Fat would also

have been supplied through dairy products or fish but, again, the consumption of these foods were restricted.

Overall, there seems to be a division between the nutritional profiles of the Benedictines, Austin Canons, and post-fifteenth century Cistercians, and the nutritional profiles of the Dominicans and pre-fifteenth century Cistercians. In general, both groups are quite well-nourished, but the levels of animal protein and their associated vitamins and minerals are very different. The supply of vitamins and minerals can impact on each other; a deficiency or excess in one may affect the intake or absorption of another. In the former group, the higher protein intake, whilst providing a good supply of iron and B vitamins, may have also caused a loss of calcium if protein levels became excessive as calcium is known to be adversely affected by high levels of meat consumption. High protein levels can also cause increased blood pressure and poor renal function although this is only likely to be a concern if levels exceed the recommended safe amount of 1.5g/kg/day (Carpenter 2000:888) which they don't appear to have done. If there was any lack of vitamin C due to overcooked vegetables, this may have caused problems with iron levels as this vitamin is needed for iron to be absorbed effectively, and may also have caused scurvy. In the latter group, a lack of B vitamins could have caused all sorts of problems such as anaemia, neurological problems, dermatitis, pellagra, and beriberi. Vitamin A, when severely depleted, can cause night-blindness, skin lesions, and a loss of taste and smell. Vitamin D deficiency can cause rickets (in children) or osteomalacia (in adults); calcium is not absorbed as this mineral is dependent upon vitamin D. Calcium is also inhibited by high protein and alcohol levels although the former certainly does not appear to have been an issue for this group. None of the possible illnesses and diseases mentioned above were observed during the course of this research. Of course, it must be remembered that although a skeleton may appear 'healthy' it does not mean that the individual did not suffer from a disease or illness during life that left no trace on the bones (Wood *et al.* 1992).

The nutritional profiles of the Benedictines, Augustinians, and post-fifteenth century Cistercians appear to be similar to that of the upper classes (see Chapter 4), whilst the nutritional profiles of the Dominicans and pre-fifteenth century

Cistercians appear to be similar to that of the poorer/rural classes. Perhaps, if the links between an upper class diet, animal protein intake, and DISH are true, we would expect there to be a higher prevalence of DISH amongst the Benedictine, Augustinian and later Cistercian orders than amongst the Dominicans and early Cistercian orders.

3.4.2.1 Obesity

One possible issue that has not yet been discussed, however, is obesity. Harvey (1993:64-65) has calculated the energy value (calories) and nutrient content of the diet of monks at Westminster Abbey for an average day, an average day in Advent, and an average day in Lent. On an average day outside of Lent and Advent (when fasting took place) 6207 calories (kcal) were consumed in the form of 20.5% protein, 27% fat, 33.5% carbohydrate, and 19% alcohol. However, on days when both dinner and supper were served in the misericord, the amount of calories consumed could jump to 7375 kcal. On an average day in Advent these figures were 5291 kcal in the form of 24% protein, 16% fat, 38% carbohydrate, and 22% alcohol. During Lent, 4870kcal in the form of 22% protein, 12% fat, 42% carbohydrate, and 24% alcohol were consumed. Harvey (1993:35) assumes that the Benedictine monks have the same energy requirements as a "rather heavy, moderately active man" whose energy intake should be 3158 kcal and made up of 15% protein, 33% fat, 47% carbohydrate, and 5% alcohol. It is clear that discrepancies exist between the amount of calories required to sustain the monks' weight and the amount of calories actually consumed, as well as differences in the proportions of protein, fat, carbohydrate and alcohol.

However, it should be noted that the monks did not consume all of their apportioned meals. They gave a portion of it to the servants within the monastery and also to the poor. Apparently the Benedictines gave away as much as 40% of their daily allowance to the needy, but even if they only consumed the remaining 60% this would still be an intake of 3723 kcal on an average day outside Lent and Advent, almost 600 kcal more than is needed (Harvey 1993:64).

We know that as little as an extra 100 calories a day can lead to weight gain of 5kg per year, so an excess of 600 calories a day would result in a huge weight gain over the course of a year (Levine *et al.* 2005). Considering the monks' daily routine of chanting, prayers, and reading, it is unlikely that they got much aerobic exercise, especially as any manual labour that was required was performed by servants, and so they would have easily gained weight. Of course, it is difficult to apply these calculations to the other religious orders used in this research as the energy intake would have differed, but it is reasonable to assume, based on the literary evidence, that the calorie intake would have been lower. However, many of the diets would still have been in excess of daily requirements as it was a common feature of religious houses to support the impoverished of their community by giving away a portion of their own meals (White 1993). Therefore, although the exact calorie amounts cannot be calculated, it is highly likely that all the religious groups were consuming calories in excess of their requirements and were susceptible to weight gain.

There is also the question of meat consumption within monastic communities. Many of the theories surrounding DISH in archaeological populations centre on not just diet and calorie intake but meat consumption. From this brief analysis, it would appear that the meat intake of the religious varied depending on which religious group they belonged to. The Benedictines seem to have had the highest protein intake, with some other religious groups consuming almost no (meat) protein at all, but even this did not exceed the recommended safe level proscribed by health organisations today. From these calculations we must conclude that there were likely no adverse affects associated with the level of protein intake for the religious groups studied here although the overall level of protein ingested was approaching the recommended safe level in some groups.

3.5 Conclusion

Although all religious groups shared some common roots there were some important differences between them. Their adherence to the Rule (St. Benedict's

or St. Augustine's) and their daily routine both impacted upon their diet and, ultimately, their health. Obviously, the above dietary analysis may not apply to all religious groups. As we have seen, there are differences in diet amongst the different groups in the literature, especially with regard to protein consumption. It remains to be seen whether these differences will be reflected in the isotope data.

Chapter 4: Medieval Diet and Lifestyle

Late medieval society was comprised of many different social classes, norms, values, and occupations. Both rural and urban populations had their own social structures and environments which undoubtedly would have influenced their access to and consumption of food as well as their health. In order to compare and contrast these lay groups with the religious groups being studied, and to make an assessment of diet as it relates to DISH, we first need to understand how these late medieval populations lived and what they ate.

4.1 Medieval Health

Although not a universal experience in the late medieval period, DISH certainly affected a small proportion of the population. The overall crude prevalence rate for the late medieval period, based on published sites, is 3.3% (96 out of 2880 individuals affected) (Roberts and Cox 2003:246) but when the monastic data are removed, this figure drops to 0.58%. These figures give a biased picture, however, as the majority of these published sites are monastic and only four of the 18 listed are non-monastic populations. There is little doubt that DISH may have caused some discomfort to those who had the condition, but as this did not affect the general health of the late medieval population it is not discussed in any great length here. Instead, the discussion on health that follows centres on the environmental and socio-economic factors that influenced the general health and well-being of the population during this period. A more in-depth discussion of DISH can be found in Chapter 2.

The late medieval period was one of growth. The population in Britain doubled during this time and with this increase in population density new diseases emerged (Roberts and Cox 2003:221). People were living in close proximity to each other and to their animals, often in a single room, and this, together with

ineffective sewerage disposal, a lack of clean drinking water and air pollution, resulted in the perfect breeding grounds for infectious diseases such as tuberculosis, brucellosis, syphilis and the Black Death (Roberts and Cox 2003:221,235). Poor hygiene is evident on the basis of parasites such as roundworm being found in latrines (Greig 1981), but it was not just the crowded and unsanitary living conditions that contributed to poor health, the climate had an effect too. The first half of the late medieval period, up to the mid-14th century, was considerably warmer than previous centuries and it has been hypothesised that this warmth helped to spread diseases such as the Black Death in 1348-49 (Roberts and Cox 2003:227). Following this warm spell was a much wetter period thought to have contributed to ergotism, a condition that causes convulsions, gangrene, and even death due to the ingestion of a fungus that grows on rye and can be incorporated into foods such as bread (ibid.).

The air was polluted, particularly in towns such as London, due to the burning of fuels for industry. Foundries and tanneries released pollutants such as lime and sodium sulphate into the atmosphere, products known to be associated with lung infections and sinusitis (Lewis *et al.* 1995). The burning of fuel, mostly sea-coal, to use in lime kilns resulted in a significant amount of pollution at ground level which peaked in summer as the extended daylight hours meant that demand for lime was greater due to the increase in building operations during this season (Brimblecoombe 1975). This industrial pollution, together with that from homes using wood and sea-coal for heating and cooking, led to complaints about poor air quality in towns being made from the 13th century onwards (Lewis *et al.* 1995, Roberts and Cox 2003:233). Evidence of sinusitis has been found in both York (at St. Helen-on-the-Walls) and Chichester (Hospital of St. James and St. Mary Magdalene, the site used in this study) with over half of the population in each cemetery exhibiting lesions (Boocock *et al.* 1995, Lewis *et al.* 1995). However, air pollution was not the only consequence of industry. Elevated lead concentrations have been found in the river bed in York dating from the 9th to 13th centuries and this is thought to have been caused not by the metal-working in York itself, but by the lead mining taking place in the Yorkshire Dales further upstream (Hudson-Edwards *et al.* 1999).

In rural agricultural populations, one of the most common signs of trauma seen are fractures. In Judd and Roberts' (1999) study of Raunds Furnells, a small agricultural community in the East Midlands, they found that rural males and rural females had a greater fracture frequency than their urban counterparts and they concluded that the location of these fractures in men and women reflected segregation in activity between the sexes. The farming environment not only predisposes to fractures, however, but to other dangers, such as the risk of tuberculosis and other zoonoses from being in close proximity to animals, and also exposure to pollen and fungal spores in the soil (Judd and Roberts 1999).

Rural populations were not the only ones to experience high fracture rates. The Hospital of St. James and St. Mary Magdalene in Chichester also had a high fracture rate but the cause is difficult to ascribe (Judd and Roberts 1998). The authors hypothesised that the high percentage of fractures might be due to overcrowded, poorly lit living quarters in which falls and trips would be frequent, a result of sensory and physical impairments created by leprosy that would lead to unsteadiness on the feet, or a reflection of the number of permanent inhabitants of the hospital who were expected to work as labourers and thus were exposed to the physical dangers of farming. Fractures were also seen in sites in York, although these were mostly fore arm fractures attributed to falls (Grauer and Roberts 1996).

Overall, the health of people living in late medieval Britain seems to have been tied to their socio-economic status (Roberts and Cox 2003:285). The conditions in which people lived, their jobs and their diet were in part dictated by their social standing and of course impacted upon their physical wellbeing, their nutritional intake, and - if the theories about animal protein intake being linked to bone formation are true - the prevalence of conditions such as DISH.

4.2 Evidence for Diet

To assess diet, researchers must rely upon documents such as tax records, wills, household inventories, funeral banquets, shopping lists and cookbooks, as well as physical remains such as archaeobotanical evidence, latrine contents, animal bones and even the chemical makeup of the bones of the people themselves (Goetz 1993:19, Riera-Melis 1999:263). Artistic representations can also be useful, for example paintings and sketches of feasts and meal times, as well as medieval writing, such as Chaucer's *Canterbury Tales*, which provides us with depictions of diet and dietary practices of the period through stories about different members of society.

Unfortunately, there are problems with using documentary and artistic evidence to interpret food and diet in the middle ages. As much of our documented evidence comes from records of household purchases for, and descriptions of, banquets and feasts, it cannot be used as a clear indicator of day to day consumption (Drummond and Wilbraham 1958:59, Hammond 1996:92). This is compounded by the fact that many of these records of feasting are of high-status people and not the ordinary peasants, servants and tradesmen of the late medieval period. Even these biased records may not be helpful as, according to Hieatt (1998:101), much of what historians have published prior to the 1980s about medieval food is incorrect. The records have either been misinterpreted or are incomplete leading to the propagation of myths about medieval food preparation and consumption. Much of the confusion stems from the miscopying or misinterpretation of the medieval culinary records as they are handwritten and in Middle English which is not always understood correctly. For example, a recipe containing 'thighs' (of a chicken or capon) was transcribed as 'cheese' because the scriber misread the 't' as a 'c' and, since the spelling was 'thees', easily mistook the entry for 'cheese' (Hieatt 1998:114). These kinds of errors can have misleading results in terms of interpreting nutrition and daily food consumption.

Problems exist with the archaeobotanical evidence as well. A large proportion of the archaeological evidence for medieval food remains comes from high status

sites (Giorgi 1997:209, Moffett 2006:43, Serjeantson and Woolgar 2006:106). This is an important point to be aware of as the choice of foods eaten during late medieval times is certainly linked to economic status, social class, occupation, and ethnic group as well as contemporary ideas on health and nutrition (Giorgi 1997:210). For example, as a food became more widely available, it would have become less desirable as a choice of food for high status individuals which means that it may be difficult to establish the importance of different foods based on the archaeobotanical evidence alone.

In order to better understand how and why these food choices were made, one has to understand the worldview that existed during the late medieval period. During the Middle Ages there was a belief that foods, just like people, were subject to the 'Great Chain of Being' (Grieco 1999:308). This was a hierarchical system that placed everything in the world into the categories of 'earth', 'water', 'air', 'fire', each of which was ordered from lowest to highest with earth and inanimate objects being the most lowly, at the bottom, and fire and God, being the most noble, at the top. The living things/people within each of these four categories were also ranked so that no two things had the same status in society. With respect to food, this system meant that plants were associated with the 'earth' category, for the obvious reason that they grew in the ground. According to Grieco (1999:309-312) however, they were subdivided further still. Those plants that produced an edible bulb underground, for example, onions, garlic, shallots, were considered to be the lowliest plants. Root crops, such as turnips and carrots, were slightly raised above the bulb plants. Plants like spinach and cabbage, whose leaves were eaten, came next, and above this were the fruit producing plants, with shrubs such as strawberry plants being ranked below the fruit trees because they grew closer to the ground. This assignment of rank continued up the 'Great Chain of Being'. In the 'water' category, mussels ranked below crustaceans, which ranked below fish, which ranked below dolphins and whales. In the 'air' category, those birds living on the water – i.e. ducks and geese – were the lowest in the chain, those that lived on the ground such as chickens and capons ranked next, then songbirds, and then birds of prey. There were no edible living things in the 'fire' category since this category contained mythological creatures. The placement of meat from livestock in this

system is difficult as they did not really fit into any category – they did not live in the ground nor in the air, so they were usually placed in between 'water' and 'air', with pork as the lowliest meat, veal the noblest, and mutton placed somewhere in between the two.

From this classification system developed the idea that certain foods were linked with certain sectors of society, thus giving a social value to foods that influenced all levels of society. The higher up a food was in the chain, the more it was associated with the upper rather than lower classes. For example, fowl were considered to be meat worthy of an upper class diet because they were high up in the chain and considered to maintain intelligence, whereas the meat of four-legged animals were considered to be more substantial and so suited to the merchant classes who were in need of sustenance, and pork and older animals who were no longer useful were sources of meat for the lower classes (Grieco 1999:311). Even within each of the four categories of the chain, the lower foods were associated with the lower classes and the higher foods associated with the upper classes. For example, the lower plants – onions, turnips – were considered to be more suited to the working classes in terms of diet, whereas those foods higher up in the 'earth' category of the chain – berries, apples – were considered to be more suitable for an upper class palate. For this reason, medical opinion was that vegetables were more suited to a lower class physiology and thus a diet based primarily on vegetables would be healthiest for the working classes and vice versa, unhealthy for the upper classes (Grieco 1999:311-312). Because a late medieval person's choice of foods communicated a certain social standing, it meant that there were rules concerning what foods could be eaten – and served – by each class of society. Thus, at banquets it was considered improper to serve foods above one's station and many communities had laws in place to enforce these rules (Grieco 1999:312). This association of social rank with food rank also means that it is possible to tell something of the social standing of the person through examination of their diet, and perhaps also something of their personality. For example, the medieval writer, Chaucer, uses diet as a metaphor for health, personality and morality in his Summoner's Tale. The Monk chooses dishes of roast swan, an item that would be high up on the 'Great Chain of Being', and

thus expensive as well as associated with noble society, which reflects his sense of pride and self-worth, and ultimately his greed (Biebel 1998:16).

4.2.1 Dietary Summaries

Unfortunately, dietary records and archaeological evidence for the sites specifically being looked at in this research is lacking. Instead, we must focus on the documentary and archaeological evidence that exists for the different socio-economic groups in medieval Britain during this time and use them as a template. That said, an attempt to link this evidence to the sites used in this research will follow later in section 4.5.

The written documentation provides us with information about the different sections of society, each with different diets according to profession and status in medieval society. A divide between rural and urban as well as peasant and aristocracy existed during the late medieval period. Prior to this period there had been only a distinction between peasant, church, and aristocracy, now a new 'urban' social group was emerging and with it came new patterns of food consumption

The archaeological evidence is dependent upon good preservation. Cereal grains, dense seeds and other robust material survive best in the archaeological record. Due to differential preservation, fruit is likely to be over-represented because its stones and seeds preserve well, whilst cereals, pulses, and vegetables seem to be under-represented according to what is seen in the dietary records where they are noted as important staples (Giorgi 1997:209). For plant remains charring is the means by which many, especially cereals, get preserved, although they are sometimes distorted (Moffett 2006:41). Unlike charring, waterlogging can preserve delicate material in addition to robust material and such deposits have yielded fruit stones, cereal bran, weeds, household rubbish, remains in faeces, flooring, bedding and building materials, leather, textiles, wooden utensils and hair (ibid.).

4.2.1.1 Rural/Peasant

Amongst rural peasant families, the majority of their diet was made up of cereals which were either consumed as pottage (a kind of stew made from cereals, root vegetables, and sometimes meat), bread, or ale (Giorgi 1997:200, Moffett 2006:51, Stone 2006:11). The types of cereals consumed depended somewhat on geographic location: wheat and rye were predominant in the north and east, oats in the north-west, and wheat and barley were predominant in the south with the exception of Norfolk where barley and rye are the dominant crops (Dyer 1998b:154). However, peasants often used a mixture of different cereals to make bread, usually barley, rye, and spelt, which produced a dark coarse bread known as treat (*ibid.*). Ale, too, was brewed using the locally available grains. After the Black Death though it contributed more significantly to the peasant diet because better brewing grains were available and peasants began to demand better quality ale (Dyer 1998b:159, Stone 2006:32).

Unfortunately, we often cannot confirm the presence of these cereals archaeologically. Most cereal remains found at archaeological sites are grains which can only be identified to the level of genus; associated chaff is needed to identify to the level of species, and even then it is uncertain that these were domesticated species (Brown 2001:310). However, aDNA analysis focusing on the origins and distributions of domestic crops is helping to address these issues (*ibid.*).

Meat was only eaten when it could be afforded. Peasants ate older animals that were no longer productive and much of this meat tended to be preserved (e.g. bacon) rather than fresh (Albarella 2006:86, Goetz 1993:19). Poultry, sheep and pigs were kept by most peasants – the pigs were kept for meat and lard, whilst the sheep were kept for wool and milk and slaughtered once they were old and no longer useful. This was often in the winter when food for feeding them was scarce (*ibid.*). They slaughtered pigs and prepared bacon at home but sold the rest of their animals to butchers and then bought back the meat in smaller quantities when needed (*ibid.*). According to Dyer (1998b:156), beef was the

predominantly consumed meat, followed by pork and then mutton. The archaeological evidence, however, demonstrates a dependence on mutton supplemented by beef (Sykes 2006:65). Meat was only eaten in moderation though, and on the religious 'meat-free' days – Wednesdays, Fridays, and Saturdays - meat was replaced with less expensive items such as cheese, dried fruit, eggs and sometimes fish (Woolgar 1999:90). If a feast day fell on a day of abstinence then further abstention may be observed, for example, not eating any dairy foods, or even very strict fasting by just eating bread and/or ale (*ibid.*). After the Black Death, meat became more affordable and fresh meat (and fish) was consumed more regularly (Dyer 1998b:157).

Marine and preserved fish were eaten more than pond/fresh-water fish albeit both were eaten in small quantities as meat was usually substituted with cheese or onions on fast days rather than fish as these foods were less expensive (Dyer 1998b:158, Riera-Melis 1999:265). According to the documentary evidence, the fish that was eaten was usually salted cod or herring (Dyer 1998b:157) but the archaeological evidence is more difficult to correlate with the documentary data. There are two reasons for this. Firstly, the historical records show a variety of names and spellings that reflect the way the fish was prepared or preserved, many of which are now obsolete or have lost their meanings, making it difficult to determine the modern equivalents (Serjeantson and Woolgar 2006:105). Secondly, as fish remains are quite small, deposits on archaeological sites should be sieved in order to collect the maximum yield of bone remains, but this is not standard practice (Bond 2001:73. Currie 1989:148). As with fruit and vegetable remains, only robust fish remains survive in archaeological contexts, and often the best preservation is found in latrines and cesspits which are predominantly found in high-status sites and thus bias the data (Serjeantson and Woolgar 2006:106).

However, the most predominant fish found in archaeological sites from the medieval period are cod and herring, and then (in no particular order) haddock, hake, ling, coalfish, and conger eel. Cod is found more frequently in sites in eastern England such as York, Norwich and London; hake is the dominant fish in south-western England in places such as Ilchester, Exeter, Taunton and Bristol;

and Conger eel is one of the main fish in the south in places such as Exeter and Winchester (Serjeantson and Woolgar 2006:115). Herring is found at both inland and coastal sites because it was often preserved and could be transported long distances from the coast. The preservation method included removing the head, or part of the head of the fish, so at those sites where the vertebrae have been found to outnumber the head bones it has been concluded that the assemblage is one of preserved rather than fresh fish (Serjeantson and Woolgar 2006:117). Remains of other fish species are also sometimes found including sharks, rays, whales, porpoises and sea basses (*ibid.*).

Other animal products such as cheese and milk were also consumed, as well as a variety of fruit and vegetables, although the latter were usually taken from their own gardens or the surrounding countryside rather than purchased. Some of the most commonly mentioned vegetables in the historical literature are garlic, onions, leeks, cabbages, spinach, beans, peas, turnips, and lentils, many of which were consumed in the form of pottage (Drummond and Wilbraham 1958:21, Dyer 2006:28, Riera-Melis 1999:265). If rural peasants had access to land, they could grow fruit such as plums, apples, cherries and pears and, if not, they could gather damsons, sloes, and other berries from the surrounding woodland (Dyer 2006:28, Watkins 1998:95).

In the archaeological record, fruit stones and pips preserve well and so are common finds at archaeological sites (Moffett 2006:54). Those most frequently seen in archaeological assemblages include plums (damsons, gages, and sloes), morello cherries, pears, apples, strawberries, grapes and figs (*ibid.*) which correlates well with the written evidence. According to Giorgi (1997:203), the most commonly seen fruit in archaeological deposits from London are figs, grapes, elderberries, blackberries, and raspberries, followed by strawberries, apples and mulberries, but occasionally, exotic fruits are also found.

Vegetables and herbs are more difficult to identify in the archaeological record because they are very closely related to the wild plants, and the cultivated/edible forms of vegetables such as carrots, parsnips, celery, and cabbage are in fact the same species as those plants that grow wild as non-edible forms (Moffett

2006:54). Vegetable seeds preserve well but are not often found, most likely because vegetables were grown for food and therefore rarely allowed to fall to seed (bar those needed to sow the following year's crop) (*ibid.*). Legumes and pulses also preserve well as charred remains but are often found in small amounts as are herbs, the most common ones being dill, fennel, coriander, celery, black mustard, and opium poppy (Moffett 2006:53)

4.2.1.2 Urban Craftsmen and Labourers

In urban areas there was a general rejection of the rural foods and patterns of consumption, especially as the new market economy meant a specialisation of food trades, with much more variety on offer. The cost of living was high in towns because there was no opportunity for self-sufficiency; even water had to be paid for (Dyer 1998b:209). The market retailers were, by law, supposed to sell to their consumers rather than other retailers so that the townspeople could have priority over those who would increase the prices and re-sell (Dyer 1998b:198). These food traders sold ready prepared foods such as pies, puddings, sauces and roasted meats, as well as foods requiring home preparation (Dyer 1998b:197, Kowaleski 2006:371). The majority of meat that was sold in towns came from young animals (as evidenced by the animal bones found in urban archaeological sites) indicating that the rural suppliers sent their best cuts to the towns, whilst they themselves ate the more mature animals (Dyer 1998b:197).

Townsmen and craftsmen ate more fish and meat than the villagers and, in general, there was a shift away from cereal foods towards meat; this certainly influenced the diet of employees who were given food as part payment for their labour. In fact, there was an Act passed in 1363 which provided the servants of noblemen, artisans and craftsmen with meat or fish once a day as well as milk and cheese (Drummond and Wilbraham 1958:54). Archaeological excavation of the cess-pits and latrines found in urban areas indicate that fruit was also important. The stones, pips, and seeds of apples, plums, cherries, grapes, gooseberries, and even wild fruit like sloes and blackberries, have been

excavated (Dyer 1998b:197). Some of this fruit likely came from those vendors with garden plots who often sold fruit and vegetables in the streets. Only small amounts of cereals are found at urban sites, possibly because they were milled outside the cities (Giorgi 1997:201). However, excavations at urban sites such as York and Chester have yielded vegetable remains, for example, fragments of onion, charred leek seeds, and garlic cloves (Moffett 2006:54).

4.2.1.3 Urban Merchants

The urban merchants aspired to be aristocracy in terms of diet and lifestyle. They ate wheat bread rather than the darker rye bread that was common in rural areas (Riera-Melis 1999:264). Like the upper classes, merchants ate large amounts of meat but, unlike their richer counterparts, they purchased it from town butchers as they did not have a supply of their own cattle (Dyer 1998b:197, Goetz 1993:19). Most of this meat was from young animals and it was always fresh meat rather than salted or cured. They also purchased fish, both marine and the more prestigious freshwater varieties, the freshwater fish usually being surplus produce from the aristocratic estates (Dyer 1988:32). As merchants resided in towns, everything had to be bought. Their fruit and vegetables were purchased from market sellers and so its supply depended upon them. As seen from the archaeological evidence, a great variety of fruit and vegetables were consumed in towns (see previous paragraph). Merchants could also purchase ready-cooked meats, puddings, and also sauces to go with, or substitute for, their own meals (Dyer 1998b:197, Kowaleski 2006:371). They drank wine and ale like the upper classes (Dyer 1998b:197).

4.2.1.4 Upper Classes

The daily diet of the wealthy aristocracy generally consisted of several dishes of meat and/or fish with white bread and wine or beer; very little or no water was drunk (Drummond and Wilbraham 1958:52-63, Harvey 1993:66). For example, the medieval noblemen have been recorded as dining on meat and fish such as

beef, mutton, pork, veal, game, venison, carp, pike, eels and lampreys, as well as fine wheaten bread (known as wastel), red and white French and German wines, ales, beer and cider (Drummond and Wilbraham 1958:53). They saw themselves as a class of 'meat-eaters' and as a separate social group to that of the vegetable and pulse-consuming poorer classes, a notion that was reinforced by the prevailing worldview at the time. Meat was more readily available to the upper classes as they kept their own livestock on their various estates and butchered them whenever it was necessary (Woolgar 1999:114). This meat was not restricted to just cattle though, as poultry and birds, such as doves, were eaten as well as venison, mutton, pork, veal, and fish, both marine and freshwater (Labarge 2003:28, Woolgar 1999:114). Most of these foods were eaten fresh (although sometimes the venison would be salted if the hunting grounds were far from the main household) and could be eaten either fried, roasted, grilled, boiled, or baked (Woolgar 1999:137). Other cooking methods included boiling meat in water with spices and herbs before roasting or frying it, usually in lard (*ibid.*).

In general, 'meat-free' religious days were observed, replacing meat with fish, which would have been fried in oil instead of lard (Drummond and Wilbraham 1958:63). This fish would have been both sea (marine) fish and fresh-water fish. Many wealthy households had their own ponds from which they obtained the majority of their fresh-water fish, buying the rest from other suppliers (Dyer 1988:32). If there was any surplus it would be sold at the local markets (*ibid.*). The main freshwater fish served in aristocratic households were eels, followed by bream, perch, pike, roach, and tench (Dyer 1988:31). Some of these fish were very expensive; for example, a mature pike could cost the equivalent of a skilled craftsman's wages for a week and, as a result, were often given as presents or served at feasts or special occasions (Dyer 1988:34). Despite the ready supply of fresh-water fish from the aristocracy's own ponds, sea-fish was still eaten in greater quantity (Dyer 1988:30). Even in places quite inland, households were able to obtain fresh seafood from the local markets. For example, a household in the midlands purchased fresh oysters, mussels, haddock and porpoise in the late 14th century from the market in Coventry (Dyer 1988:30).

An extreme example of an upper class diet would be that of the royal family. King Henry VIII's diet in the early 1500s was very similar to that of earlier centuries of the late medieval period (Hammond 1996:93). The King was presented with 23 dishes in two courses for dinner every non-fasting (meat) day, and even more dishes on a fasting (fish) day and, although no vegetables are mentioned in the royal accounts, they certainly would have been present in the stews and pottages served (*ibid.*). Despite descriptions of such huge feasts, there is, however, some evidence that checks were placed on excessive eating so that only a certain number of dishes were allowed to be consumed at one sitting (Drummond and Wilbraham 1958:60-61). Meals ended with fruit, sometimes apples, but always oranges. In addition there was also bread, ale and wine available, as well as butter and eggs (*ibid.*).

Vegetables were certainly secondary to meat at the dinner table and, according to Riera-Melis (1999:259), fruit was absent although many other authors stress its presence (Dyer 1998, Dyer 2006, Giorgi 1997, Hammond 1996). It is known that aristocratic households had large, well-stocked, professionally managed gardens, whilst most peasants and urban households had access to smaller pieces of land near to where they lived (Dyer 2006:33). The larger aristocratic gardens were not necessary to the survival of the households to which they were attached, and often sold their surplus produce (Dyer 2006:29, Stone 2006:13). Although many of the historical documents give the impression that the upper classes did not eat much fruit, it was apparently highly regarded amongst the elite and was eaten prevalently in winter, especially at Christmas (Dyer 2006:35). Apples were a particular favourite amongst the elite as they fit in with the world view that fruit growing high up away from the ground were more suitable for an upper class diet (Riera-Melis 1999:309). However, much of this fruit may have been preserved rather than fresh as there is evidence that dried and preserved fruit such as raisins, figs, dates, and peaches were imported from the continent from the 13th century onwards (Dyer 2006:34, Giorgi 1997:203). The archaeological evidence for distinctly upper class consumption of fruit and vegetables is sparse, although sites in London show evidence of imported fruits like dates, quinces, and citrus fruits (Giorgi 1997:203) which must have been destined for high-status households.

Upper classes also marked their status by consuming young animals in their prime (rather than older animals that were no longer useful or productive), and this is supported by the archaeology. From the mid-12th to mid-14th century, 14% of cattle and 12% of sheep at high-status sites were slaughtered by six months of age and there is also evidence that neonates and foetuses were eaten (Sykes 2006:68). On the basis of the archaeological evidence, pork was the second most commonly eaten meat during the medieval period, with peasants eating it in preserved form and the upper classes consuming it fresh (Albarella 2006:86). Although there is archaeological evidence that pork was consumed in quite large quantity at high status sites, its popularity appears to have declined amongst all levels of society towards the end of the medieval period (Albarella 2006:80).

4.3 Dietary Assessments

It is difficult to assess the diet of medieval people because the exact quantities of food eaten are not known. It is especially difficult when it comes to looking at vitamins because these are adversely affected by heat, light and water so cooking, drying, salting, and storing foods all have an impact on the resulting vitamin content of foods (Crawford 1993:90). The discussions of nutrition that follow are merely attempts to gain an idea of how well nourished people were, what the major components of their diet were, and to highlight any possible deficiencies.

The major components of any diet include carbohydrates, sugars, starches, fats, protein, and several vitamins and minerals including vitamin A, B, C, D, E, K and calcium, iron, and iodine. For an in-depth discussion of these components and their roles in terms of nutrition refer back to the "components of a healthy diet" in section 3.4.1 of Chapter 3.

In general terms, late medieval dietary records tell us that the late medieval diet was based on cereal grains such as wheat, barley, oats, and rye, which were made

into bread, pottage and beer, as well as being used for animal feed (Goetz 1993:19). Meat was consumed in greater quantity than is eaten today (Cortonesi 1999:270), with a whole range of animals being consumed including fish, poultry, game, cattle, pigs and sheep. This was supplemented by eggs, cheese and a variety of vegetables which include broad beans, peas, lentils, tares, vetches, cabbage, onions, turnips, and spinach. Fresh fruit was considered by some to be unhealthy and was associated with dysentery and diarrhoea, but it was still eaten, either fresh; cooked and made into pies, pastries, or puddings; or else fermented and converted into alcohol (Giorgi 1997:203).

During this period there was also a shift from a subsistence economy to a market economy, a result of the rising population (Montanari 1999:247). This meant that there were some choices in terms of what foods were consumed and the foods that were selected reflected people's class and social status.

4.3.1 Rural/Peasant

The peasant diet was in general quite well balanced with a variety of foods consumed (see Table 1). Although the bulk of the diet was made up of cereals, there were also a lot of vegetables consumed, some fresh and dried fruit, small portions of meat and fish, and some cheese, milk and eggs. Carbohydrates would have been provided by cereals, mostly bread and pottage, but also ale. Fruit and milk would have provided some sugars, and fats would have been gained from meat, fish, dairy products, and lard. Protein was certainly readily available from the meat and fish being eaten although more may have been gained from beans and oats, which are moderately high in protein, than meat/fish since they were consumed regularly and meat/fish may not have been an everyday food. Vitamin A, of which the precursor β-carotene is found in green leaves, yellow and red vegetables, would have been sufficiently available as the peasants regularly consumed leeks, cabbages, spinach and also beans. The preformed vitamin A would also have been gained in sufficient quantity through the consumption of meat, especially livers and kidneys, fish and dairy products. Because the peasant

diet was so varied and seemingly well balanced, there should have been enough meat, milk and dairy, green vegetables, and fruit to supply all the B vitamins required by the body. Vitamin C, however, is more difficult to assess. Although the rural diet certainly included sufficient quantities of vitamin C foods (cabbage, possibly citrus fruits) it depends on how these foods were cooked or prepared as to whether any vitamins remained upon consumption. One author (Spencer 2000:1221) tells us that medieval people believed vegetables to be a source of disease, especially when raw, and so long cooking times were recommended. If this is the case then many water soluble vitamins might have been lost, not just vitamin C. Vitamin D was probably gained, in part, from eggs and fatty fish such as herring. However, since peasants spent long periods of time outside tending the fields, they would have had plenty of exposure to the sun and been able to synthesise vitamin D more than adequately. Vitamins E and K were probably gained from vegetable oils but vitamin E would also have been available from lard and wheat germ, and vitamin K from the leafy green vegetables eaten. Calcium levels may have been low as it is most abundantly found in milk, which was not drunk much, although peasants may have gained sufficient calcium from the cheese that was consumed. Even if low levels of calcium were consumed, there would have been no problems with absorption as this relies on vitamin D intake of which there should have been plenty. Iron levels should have been sufficiently high as, although found in red meat and eggs, both of which were eaten by the rural peasants, it is also found in oatmeal which was consumed daily in the form of pottage, and in brown bread which was also consumed on a daily basis. The overall picture of the rural peasant's diet is that it seems to be well-balanced and not sufficiently lacking in any essential vitamins or minerals. One possible exception would be calcium and vitamin C which may have been lacking and/or depleted; if so, the medieval peasants may have experienced problems with bone density (osteoporosis) or scurvy. There is also the possibility that these individuals had a high salt intake as most of the meat and fish that they ate was preserved, usually with salt, rather than fresh because it was much cheaper to buy.

4.3.2 Urban Craftsmen and Labourers

The urban working class diet was not all that different from their rural counterparts with the exception that they may not have consumed quite as many vegetables and fruits. They were said to be consuming wheat bread and fresh meat whilst their rural counterparts were subsisting on dark bread and salted meat (Montanari 1999:249). According to Dyer (1998b:220), craftsmen were consuming a 70:30 ratio of cereals to non-cereals so the majority of their carbohydrate intake would have come from grains, especially bread and ale. Sugars would have been supplied by milk and fruit, and fats by meat and fish, dairy, and lard, and purchased puddings and sauces from town vendors. Not all urban dwellers had plots of land on which to grow fruit and vegetables so they were reliant upon street vendors for these foods, which may have meant lower intakes of vitamin A in the form of β-carotene. However, since we know that townsmen and craftsmen were supplied with meat, milk and cheese once a day, they were likely to have been able to get their vitamin A in the preformed version through consumption of these foods. This daily supply of meat, milk and cheese also meant that calcium and iron levels would also have been sufficient to sustain a healthy life. The urban working class appears to have been eating a wide enough variety of meat, dairy, vegetables and fruit to cover all the B vitamins, and also vitamin C, although, as discussed in the peasant diet, this depends upon the cooking methods employed. Vitamin D synthesis would likely not have been a problem as many urban occupations still relied upon outdoor activities and there would also have been some input from fish, and possibly liver. Since wheat bread was a daily food for the urban dweller, this would have provided a good source of vitamin E (and iron) along with fish and lard (animal fat). Vitamin K may have been consumed in lesser quantities in the towns and cities than in their rural counterparts as it relies upon green leafy vegetable consumption which may not have occurred in those without garden plots. As meat was supplied daily to urban tradesmen, it would be expected that they would gain most protein from meat and fish, although some of this would have been supplied by dairy products and oats as well. Overall, the urban working class diet was not that dissimilar to that of their rural counterparts. However, since they were able to buy products

such as sauces and puddings from the markets, their diets may have been higher in fat.

4.3.3 Urban Merchants

The urban merchants aspired to a lifestyle similar to that of the nobility and that certainly influenced their diet. Unlike rural peasants, urban merchants ate large quantities of fresh meat and fish and fewer vegetables and fruit. The majority of their carbohydrates were supplied by wheat bread and ale or wine; their sugars by whatever fruit and milk was consumed, and probably wine also made a contribution; and their fats by the meat, fish, lard, dairy, and rich sauces and puddings they consumed. Protein was more than sufficient as meat and fish were eaten in large amounts. Vitamin intake is difficult to assess but most sources indicate that fruit and vegetables were bought from local vendors so they were presumably consumed regularly, albeit perhaps not in the same quantity as the rural poor who depended on them as a food source. If fruit and vegetable intake was low then they may have been deficient in vitamin C, folic acid, and vitamin K which could have caused problems of scurvy, in pregnancies, and haemorrhaging, respectively. The combination of meat, fish, fruit, vegetables and some dairy, however, should have provided the merchants with enough vitamins and minerals to live healthily. The meat and fish, providing it was the fatty variety such as herring, mackerel, or salmon, which it most likely was, would have provided vitamins A, B, D, and E as well as iron. Fruit and vegetables should have provided sufficient vitamin C and K although the cooking times would have had a bearing on this, and dairy should have provided some calcium and vitamin D (from egg yolks).

4.3.4 Upper Classes

The diet of the aristocracy was a step further removed from the rural peasant diet than that of the merchants and was, in general, very high in calories and high in animal protein. According to Dyer (1998a:64), it was high in fat and low in fibre,

with only small amounts of vitamins A and C due to the low dairy, fruit and vegetable consumption. Whilst it is true that fruit and vegetables were not consumed in as large a quantity as for the poor, they were nevertheless still present at mealtimes. Most of the carbohydrates would have been supplied by wheat bread and also wine and ale. Sugar was an expensive item to buy, but was still consumed in large amounts by some wealthy households, particularly the royal household (Hammond 1996:11), and would have been the major contributor to sugar intake. Fats were contributed to by meat and fish, lard, dairy products, rich sauces and puddings, and also nuts. Since the aristocracy consumed large quantities of meat and fish, protein needs were certainly sufficiently met. Providing that they did eat sufficient vegetables, vitamin A, C, and K levels should have been adequate. The meat and fish intake would certainly have provided sufficient amounts of vitamins A, B, D, E and also iron.

If, however, fruit and vegetable levels were as low as is suggested by some researchers, then the aristocracy could certainly have been deficient in vitamin C and may have exhibited signs of scurvy. Folic acid may also have been low as this is gained from green vegetables and fruit and may have resulted in difficulties in pregnancy and anaemia in pregnant women. Vitamin K deficiency may have been an issue though as this is found in green leafy vegetables, and also soyabean, rapeseed and olive oils; and with none of these oils being regularly consumed, a low intake of vegetables could certainly contribute to a deficiency resulting in difficulties in blood coagulation. Calcium, whilst it would not have been gained much from milk as this was thought by the nobility to curdle in the stomach and so was rarely drunk, should have been sufficiently available in the cheese that was consumed. However, since calcium absorption is adversely affected by high protein intake and excessive alcohol consumption it may not have been supplied to the body in adequate amounts. This high protein intake certainly meant that iron levels were sufficient, although possibly not efficiently absorbed if vitamin C levels were low. Mostly, this protein is gained from animal products with very little contribution from beans or grains, and there is evidence that checks had to be placed on meat consumption at meal times because it was being consumed in excessive amounts. This excess of protein could lead to calcium loss and also kidney malfunction. If calcium levels were

also low (as they did not drink much milk, and cheese was the only major source), then they may have also exhibited signs of osteopenia.

Overall, it would seem that the diet of the aristocracy and merchants was much less well balanced, and less healthy, than that of the urban and rural working classes. There were possible deficiencies in vitamins A, C and K and possibly an excess consumption of protein which, in contrast to the working classes, is gained in greater proportion from meat rather than grains. Unfortunately, as economic situations improved for the working classes during the late 14th and 15th centuries, their diets declined. They began to emulate the wealthier classes – consuming white bread instead of brown, more meat and less vegetables, and more ale and less water – resulting in a less balanced overall diet (Dyer 1998a:69). However, the positive side of this change in diet was that different cereal crops were being consumed, meaning less reliance on one type of crop which alleviated the problems of famine during harvest failures – those crops that were now in less demand as food became animal feed but could be also put aside for human consumption during a food shortage (*ibid.*).

Table 8. Nutritional Assessment of Medieval Lay Diet

	Rural Peasants / Poor	**Urban Townsmen / Craftsmen**	**Urban Merchants**	**Aristocracy / Upper Classes**
Carbohydrates	Coarse brown bread,	Wheat bread	Wheat bread	Wheat bread
	pottage	Pottage?		
	Ale	Ale	Ale, wine	Ale, wine
Sugars	Fruit and milk	Fruit and milk	Fruit and milk	Fruit and milk
				Sugar
Fats	Some meat, some fish	Meat, fish	Meat, fish	Meat, fish
	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs
	Lard	Lard, puddings, sauces	Lard, puddings, sauces	Lard, sauces, nuts
Protein	Some meat, some fish	Meat, fish	Meat, fish	Meat, fish
	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs	Milk, cheese, eggs
	Pottage	Pottage?		
Vitamin A	Green/red vegetables	Green/red vegetables	Green/red vegetables	Some green/red vegetables
	Animal livers/kidneys	Animal livers/kidneys	Animal livers/kidneys	Animal livers/kidneys
	Some fish	Fatty fish	Fatty fish	Fatty fish
	Milk, butter, eggs	Milk, butter, eggs	Milk, butter, eggs	Milk, butter, eggs
Vitamin B	Meat	Meat	Meat	Meat
	Dairy, green vegetables	Dairy, green vegetables	Dairy, green vegetables	Dairy, some green vegetables
Vitamin C	Citrus fruits, cabbage	Citrus fruits, cabbage	Citrus fruits, cabbage	Citrus fruits, cabbage
Vitamin D	Herring, animal livers	Fish, animal livers	Fish, animal livers	Fish, animal livers
	Eggs	Eggs	Eggs	Eggs
Vitamin E	Fish, lard	Wheat bread, fish, lard	Wheat bread, fish	Wheat bread, fish
Vitamin K	Green vegetables, oils	Green vegetables, oils	Green vegetables, oils	Some green vegetables, oils
Calcium	(Milk), cheese	Milk, cheese	Milk, cheese	Milk, cheese
Iron	Red meat	Red meat	Red meat	Red meat
	Pottage (if oats)	Oats, bread	Oats, bread	Oats, bread
	Eggs	Eggs	Eggs	Eggs

4.4 Sites Analysis

It is difficult to apply this dietary analysis to the sites being used in this research. The four non-monastic sites, Fishergate House in York (a parish cemetery), the Royal Mint site in London (a Black Death cemetery), Hereford Cathedral in Hereford (a parish cemetery), and the Hospital of St. James and St. Mary Magdalene in Chichester (a hospital cemetery) - plus Blackfriars Friary, Ipswich, which may contain non-monastic burials (lay benefactors) rather than friars - are all composed of different sections of the population. Without detailed burial information, it is not possible to ascribe a definitive social class or a definitive diet to the individuals buried there. However, we can make some inferences based on the bioarchaeology.

It is uncertain what parish the cemetery of Fishergate House, York belonged to. It may have been associated with one of the churches known to have been in the area: All Saints, St Helen, St Stephen, or St Andrew. The individuals buried within the Fishergate House Cemetery have been suggested as being of low socio-economic status on the basis of their poor general health, the lack of high status grave goods, and the absence of coffin burials (Holst 2004, accessed September 2006). If this is the case, then the skeletons from Fishergate may have had a similar diet to those in the "rural/peasant" category as described above.

The Royal Mint Black Death cemetery was used for burial of those who died during the Black Death epidemic in London. The parish cemeteries filled up quickly during the epidemic so the Royal Mint site cemetery was sourced as an additional space for burial. As a result, the Royal Mint site likely contains individuals from all socio-economic groups in London at the time (Hawkins 1990). Therefore the diets from this site should be mixed.

Hereford Cathedral most likely contained a wide diversity of social classes within its cemetery. Amongst the individuals analysed, there was little sign of

poor nutrition in the skeleton (Stone and Appleton-Fox 1996). Hereford Cathedral may also have been the burial ground for another parish in Hereford, St John's, which represented people of all social classes (*ibid.*). Consequently, there should have been all types of diets represented; from rural/peasant to the upper classes. The principal cereal crop found here was barley, complemented with oats and a smaller amount of wheat. Locally available fruits included apples, pears, blackberries, elderberries, sloes; imported figs were also available. Cows, sheep, pigs, rabbits, and deer where known to have been eaten and likely supplied most protein, along with hazelnuts (*ibid.*).

The Leprosy Hospital of St James and St Mary Magdalene in Chichester catered for the "poor and pilgrims" (Magilton and Lee 1989:249) or, as Judd and Roberts (1998:52) relay, the "wanderers,...foreigners, and seafarers". The base diet of these people was likely similar to that of the rural/peasant population with lots of cereals and vegetables. However, once admitted into the hospital, all individuals would have been fed the same foods, which, according to Judd and Roberts (1998) is suggested to have been comprised of bread and beer, complemented by meat, herrings, cheese, eggs, butter, and fresh vegetables.

Blackfriars Friary in Ipswich, although a religious house, is thought to have yielded skeletons that were more likely to be lay benefactors than monks. According to Mays (n.d:62), the skeletons appear to have been "privileged in terms of diet and general health"; there is no evidence of chronic periostitis and juvenile health is good. Mays concluded that the Blackfriars burials were probably from the wealthier section of the population. Therefore they should have had diets similar to those described for the "urban merchants" or "upper classes".

We can also say something of the foods that were available in the region, in particular fish and cereals. With the exception of Chichester, all the sites likely contain individuals who were resident in the local area and so were likely to be consuming local produce. In York and London the most frequently found fish remains are cod, in the region near Hereford it is hake, and in the Chichester region it is conger eel, but herring (salted and preserved) would also have been

eaten in all these locations (Serjeantson and Woolgar 2006:115). As for cereals, according to Dyer's (1999b:154) geographical summaries, the dominant cereal crops in York would have been wheat and rye, and in London, Chichester, and Hereford wheat and barley. It is likely that wheat bread would have been available and also rye and barley bread for those unable to afford wheat. Pottage would likely have been made out of a mixture of those cereals, and ale, most likely from barley, where it was available, or possibly wheat. Meat, dairy, fruit, and vegetable consumption would, presumably, have been similar for all areas but would differ according to social class.

In terms of nutrition, the slight differences in local foods does not really alter what has been discussed previously; the basic descriptions of diet as they pertain to social class would still apply to these sites even if the actual socio-economic make-up of the cemeteries cannot be precisely determined. We could infer from the above data that the individuals from Fishergate House had a diet similar to the rural/poor, the individuals from the Royal Mint Black Death Cemetery and Hereford Cathedral had diets representing all categories, and the Blackfriars individuals had a diet similar to the urban merchants or upper classes. On the basis of the labels of the categories alone, we might expect that a "rural/poor" diet would be nutritionally lacking in comparison to an "upper class" diet simply because of the differences in purchasing power. However, when you begin to match the documentary and archaeological evidence for food with their nutritional values, it becomes clear that the upper classes were more likely to be suffering from nutritional deficiencies than the lower classes (as discussed in the previous section). This makes interpretation of the skeletal data more difficult because, quite often, a greater number of markers of nutritional stress are associated with a poor diet and with a lower socio-economic group. Of course, those without any skeletal lesions could also have been suffering from poor nutrition or other health problems but may not have exhibited them in their hard tissues. Therefore, the above assumptions about the social classes represented in the cemeteries, based on their skeletal health, may not be correct. However, for the purposes of this discussion, we will assume that the social classes assigned to these cemeteries are correct.

Individuals from Fishergate House should have been eating a well balanced diet based on cereals such as wheat (if it could be afforded) and rye, small amounts of meat and fish (cod, herring), fruit, vegetables, and some dairy. Some of the individuals from the Royal Mint Black Death cemetery should have been eating a similar diet, but with barley substituted for rye, whilst others from wealthier backgrounds would have been eating more wheat than barley/rye, more meat, and fewer vegetables and dairy. Those from Hereford Cathedral would be similar to the Royal Mint site but with hake substituted for cod. The individuals from Blackfriars, Ipswich, should have been eating a high status diet consisting of wheat, greater amounts of meat and fish than the lower classes, and fewer vegetable and dairy products.

It could be argued that the Hospital of St. James and St. Mary Magdalene may have had a different diet as, being an institution, everyone would have been fed the same foods regardless of class. Judd and Roberts (1998) suggest that this basic diet was comprised of bread and beer, complemented by meat, herrings, cheese, eggs, butter, and fresh vegetables. This is quite similar to the basic components of a late medieval diet anyway, although the proportions may differ, so it is unlikely that the residents at Chichester were much different in terms of nutrition to the other sites analysed.

4.5 Conclusion

Although the types of food and their overall availability were similar throughout the country, there were differences in consumption depending on the individual's social class. Essentially, as a person moved up the scale from peasant to aristocracy their diet became less well balanced and more detrimental to their health; the ratio of vegetables to meat inverted so meat became the dominant food source. If DISH is linked to high intakes of animal protein then, on the basis of this brief analysis, the upper classes should certainly be more prone to developing DISH. However, all of the above sites contain individuals with and without DISH so even the poorer sections of society, as represented by

Fishergate House, who should not have been able to access and consume large amounts of animal protein, show signs of the condition.

These differences in diet between different social groups mean that, when discussing the diets of late medieval people, one has to be conscious of the social context of the individual or population. It will be interesting to see whether any of these social divisions appear in the isotope data although, as discussed earlier, the differences in diet are mostly in terms of proportions of food eaten, rather than the consumption of different foods altogether, which is difficult to differentiate using stable isotope data. Even the standardised diet fed to the inhabitants of the hospital in Chichester may not be differentiable; since stable isotope analysis is an indication of long term diet (Muldner and Richards 2005, Richards *et al.* 1998), the individuals would have to be hospitalised for several years before this would impact upon their $\delta^{13}C$ and $\delta^{15}N$ results.

Chapter 5: Stable Isotopes and Diet

One of the analytical techniques used in this research project is carbon and nitrogen stable isotope analysis. This chapter will give a brief overview of the technique, how it is used and how it can be applied to the research questions posed in this study – whether DISH is related to high protein intakes.

5.1 Stable Isotopes

Isotopes are atoms of an element that still have the same number of protons but a different number of neutrons, resulting in atoms of different masses (Katzenberg 2000:307). Stable isotopes, unlike radioactive isotopes, do not decay over time and hence the amounts present in an organism will remain constant, even after death (Katzenberg 2000:307). Carbon and nitrogen are the commonly used isotopes when looking at diet and make up two-thirds of bone collagen by weight (Mays 2000a:425).

Stable isotope ratios are written as delta units, a measurement of the deviation in the isotope ratios from that of the standard – for carbon this standard is vPDB (Vienna Peedee belemnite), for nitrogen it is AIR (atmostpheric air) (Coplen 1994, Mays 1998:182). Delta units (δ) are expressed in parts per thousand, or *per mil*, using the symbol ‰. Delta ^{13}C is calculated using the following formula:

$$\delta^{13}C = \{[(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{standard}] -1\} \times 1000$$

Delta ^{15}N is calculated as follows:

$$\delta^{15}N = \{[(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{standard}] -1\} \times 1000$$ (Mays 1998:182)

Most biological materials have less ^{13}C than the standard and so their delta values are negative whilst there is usually more ^{15}N than the standard in these materials and so delta values for this isotope are positive (Mays 2000a:425).

Much of the stable isotopes that are investigated for diet are isolated from collagen. Bone is composed of approximately 70% inorganic and 30% organic tissue, of which 85-90% of the organic portion is collagen (Katzenberg 2000:308). Collagen is a protein and has a turnover rate of at least ten years in adults; therefore its stable isotopes give a good indication of long term diet (Müldner and Richards 2005, Richards *et al.*1998). However, although collagen can survive for thousands of years, it does eventually break down after burial so only bones with 5% or more intact collagen can be used for isotope studies (Katzenberg 2000:308, Mays 1998:184). Another source of carbon is bone carbonate found in the mineral portion of the bone. Bone mineral is largely made up of hydroxyapatite, a mineral salt that is primarily calcium phosphate and forms small crystals that combine to form a lattice-like network (Burns 1999:14). Carbonate is derived from dissolved bicarbonate in the blood and hence is made up of dietary carbohydrate, lipid, and protein which have been used by the body for energy (Katzenberg 2000:309). The mineral is also the source of oxygen and strontium for further isotopic studies on palaeoclimate, mobility, and palaeodemography.

5.2 Carbon and Nitrogen Isotopes

The use of carbon and nitrogen isotopes is based on the assumption that 'you are what you eat', in other words, that the carbon or nitrogen consumed is reflected in body tissues (Ambrose and Norr 1993:1). Carbon isotopes can be used to differentiate between a terrestrial and marine diet and also a diet of C_4 and a diet of C_3 plants – those that produce 4-chain and 3-chain carbon molecules during photosynthesis, respectively (Ambrose *et al.* 2003, Ambrose and Norr 1993:2). C_4 plants generally grow in warm climates and include sorghum, millet, sugar cane, maize, and tropical grasses whereas C_3 plants generally grow in temperate climes and include wheat, rice, root crops, legumes, vegetables, trees, and shrubs (Ambrose and Norr 1993:2). The average $\delta^{13}C$ value for a C_3 plant diet is -26‰ and the average for a C_4 plant diet is -12.5‰ (Ambrose *et al.* 2003). In terms of

bone collagen, which is usually about 4-6‰ less negative than diet, this equates to an average $\delta^{13}C$ value of -21‰ for a C_3 diet and a $\delta^{13}C$ value of -8‰ for a C_4 diet (Mays 1997, Ambrose and Norr 1993:23). The $\delta^{13}C$ value for marine diets usually falls somewhere in between the C_3 and C_4 values although, according to Richards *et al.* (1998), values for bone collagen would be close to -12‰ for those consuming >90% marine protein. Because it is sometimes difficult to tell whether the $\delta^{13}C$ values are a combination of C_4 and C_3 plant consumption or a mixture of terrestrial and marine foods, nitrogen is often used to help clarify the dietary components (Ambrose and Norr 1993).

Nitrogen isotopes are often used to look at sources of dietary protein, usually marine or freshwater foods, as well as the organism's place in the food chain. Due to what has been named the 'trophic level effect', whereby consumer tissue $\delta^{15}N$ values are elevated by approximately 2-4‰ over that of the dietary protein source, measurements of the consumption of animal protein has been possible (Ambrose *et al.* 2003, Fuller *et al.* 2003). This has also allowed weaning studies as breastfeeding children will have tissue $\delta^{15}N$ values 2-3‰ higher than their mothers, since they are in effect consuming their mother's tissues (Fuller *et al.* 2003). The consumption of aquatic foods can be identified through nitrogen isotope analysis since they exhibit significantly enriched $\delta^{15}N$ values over terrestrial animals. This is most likely due to the existence of longer food chains in aquatic environments that result in the accumulation of more trophic levels (Privat *et al.* 2002). Studies on historic populations who subsist primarily on marine foods, for example, Eskimo, Haida, and Tlingit groups, show bone collagen $\delta^{15}N$ values of between 17‰ and 20‰ (Schoeninger *et al.* 1983). Those groups that are primarily agriculturalists and subsisting on terrestrial food sources were found to have $\delta^{15}N$ values ranging from 6‰ to 12‰ and those that have mixed marine and terrestrial diets have bone collagen $\delta^{15}N$ values that are in between the two extremes (Schoeninger *et al.* 1983). However, when using nitrogen to examine protein consumption, some caution must be used as this ratio is often more greatly influenced by animal protein consumption than plant protein consumption since the percentage of protein in animal tissues is much greater than that found in plants (85-90% versus 10-25%) (Ambrose *et al.* 2003).

In general, though, a high $\delta^{15}N$ value reflects a high consumption of animal protein.

5.2.1 Scrambled and Routing Models of Carbon

Two models exist for the incorporation of carbon atoms from the diet into the tissues of the body – the linear, or scrambling, model and the routing model. These models have been refined through various animal feeding experiments conducted in the late 1980s that examined the different biochemical pathways of nutrients from diet to tissue. Essentially, all nutrients are broken down into their component parts during digestion so that starches become sugars, proteins are hydrolyzed into amino acids, and fats are broken down into glycerol and fatty acids (Schwarcz 2000:190). These basic components are eventually incorporated into the collagen and carbonate of bone following either the linear/scrambled or routing models. The linear, or scrambled, model proposes that all carbon atoms from the diet, no matter whether the source is protein, fat or carbohydrate, are incorporated equally into both collagen and carbonate tissues of the body; the routing model proposes that dietary proteins are directed into bone collagen (tissue protein) and dietary energy (fat and carbohydrate) is directed into bone apatite carbonate (Ambrose *et al.* 2003).

Ambrose and Norr (1993) tested these models by tracing the pathway of carbon from the diet to tissue to see whether it was "routed" or "scrambled". Prior to these experiments, no researchers had ever tested the routed and scrambled models to see which one was reality. Ambrose and Norr (1993) conducted nine experiments investigating the effects of protein nutrition on biochemical routing of carbon by raising rats on food pellets consisting of a variety of protein and energy levels, combining both C_3 and C_4 foods. They found that those fed on a pure C_3 diet had a $\delta^{13}C$ of -21.4‰ but that, if they changed the diet slightly so that 5% of the diet was protein from a C_4 source, the $\delta^{13}C$ increased to -14.7‰ and also resulted in the C_4 protein carbon atoms contributing to 51% of the C in the collagen, despite protein being only a small part of the diet (Ambrose and

Norr 1993:23). They found that a similar collagen contribution occurred with a 5% C_3 protein diet and that the same was true of both animal and plant protein, although plant protein was slightly less effectively assimilated.

Tieszen and Fagre's (1993) experiments were undertaken to explore the dietary factors influencing the differences between diet and collagen or bioapatite isotope values. They used mice fed on eight different diets that were a combination of C_3 and C_4 foods with varied amounts of protein. Through their experiments they found that the enrichment of collagen in comparison to diet was often less than 4‰, ranging from less than 1‰ to as much as 8‰, and was not the 4-6‰ enrichment that had been reported as standard in previous studies (Tieszen and Fagre 1993:151). They found that the isotopic signal in collagen was derived mostly from protein but also included lipids and sometimes starches, and that bioapatite contained the signal derived from respiratory CO_2 which contains all the biochemicals for energy metabolism.

These controlled diet experiments on rats and mice showed that collagen preferentially incorporates carbon from dietary protein whereas bone apatite carbonate incorporates the carbon from the whole of the diet (Ambrose and Norr 1993:27, Tieszen and Fagre 1993:153). As rats and mice have similar digestive systems to humans, these results were considered to be analogous to human routing of carbon from diet to tissues.

5.2.2 Determining Components of Diet

Separating human palaeodiets into broad categories such as 'marine foods' and 'terrestrial foods', whilst useful, may not provide the level of detail required in order to truly understand what different groups of people were eating and the impact on health and disease. To be able to break these categories into smaller parts and actually identify the percentage contribution of each type of food to the diet would provide much more information. Thanks to the use of stable isotope

mixing models it may be possible to do just that - to quantify the relative proportions of various food resources in past diets.

These techniques first appeared in the literature about three decades ago with the emergence of two key papers. DeNiro and Epstein (1978), famously credited with exploring the concept of 'you are what you eat', were feeding specific diets to different animal species to determine what influence diet had on carbon isotope distribution and suggested that it would be possible to use stable isotopes as palaeodietary indicators in human populations. Around the same time, Vogel and van der Merwe (1977) were actually applying a single stable isotope, carbon 13, to the investigation of the consumption of maize in New York State, USA. Here, it was assumed there was a linear relationship between the $\delta^{13}C$ value and maize consumption, thus allowing the proportion of maize actually consumed to be estimated. Following this study were attempts to determine the proportions of terrestrial and marine protein in the diet, also using carbon isotopes. Chisholm *et al.* (1982) compiled a list of animal species that they thought would have made the largest contribution to the protein in the diet of an aboriginal population off the coast of British Columbia, Canada. They took samples from these animals, analysed them and compiled a list of isotope values that could be compared to that of the human population. By doing this, Chisholm *et al.* were able to estimate the percentage contribution of terrestrial and marine protein in the diet of this population.

Since the late 1970s, early 1980s, techniques have moved forward and the use of the linear mixing model is now standard, usually with the incorporation of added techniques such as concentration dependence. A standard linear mixing model allows the determination of the relative contributions of n+1 food sources to the diet, where n is the number of isotope systems ($\delta^{13}C$, $\delta^{15}N$ etc.) used (Bocherens *et al.* 2006, Schwarcz 1991). This means that if ^{13}C is being used to analyse diet then the proportions of two different food types can be determined; if both ^{13}C and ^{15}N are being used, the proportions of three different food types can be determined. Obviously, there are limitations with this technique when attempting to apply it to human populations as the number of potential food sources is often far greater than the number of stable isotope systems used. To

combat this problem, Newsome *et al.* (2004) used a 'source-partitioning' model to constrain the relative proportions of the various food sources in the diet being studied. This allows the assessment of all the different combinations of each source (from 0% to 100%) that add up to the known isotopic signature, i.e. the isotope value of the human bone collagen. From these data, a distribution of the frequency and range of the potential source contributions can be created. Another problem that must be combated is that the food sources do not necessarily contribute to each element equally; in other words, the nitrogen proportion of a certain food may not be equal to the carbon proportion for that same food. This leads to the creation of a "concentration-weighted" model whereby the contribution of each source is proportional to the contributed mass times the elemental concentration in that source (Phillips and Koch 2002). Using this model, Philips and Koch (2002) were able to identify food sources even when they had widely varying differences in C and N concentration.

Little and Little (1997) adopted a more mathematical approach to linear mixing models by using linear programming to analyse the isotopic data. They set out to address some of the issues with using isotope studies to reconstruct diet, namely the issue of ambiguity that creates a range of possible diets that are all consistent with the isotope values obtained from the human bone collagen being studied. Little and Little (1997) demonstrate how linear programming can be used to narrow this range of ambiguity by setting upper and lower bounds on the percentage of each food group in the diet. Through this method it is possible to determine how much or how little or a particular food group could possibly be in the diet. The advantage of this technique is that several different food groups can be considered, i.e. greater than one more than the number of isotope systems used; the disadvantage is that the isotope data being used to generate the dietary data is limited in its accuracy due to measurement errors. However, the calculations can of course be rerun several times using values considered to be within the range of measurement error in order to generate more accurate results.

Newsome *et al.*'s (2004) study attempted to determine the proportions of seven different foods (marine fish, shellfish, pinnipeds, terrestrial meat, leafy plants, nuts, and seeds and grains) in the diet of early and middle Holocene groups from

the Californian coast. They used modern marine fish and shellfish to obtain isotope values, adjusting them for changes in $\delta^{13}C$ due to modern pollution, and for the remaining foods used data obtained from animal bones found at the site. In total, the calculations produced 11, 345 possible combinations of the seven food sources for the early Holocene group, and 35, 021 combinations for the middle Holocene group, each with a minimum and maximum percentage contribution and a mean, similar to Little and Little's (1997) linear programming method. Using these minimum and maximum values, the authors were able to make comparisons between the two groups and establish which foods had increased or decreased in terms of consumption from the early Holocene to the middle Holocene.

Bocherens *et al.* (2006) also used linear mixing models to reconstruct diet, in this case the diet of ancient people from south-west Turkmenistan. The researchers had several related animal species that they wanted to combine into one category for the purposes of analysis and they explored two ways of doing this in order to find out which was the best method. The first method is based on the percentage of remnants of each species; the second is to weigh the bones from each species. Since the study included cows, goats and sheep the weighing method produced a much higher value for cattle as they are bigger animals, whereas for the percentage of remnants the caprines produced the higher number, although this method can obviously be biased due to fragmentation. Bocherens *et al.* (2006) decided that the best way to determine the plant food isotope composition was to calculate the signature of an animal consuming the same type of plants that humans would have consumed and then adjust the value to accommodate the isotopic shift between plant and animal tissue. For this they used a pig as it was less likely to be consuming wild plants that would not have been part of the human diet. The authors concluded that using the value for the percentage of remnants or the weight of the bones had little influence on the final results (which showed that plant foods were dominant, pig meat was optional, and that cattle, sheep and goats needed to be included in the diet in order to fit the isotopic data). They felt the weighing method to be more realistic so long as the animals were consumed as meat and they cautioned that is was not possible to quantify the consumption of dairy products in the same way. Ultimately, this

analysis allowed Bocherens *et al.* to compare the minimum contribution of ruminant meat and dairy in humans from the Historical period with those of the Iron Age.

All of these studies present methods and techniques that allow dietary reconstruction on a more specific level than is provided by the usual carbon and nitrogen isotope analysis. Although linear mixing models have the potential to tell us exactly what past populations were eating and how much, the technique relies on two unknowns, firstly in identifying potential sources of food and secondly in calculating the isotope values of these foods precisely. Thus, there will always be a degree of error and uncertainty in the results. It seems that the strength of the linear mixing model lies in its ability to generate comparative results for populations or groups of individuals. By using it as a method of comparison it is possible to see which groups were eating more, or less, of a particular food in comparison to others at the same site, a very useful tool for analysing status or social groupings. Unfortunately, as there is a lack of detail about the specific foods being consumed by the monastic and lay populations at the sites used in the study, this method of analysis is not possible.

5.3 Limitations

Several factors limit the ability to use and interpret stable isotopes for dietary analysis. One of the most influential factors is that of bone collagen diagenesis, for without a reliable source of carbon and nitrogen to study there can be no stable isotope analysis. After burial, bone collagen breaks down into large peptide units, which in turn break down into amino acids, which will eventually leach out of the bone potentially changing the amino acid composition (Mays 1998:184). The original amino acid composition of the bone collagen will remain until approximately 5% of the original protein remains but, once degradation of this final 5% occurs, a new amino acid composition predominates, thus changing the outcome of the stable isotope ratios (DeNiro and Weiner 1988, Mays 1998:184). Bone carbonate is often sought out when there is not enough

remaining bone collagen to be analysed as it is seen to be more stable and longer lasting. However, this too can be altered in the burial environment as the ions that make up the hydroxyapatite crystals in the bone can be substituted for those in the soil sediments, thus changing the carbon isotope ratios (Katzenberg 2000:308). Fortunately, laboratory preparation methods have been developed that can remove the soluble carbonates likely to be the source of such contamination. The C/N ratios of collagen can also be used as an indicator of diagenesis; if they fall within a certain range the samples are considered to be unaffected. Different authors have reported different ranges, for example DeNiro (1985) states that the acceptable range is between 2.9 and 3.6, whereas Schoeninger *et al.* (1989) state that the range is between 2.6 and 3.4.

Once the potential problems of bone diagenesis are overcome, the researcher is faced with problems of interpretation of the stable isotope data obtained. Although it is reported in the literature, and proven through experimentation, that consumption of marine resources will produce a dietary signal with a high $\delta^{15}N$ isotope value, it is not always the case. For marine organisms that use nitrogen fixation, such as those existing in coral reefs, nitrogen isotope values will be low, resembling those of terrestrial animals. Therefore any groups of people subsisting on tropical reef organisms will not produce a marine signal for their $\delta^{15}N$, despite having a diet that is primarily marine in origin (Schoeninger *et al.* 1983). However, subsistence on this type of diet should be evident from the $\delta^{13}C$ data.

There are also problems with identifying the source of protein in the diet. As isotope values only separate protein input to the diet into broad categories of plant, herbivore, and carnivore protein from either marine or terrestrial environments, it is not possible to distinguish between protein sources from the same animal as they are isotopically identical (Müldner and Richards 2005). Therefore, meat and dairy products would give the same isotope values even though they are nutritionally very different and are also likely to carry very different weightings in terms of cultural value. If meat is the only protein source being consumed then it is also difficult, if not impossible, to distinguish between someone who eats only a small amount of meat, for example, 100g, and someone

who eats a large amount of meat, for example, 500g (van Klinken *et al.* 2000:51). This could cause difficulties in assessing the overall nutrition and health of a person and their diet.

There is evidence that isotope values are influenced by climate as well as diet. Those people consuming a terrestrial diet and living in the warmer regions of Europe are found to have $\delta^{13}C$ values that are 1 or 2‰ more positive than those people living in the colder northern countries (Richards *et al.* 1998). Therefore, $\delta^{13}C$ results that seemingly indicate a small marine influence (i.e. are more positive than the average), but are not supported by high $\delta^{15}N$ values (as would be expected for a marine diet), could be due to migration from a warmer climate shortly before death. This example also demonstrates the advantage in using both carbon and nitrogen isotopes to reconstruct diet; if the two values do not concur then new explanations must be sought out. Care must also be taken when comparing modern $\delta^{13}C$ values with those from antiquity as atmospheric pollution has meant that the modern carbon isotope values are approximately 1.5‰ more negative than they were in the past (Mays 1997).

One last issue to be addressed that could alter stable isotope values and interpretation of diet is pathology. Katzenberg and Lovell (1999) investigated the effects of fracture, periostitis, atrophy, and osteomyelitis on stable isotope values from several individuals by comparing values obtained from the pathological bone with that of normal bone from the same individual. They found that, during times of stress, the body would be in 'negative nitrogen balance' as a result of preferentially recycling ^{14}N from body tissues to replace the lack of nitrogen being ingested, resulting in an enrichment of ^{15}N in the remaining tissue. Therefore, newly formed/repaired tissues may have a slightly different isotope value than would be found in normal tissues and may not reflect the average diet accurately. None of the samples used in this research study were taken from bones showing evidence of new bone growth and/or lesions.

5.4 Stable Isotopes and Diet

To date, very few studies have used carbon and nitrogen stable isotopes to examine later medieval diet, especially on a broad scale. Such studies often explore very specific topics such as weaning age, using dietary isotopes to help interpret radiocarbon dates, or looking at the contribution of marine resources to diet (Bayliss *et al.* 2004, Fuller *et al.* 2003, Mays 1997) rather than producing a general overview of diet in the middle ages. In those studies that do take a broader approach to the exploration of late medieval diet, the emphasis is on examining marine versus terrestrial diets, and excludes the examination of C_4 versus C_3 diets since C_4 plants were virtually nonexistent in England until sugarcane and other C_4 plants were introduced in the post-medieval period (Mays 1997).

One such example of an overview of late medieval diet is that of Müldner and Richards (2005). They examined the range of isotopic variation amongst northern English medieval populations by examining three sites – St Giles Hospital in North Yorkshire, an Augustinian Friary in Warrington, Lancashire, and a mass grave from the village of Towton, also in North Yorkshire. These sites were all from the same time period yet were selected to represent very different sections of society, in the hope that it would provide a more complete picture of late medieval diet. Samples of bone collagen were prepared from each of the sites and analysed in terms of ^{13}C and ^{15}N ratios. It was found that the $\delta^{13}C$ ratios from all three sites ranged between -20.6‰ and -18.1‰ and that the $\delta^{15}N$ ratio values ranged between 10.5‰ and 14.9‰. According to the authors, this was a surprising result as the $\delta^{13}C$ values suggest a terrestrial C_3 based diet with no, or only minor, marine input and yet the $\delta^{15}N$ values were too enriched to be explained by consumption of terrestrial herbivore protein alone. Müldner and Richards hypothesised that the $\delta^{15}N$ ratios could be explained by consumption of omnivore protein such as pigs or the consumption of freshwater resources, possibly eels or other river fish. Despite the social diversity of the three sites, the diets showed little variation in terms of isotope ratios.

Müldner and Richards (2007b) also looked at late medieval diet in 155 adults from the priory of St. Andrew, Fishergate in York. They attempted to differentiate between individuals buried in different locations within the cemetery on the basis of their carbon and nitrogen isotope results. The $\delta^{13}C$ values ranged from -20.8‰ to -16.5‰ with a mean of -19.1‰ +/- 0.6‰. The $\delta^{15}N$ values ranged from 9.1‰ to 17.2‰ with a mean of 12.8‰ +/- 1.3‰. They found that the isotope values from males and females overlapped, but that males had statistically significantly higher values for both carbon and nitrogen, indicating that males ate more marine foods than females. They also looked at four male individuals with DISH, all of whom plotted with the isotope values for the other males, but all of whom also plotted above the male means of -18.9‰ for $\delta^{13}C$ and 13.0‰ for $\delta^{15}N$. Müldner and Richards inferred from this that those individuals with DISH were eating a diet rich in animal protein and marine foods. There were few differences between individuals buried in different locations and no differences between lay and monastic individuals. Overall, they concluded that stable isotope analysis may not be sensitive enough to deal with the complexities of medieval diet.

Müldner and Richards (2007a) also looked at diet in York through time, spanning 1500 years of diet from the Roman to the post-medieval period. Their analysis of the late medieval samples demonstrated that just prior to this period, in the 11th and 12th centuries, there was a transition from terrestrial to terrestrial and marine protein diets. As a result, the later samples showed enriched $\delta^{13}C$ and $\delta^{15}N$ over earlier periods indicating that Christian fasting regulations were being adhered to. They also found high $\delta^{15}N$ values amongst some of the samples which, for the late medieval period, could be explained by high protein diets leading to a larger than normal trophic enrichment, consumption of freshwater fish, or consumption of omnivore protein (pork or poultry).

Another late medieval dietary overview is that of Polet and Katzenberg's (2003) reconstruction of diet in a monastic community on the Belgian coast. They studied skeletons from the Dunes abbey of Koksijde, a Cistercian monastery in Belgium where approximately 2000 people were buried, mostly monks and lay-brothers. The authors analysed 29 skeletons using bone collagen to obtain the

average $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of -19.1‰ and 11.1‰, respectively. They determined that these individuals had a high $\delta^{15}N$ stable isotope ratio accompanied by a high $\delta^{13}C$ ratio, indicating marine influence. As this population were living on the coast this finding was not surprising, but nevertheless it was supported by the fact that there was a low caries rate (marine diets are rich in fluorine) and also that the middens contained a high proportion of marine fish bones, and mollusc shells. Polet and Katzenberg (2003) also looked at children from the monastery to see whether their isotopic values would differ from the adults. They could not be differentiated. They decided that the reason for this lack of distinction between adult and child diet was either due to the fact that the children were boarders at the monastery, and hence ate the same food as the monks, or because the surrounding populations that the children came from also had similar diets to that of the monks. It was also discovered that those individuals buried in the more privileged areas of the church (the nave, or the chapels) exhibited fewer skeletal stress indicators and also had higher $\delta^{13}C$ and $\delta^{15}N$ values, perhaps indicating that they ate more terrestrial or marine animal protein. Their Sr levels were also examined and found to be lower than the other individuals and this was taken to indicate that they had a diet 'richer in meat'.

Bayliss *et al.* (2004) took a slightly different approach to dietary reconstruction, using it as a means to a different investigation altogether. They examined the diet of a medieval site in Norwich, the cemetery of St John the Baptist, Timberhill, in order to use the stable isotope data to help interpret radiocarbon dates at the site. On the basis of archaeological and documentary evidence, it was interpreted that the cemetery was in use from the early to mid 12th century until the late 13th-14th century, and was therefore founded post-Norman conquest. However, on the basis of radiocarbon evidence, it was interpreted that the cemetery was founded sometime between 980-1030 AD and ended in 990-1050 AD, being in use for a maximum of 65 years and therefore founded pre-Norman conquest. This difference was quite significant because the cemetery contained a number of individuals with leprosy and could have been a leprosy hospital, something which was thought to be a Norman invention.

Bayliss *et al.* (2004) declared that as carbon and nitrogen isotope values are measured from bone collagen, which is derived from protein in the diet, it is essential to know where the protein in the diet comes from and how it might influence the radiocarbon data; it is the radiocarbon age of this protein that is measured when dating skeletons. The authors produced several different dietary models based on documentary, archaeological (i.e. faunal remains), and stable isotope data which they used to re-interpret the chronology obtained from the radiocarbon data. Three of the models – one which assumed a purely terrestrial C_3 diet, one that used documentary evidence, and one that used nitrogen isotope values - showed good overall agreement with the radiocarbon dates and the stratigraphic evidence, thus producing three different interpretations of the stable isotope and radiocarbon data, each of which produced a different estimate for the chronology of the Timberhill site, differing by 50 years. This presented problems for the researchers as this small shift of 50 years in the chronology of the site had major implications for the interpretation of the archaeological evidence. Although this study could not settle the issue of correcting radiocarbon dates for the effects of diet, it was concluded that measurement of carbon and nitrogen stable isotopes should be taken whenever human bone was dated so that the possible effects of diet could be assessed and taken into account in the interpretation of the radiocarbon results.

Many of the other medieval-focused studies of diet centre on specific topics. One such example is Mays' (1997) study of marine contribution to diet at medieval sites in north-east England. He looked at skeletons from seven different sites, taking samples from one monastic and six lay populations with the intention of comparing diets of monastic and lay peoples, and also coastal diets to those inland. Mays found that the monastic and lay groups from St. Andrew Fishergate, York differed in terms of $\delta^{13}C$ values, indicating that marine foods made a greater contribution to the monastic diet than to the lay diet. This was also supported by the lower rate of dental caries found amongst seafood consumers, although it could also be due to consumption of lesser amounts of cariogenic foods high in carbohydrates. The lay people from St. Andrew Fishergate, York still consumed marine foods, although in comparison to those from Wharram Percy, a small village nearby made up of rural peasants, there was

little difference in seafood intake despite having the money and resources to buy and access seafood. Significant differences did exist, however, between the coastal sites of Hartlepool Greyfriars and Newcastle Blackfriars, both northeast of York, where seafood made a greater contribution to the lay populations sampled there than to those from the inland sites.

Another such example of an isotopic study used to investigate a specific dietary question was that of Fuller *et al.*'s (2003) study of weaning at medieval Wharram Percy in Yorkshire. As breastfeeding children have tissue $\delta^{15}N$ values 2-3‰ higher than that of their mothers due to the 'trophic level effect', any decline in these values will represent weaning and the consumption of foods other than their mother's milk. Once the child is fully weaned, that is, no longer consuming any breast milk, and is consuming the same foods as its mother, the $\delta^{15}N$ values of both parent and child are nearly identical. Fuller *et al.* used tooth sections and compared the isotope data to that obtained from ribs in order to track the changes in diet from infancy to childhood and beyond. They believed that there may be differences in feeding practices for those that died before reaching two years of age, and those that survived into childhood that would account for infant deaths. This hypothesis was not supported though as there were no statistically significant differences in the $\delta^{13}C$ or $\delta^{15}N$ isotope ratios for the two age groups. However, the authors did discover that there was an enrichment of ^{13}C in breastfeeding infants - a carbon trophic level effect. In previous studies, this had been postulated as being due to the presence of C_4 foods during weaning, but C_4 foods could not account for the changes in the Fuller *et al.* study as there were none in existence at Wharram Percy. Interestingly, through analysing teeth and ribs formed later in life, Fuller *et al.* found that diet changed with age and, in some of the individuals they examined, that there was a decrease in animal protein later in life.

There are, of course, numerous studies of diet using stable isotope analysis from other periods in England besides the medieval, and from other regions of the world. Richards *et al.* (1998) used stable isotopes to reconstruct diet from an Iron Age/Roman site in southwest England, with the hope of being able to find differences in diet between individuals that they could attribute to social class.

They found there was little variation in the isotope values of the Iron Age/Early Roman individuals and that they consumed a terrestrial based diet. Amongst the Late Roman burials however, most of which were in lead coffins or from mausolea found in the cemetery, $\delta^{13}C$ values were more positive, as were the $\delta^{15}N$ values, indicating a marine contribution to their diet. As the authors had already hypothesised that these individuals were of a higher class (due to the mode of burial) they took the isotope data to be a reflection of a high status diet, thus concluding that fish was a food consumed by members of the upper class only.

A more in-depth study of social status was conducted by Ambrose *et al.* (2003) at the Cahokia site in the Mississippi River valley of Illinois, United States. This mound site was dated to the Lohmann and early Stirling phases of the Mississippian period (1050-1150AD), comparative in date to the medieval period in England, and the burial styles of the graves are suggestive of status and gender differences due to the presence of grave goods, the use of mass graves, and differences in frequencies of pathological indicators of nutritional stress. The authors' aim was to correlate the skeletal and archaeological data with data from stable isotope analysis, and is one of the few studies to link palaeopathological data with stable isotope data. They found that those individuals thought to be of high status because they were buried in single graves with grave goods and exhibited fewer indicators of nutritional stress in their skeletons had stable isotopes values suggesting higher animal protein consumption and less maize consumption (although still a high proportion of the diet) than those of the lower status individuals. The low status individuals, most of whom were young females buried in mass graves and exhibiting skeletal and dental signs of nutritional stress, had stable isotope values that indicated substantially less animal protein and greater maize consumption than those in the high status group.

Privat *et al.* (2002) used both human and animal remains in their stable isotope analysis in order to identify dietary trends within and between groups at an Anglo-Saxon cemetery at Berinsfield, Oxfordshire. Unlike other studies of diet, Privat *et al.* used the stable isotope analysis of faunal remains recovered from the

site to first establish a baseline for comparison with the human data. Their results for the ^{13}C analysis indicated a diet based on terrestrial C3 and possibly freshwater resources whilst the $\delta^{15}N$ values were enriched over the faunal baseline indicating consumption of a significant amount of animal protein. There were some adults with "highly enriched" $\delta^{15}N$ values, up to 6‰ above the herbivore faunal material, and it was concluded that these individuals either consumed aquatic animals regularly or omnivore protein (for example, pigs). A comparison was made between male and female isotope ratios but no sex-dependent dietary trends emerged despite there being very distinct roles for the sexes in this time period. However, the authors do caution that isotope analysis cannot distinguish between protein derived from the flesh of an animal and protein derived from that animal's secondary products nor can it determine the quality of the food consumed, therefore differences in diet may have existed in actuality. A comparison between the "poor", "intermediate", and "wealthy" classes (categories were determined by number and type of grave goods) showed elevated $\delta^{15}N$ values amongst the "poor" group which suggested either that they consumed more aquatic and/or omnivore protein or that the wealthier classes consumed more herbivore meat/and or dairy products . Although the consumption of fish, and high $\delta^{15}N$ values, is usually associated with upper classes in stable isotope studies, here the data can be explained by the fact that the site is located near the Thame and Thames Rivers which would have meant riverine resources were readily and cheaply available whereas raising sheep, cattle or goats would have been expensive and therefore highly desirable.

5.5 Conclusion

Although the study conducted by Katzenberg and Lovell (1999) looked at the affect of disease on stable isotopes it was not using stable isotopes to investigate the patterns of disease in the skeleton. This is an area that seems to be neglected in palaeopathology and yet could be a very useful avenue of research. Many diseases are deemed to be related to diet, DISH included, yet there are only a few studies that link palaeopathological indicators of disease with stable isotope

analysis. Ambrose *et al.*'s (2003) research, discussed above, is one such study; using stable isotope analysis to support their findings of nutritional stress indicators in the skeleton and also as a basis for establishing status differences. White and Armelagos (1997) also used isotopes to look at pathology, in their study, the focus being on osteopenia in Nubians. They found that osteopenic females had higher $\delta^{15}N$ values than non-osteopenic females and suggested this was linked to water stress and kidney function.

The other, more general, studies of diet and stable isotopes discussed here present some interesting results yet they are all too individually focussed to draw generalised conclusions from with regards to age, sex, and social class. With regard to age, Polet and Katzenberg's (2003) study showed that children were isotopically indistinguishable from adults, yet Fuller *et al.* (2003) found that diet changed with age which contrasts with the absence of any difference between the adult monks and the child oblates of the Belgian monastery. In terms of sex, it was found by Privat *et al.* (2002) that, despite their site having evidence for distinct roles for males and females, that they thought would have been reflected in diet through isotope values, no such pattern emerged. However, this was not a concrete result as it is not possible to distinguish between meat and dairy products or determine the quality of food eaten so a sex-based dietary trend may yet still exist. With respect to social class, the results are a little more muddled. Müldner and Richards' (2005) study, which included a monastic site, a hospital, and a mass grave from a battle site, found that there was very little isotopic variation (and hence very little dietary variation) between these three socially diverse sites. However, Mays' (1997) study of a monastery in York concludes that monks ate more seafood than lay populations. Fish was deemed to be a high status food by both Mays (1997) and Richards *et al.* (1998), yet Polet and Katzenberg's (2003) study of Belgian monks concluded that the more privileged members of the monastic community ate more meat than those in the lay population. Privat *et al.* (2002) also found that the poorer classes at their site consumed more aquatic protein than did the wealthier classes. However, they explain that this site was located close to a river so it was thought that the ease of access to fish deemed it less socially valued, with consumption of the more resource-heavy meat products being more indicative of wealth. This may also

explain the findings of Polet and Katzenberg (2003) as their site was located on the Belgian coast.

It would seem then that making generalisations about diet and expectations of stable isotope findings is difficult. The outcomes of the stable isotope analysis, and the conclusions drawn, appear to be dependent upon the context of the population studied and its location. Monastic populations seem to be particularly problematic as they are difficult to place on a social scale; they are meant to be living humble simple lives, consuming foods similar to lower class lay populations, yet often are described as being closer, in dietary terms, to the upper classes. However, so long as the environmental and social context of the population being analysed is known, stable isotope analysis should provide some informative results.

Chapter 6: Ancient DNA (aDNA) Analysis

The other analytical technique used in this research project is ancient DNA (aDNA) analysis. This chapter will give a brief overview of aDNA analysis, how it has been used and how it can be used to address the questions posed in this study – whether DISH has a genetic component.

6.1 DNA and Ancient DNA

Deoxyribonucleic acid (DNA) is the genetic material for nearly all organisms, with a few viruses being the exception. It is composed of four types of nucleotides which differ in terms of what bases are attached to them. These four bases are adenine, guanine, cytosine, and thymine, often abbreviated as A, G, C, and T, respectively, and it is the sequences of these bases that provides the genetic information for the organism (Jobling *et al.* 2004:22). Each of these bases is joined to the sugar molecule deoxyribose and each deoxyribose has a phosphate group attached to it. This interaction between deoxyribose and phosphate molecules is what gives DNA its stability, forming the 'sugar-phosphate backbone', and also its polarity, creating a 3' end and a 5' end which are important for the processes of DNA replication, transcription (into RNA), and translation (of RNA into protein), as the enzymes that carry out these processes will only proceed in one direction along the molecule (Griffiths *et al.* 1998:388-89). Within a cell, DNA is mostly in a double-stranded form, with two ends of opposing polarity joined together – i.e. 3' and 5' – and intertwined to form a double helix. The bases of one strand pair together with those of the complementary strand so that A always pairs with T, and C always pairs with G (Strachan and Read 1996:5). DNA has two important functions – it carries the codes for making different proteins which themselves manufacture different components of the cell, and it provides a means for these codes to be passed on to the next generation of cells when cell division occurs (Jobling *et al.* 2004:22).

There are two types of DNA in the human genome – nuclear and mitochondrial. In each diploid, somatic cell (non-reproductive body cell) there is only a single nucleus containing 46 linear molecules known as chromosomes, consisting of two copies of each of 22 autosomes and a pair of sex chromosomes – XX if female, XY if male. A single cell holds approximately $3.2x10^9$ base pairs (bp) of genetic material (Hummel 2003:20). Two to ten copies of mitochondrial DNA, which are circular molecules, are found in each mitochondrion of which there can be as many as one thousand per cell (Jobling *et al.* 2004:22). Mitochondrial DNA (mtDNA) is only passed on from the mother's side of the family and is much smaller than nuclear DNA with only 16,569bp. The entire sequence of the mtDNA is known and the segments known as hypervariable region I and hypervariable region II (HVRI and HVRII) can be differentiated from the many polymorphisms that occur elsewhere in the mtDNA (Jobling *et al.* 2004:40).

In archaeological studies, any genetic material found is referred to as ancient DNA. Ancient DNA (aDNA) is considered to be any DNA molecules that are preserved in ancient biological material or, put another way, any biomolecule that can be extracted from ancient tissues (Brown and Brown 1992, Wayne *et al.* 1999). In human remains, this aDNA can be extracted from the roots of teeth, coprolites, bone, and the cellular remains of soft tissues that have been frozen, mummified or preserved in water (Stone 2000:351). Ancient DNA analysis is now used to answer questions of biological sex, migration, plant domestication, and relationships between individuals to name but a few examples. Generally, mtDNA is used in aDNA studies, as it is circular and thus more robust against degradation and there is a higher copy number per cell than nuclear DNA and so a better chance of survival and recovery. Mitochondrial DNA is maternally inherited, does not recombine and has a higher mutation rate which makes it ideal for tracing lineages (Stone 2000:354, Rubicz *et al.* 2007). Nuclear DNA is also used in archaeological studies but it is often more degraded and, hence, difficult to study. If nuclear DNA is recovered, however, it can be used to identify biological sex, and also relatedness between individuals (Faerman *et al.* 1995, Faerman *et al.* 1998, Gerstenberger *et al.* 1999, Hummel *et al.* 1999, Hummel and Schultes 2000).

6.2 The History of Ancient DNA Studies

The first attempts at aDNA analysis took place in the early 1980s. These first experiments were merely attempts to prove that extraction of DNA from ancient biological material was possible, rather than attempts to answer any particular question or hypothesis about the material being studied. Many of them focused on using museum specimens rather than material taken directly from an archaeological site because this material was readily available and also provided access to preserved soft-tissue remains.

The first successful extraction and cloning attempt was made by Higuchi *et al.* (1984) on a 100 year old piece of museum muscle tissue from an extinct quagga. The DNA of this horse-like animal was cloned and its sequences compared to animals thought to be its modern relatives. Higuchi *et al.* found that the quagga was closest in sequence to the modern zebra and that the two animals likely shared a common ancestor 3-4 million years ago. The success of this study led to more extraction attempts from museum material, most notably that of Pääbo (1985) who extracted human DNA from 23 museum-curated mummies. He sampled different tissues from these mummified bodies and concluded that the best aDNA preservation was in the superficial tissue layers rather than the subcutaneous tissues. This was likely due to the way that the tissues preserved during mummification; the outer layers would have desiccated first, thus inhibiting hydrolytic processes that act to degrade DNA. One of the most interesting findings of this study, however, was not that some tissue layers yielded better preserved DNA than others but that long fragments of DNA could be recovered from ancient material. Pääbo was able to extract fragments up to 5000bp long which gave biological researchers hope that it might be possible to identify genes in ancient remains.

The studies of museum material firmly established that DNA survived in ancient tissue but would the same be true of bone? Hagelberg *et al.* (1989) tested a range

of archaeological bone varying in age from 300 to 5500 years old. With the help of polymerase chain reaction (PCR) (see section 6.3.1), they were able to successfully extract and amplify DNA from bones of all ages although the fragments recovered were not as long as those from soft-tissue. Thus, they concluded that the recovery of DNA was dependent upon the burial conditions of the sample and not its age. Most importantly, their results were reproducible - they were able to produce the same results from different extracts of the same bone. Once it was established that DNA extraction was possible (and reproducible) from archaeological material thousands of years old and that long fragments could be identified, research expanded to test what the age limits of DNA recovery were. The early 1990s saw the publication of several studies with materials that were millions of years old. Extraction of DNA from a fossilised magnolia leaf 17-20 million years old (Golenberg *et al.* 1990) led to several attempts at extraction from material trapped in amber (for e.g. Cano *et al.* 1993, De Salle *et al.* 1992, Poinar *et al.* 1993). Amber preserved materials were seen as likely targets for DNA survival because of the speed with which amber desiccates the material and prevents degradation by bacteria (Wayne *et al.* 1999). One of the most exciting of these attempts on material beyond the one million year mark was that of Woodward *et al.* (1994) who extracted DNA from an 80 million year old dinosaur bone. This study seemingly removed the barriers of age from the ability to recover ancient DNA; if it was possible to extract and amplify DNA from the Cretaceous period then it must be possible to extract and amplify DNA from any archaeological period.

Unfortunately, the bubble of excitement that had formed over these early aDNA studies soon burst. Later attempts to reproduce the data from fossilised plants, the amber insects, and the dinosaur bone failed. It was soon apparent that the DNA simply did not survive that long and that the sequences that had been amplified were contaminants from modern species and, in the case of the dinosaur DNA, not even an animal sequence but human (Zischler *et al.* 1995)! Even the long fragments of DNA from the museum mummies that had prompted talk of gene reconstruction were found to be a long repeat sequence of uninformative DNA (Del Pozzo and Guardiola 1989). Several important lessons were learned from these early studies. Firstly, that DNA survival had time limits;

and secondly, that contamination from modern DNA was a big concern (see sections 6.4.1 and 6.4.2 later in this chapter for discussion).

The mid-90s, and beyond, represented a new phase in aDNA research. Having established that DNA survived in ancient remains, and that it was possible to extract it, research turned to more question-based hypotheses such as tracing the origins of and identifying disease, investigating migration and dispersal of different groups of people, and attempting to establish genetic relationships between different individuals and groups. Attempts were also made to follow the newly established authenticity and anti-contamination criteria that were an outcome of the early studies on aDNA.

6.3 Techniques of DNA Extraction and Analysis

6.3.1 Extraction of aDNA

The techniques of aDNA extraction and analysis have been borrowed from molecular biology and forensic science and slightly modified to accommodate low yields of genetic material. In terms of the extraction process, the key factor with ancient DNA analysis is minimising the risk of contamination. This is done in a variety of ways but generally involves the use of separate extraction and analysis laboratories, use of disposable lab consumables, UV irradiation (or use of DNA removal solutions) of work surfaces and equipment, and negative extraction and PCR controls so that any contamination can be identified at each step (Adachi *et al.* 2003, Cooper and Poinar 2000, Gerstenberger *et al.* 1999). The bone or tooth sample itself is also commonly subjected to irradiation with UV after the outer layers (and surface contaminants) have been removed (Adachi *et al.*. 2003, Bouwman and Brown 2002, Gerstenberger *et al.* 1999, Hummel *et al.* 1999). The actual DNA extraction usually involves grinding up the bone or tooth material or drilling into the interior of the sample to remove the material from inside the bone or tooth.

Once the bone or tooth has been cleaned and ground up or drilled, there are several different methods that can be applied to extract the DNA. The methods chosen for extraction may depend upon the condition/preservation of the material being use, the quantity of DNA desired, and what processes will be carried out after extraction – i.e. storage or more analysis. Essentially, all the extraction methods attempt to do the same thing: to break up the cells to release the DNA and to separate the DNA from the proteins and impurities in the sample (Stone 2000:352). The final aim is to obtain purified aDNA.

As soon as the purified aDNA has been obtained, a polymerase chain reaction (PCR) is performed to increase the amount of DNA for analysis. Since ancient DNA is often degraded and in short fragments, the use of PCR is crucial for detection of these small samples. In simplified terms, PCR is a three-step process involving denaturation of the double stranded DNA, primer annealing, and DNA extension (Stone 2000:352). During the denaturation phase, the DNA is heated (usually to 94°C) in order to separate or denature the double-stranded DNA molecule into two single strands. The DNA is then cooled slightly to allow the primer (a short sequence of bases that complement those of the DNA strand in question) to anneal or hybridise to its recognized site on the single stranded DNA molecule. These primers are designed specifically to target a particular site on the DNA strand and, in theory, fully match these hybridisation sites as well as meeting several other criteria such as a specific annealing temperature, and being unique so that they will only anneal to one site with the targeted genome (Hummel 2003:90-91). Once the primers have annealed, the extension phase begins. This phase is where the strands of DNA are extended through the use of an enzyme known as Taq polymerase which adds complementary bases onto the primer by using the DNA strand it is adhered to as a template (Griffiths *et al.*. 1998:449). The entire process starts again from denaturation and after several cycles of denaturation, primer annealing, and extension, the number of DNA molecules will have increased exponentially so that millions of copies of DNA are present for analysis (Stone 2000:353).

6.3.2 Analysis of aDNA

Electrophoresis is commonly used to determine the size of the PCR products obtained from the extraction process and also to confirm the presence of DNA. This method uses an electric current to separate out DNA fragments according to their size as they pass through an agarose or polyacrylamide gel. As DNA is a negatively charged molecule, all the samples are loaded onto the gel at the negative end (anode) so that they will be pulled by the electric current towards the positive end (cathode). The larger the DNA fragment is, the slower it will be able to move through the gel; the smaller the DNA fragment, the faster it will be able to move through the gel (Hummel 2003:113). However, this speed of movement and separation also depends on the percentage of agarose in the gel (lower percentages result in faster movement), the length of the gel (the further the DNA molecules have to travel, the better they will be separated out according to size), and the thickness of the gel (the thinner the gel, the better the separation) (*ibid.*).

Sequence analysis, or sequencing, is used to determine the order of bases within a particular amplified fragment of DNA. In circumstances whereby a mixture of sequences may be present in a sample, such as in archaeological specimens, direct sequencing cannot be used. Instead, clones must be produced, and each of these clones sequenced, in order to determine the authentic sequence. A PCR is carried out, but it differs from the amplification PCRs in that only one primer is used, thereby producing only elongated single strands of DNA, and that some of the bases that are added to the mix are fluorescently labelled (known as ddNTPs) (Hummel 2003:120). Elongation of a strand is stopped as soon as a ddNTP is added (because it lacks the hydroxyl group that the next nucleotide would bind to) and therefore the end result is a series of single-stranded products representing all possible lengths of the DNA fragment. These labelled strands are then sorted by length via electrophoresis and eventually produce a graph, known as an electropherogram, with a succession of coloured peaks, each of which represents a base in the DNA sequence which can then be read from start to finish (Hummel 2003:121).

6.4 Problems and Limitations

There are two key issues with using and analysing ancient DNA. The first is that DNA from archaeological specimens is often very degraded, the second is that, because it is very degraded, it can be easily contaminated by modern DNA during the analysis stage.

6.4.1 Degradation and DNA preservation

Degradation of DNA occurs because the monitoring and repair system that protects DNA from damage whilst we are alive ceases to function once the body has died (Jobling *et al.* 2004:110). After death, the DNA of an individual is rapidly degraded by endonucleases in the body but, eventually, other processes take over, mainly oxidation and hydrolysis. These processes severely damage the sugar-phosphate backbone of the DNA resulting in short fragments through cleavage, loss of bases, chemical modification of bases, and cross-linking of sugar-phosphate backbones (Burger *et al.* 1999, Jobling *et al.* 2004:111). Not only does this make detection and amplification of aDNA difficult, but the oxidized derivatives of cytosine and thymine will actually block the action of polymerase during replication attempts, resulting in short 100-200bp fragments. For these reasons, mitochondrial DNA is often favoured in aDNA studies because its higher copy number per cell means that the possibility of longer segments of DNA surviving is greater. It is thought that environmental factors such as temperature, humidity, and pH of the surrounding burial environment play a bigger role in DNA preservation than does the amount of burial time (Burger *et al.* 1999, Colson and Bailey 1997, Smith *et al.* 2001, Smith *et al.* 2003). Low temperatures may yield good DNA preservation as has been seen at high altitude environments and in cave sites where the temperature remains constant and below ten degrees Celsius (Burger *et al.* 1999, Smith *et al.* 2001). Such sites have been proven to show a 60-90% success rate for PCR

amplification. More humid environments will produce lower quantities of DNA, and also lower quality of DNA, resulting in a decrease in reproducibility. In terms of pH, DNA has been shown to survive best in either neutral or slightly alkaline (pH7.2-8.4) conditions (Burger *et al.* 1999). Smith *et al.* (2003) dismiss the influence of pH and humidity believing that 'thermal age', which they define as the time taken to produce a given degree of DNA degradation when temperature is held at a constant 10°C, is the one key factor in DNA preservation. Many researchers state that, with optimum conditions, DNA can survive, and be in a condition to be amplified, up to 100,000 years and that a maximum limit of one million years is likely for any DNA to be present in a sample (Hofreiter *et al.* 2001, Jobling *et al.* 2004:111).

Macroscopic analysis of the bones themselves will not yield any information on DNA preservation. Burger *et al.* (1999) determined that complete bones with dense cortical bone yielded poor DNA in comparison to brittle bone material that appeared to be less well preserved. Not only that, but bone preservation was deemed to differ from bone to bone of the same individual; long bones, cranial bones, and dental pulp were determined to be good sources of DNA whilst rib samples were not. Various methods of detecting the degree of DNA preservation have been suggested, including amino acid analysis and pyrolysis. Amino acids can exist in two different forms which are stereoisomers (chemically identical but mirror images of each other) known as the D-form and L-form. In living systems, amino acids are present only in the L-form but upon death a process known as racemization comes into effect which changes some of these L-forms to D-forms of amino acids until an equilibrium is reached. The speed at which this transformation happens depends on the burial conditions of the material, and so examining the D/L ratio gives an indication of these burial conditions and, ultimately, an estimation of how well any DNA present might have survived (Jobling *et al.* 2004:112). It can also give weight to the authenticity of aDNA sequences by demonstrating that preservation of such material is possible in a particular sample (Hofreiter *et al.* 2001). Histological analysis of thick-sections have also been used to estimate DNA preservation and there appears to be a correlation between histological preservation and DNA preservation (Colson and Bailey 1997). Pyrolysis, together with gas chromatography and mass

spectrometry, is another method that could be used to estimate DNA survival. This method looks at the condition of proteins in the sample by examining the hydrolysis of peptide bonds in amino acids but it has not yet been confirmed as an appropriate technique for authenticating aDNA sequences (Hofreiter *et al.* 2001, Jobling *et al.* 2004:112).

6.4.2 Contamination

The problem of contamination is a key issue for ancient DNA analysis because it can bring into question the authenticity of the sequences obtained. Because ancient DNA is so fragmented, any modern DNA that is present will always be preferentially amplified by PCR. It only takes a few skin cells or a drop of saliva to introduce modern human DNA into an ancient sample and, of course, there is the possibility of introducing bacterial DNA from the environment as well.

Brown and Brown (1992) identify four potential sources of contamination for ancient DNA, each occurring at different stages. The first contamination source occurs between death of the individual and inhumation. Burial rites that involve exposing the deceased individual to human blood, or other bodily fluids of other human beings, risk being contaminated by DNA other than their own. There is nothing that can be done to combat this issue other than to be aware of it during the analysis. After inhumation, but before excavation, there is a possibility, albeit very small, that DNA may migrate from one individual to another through the surrounding soil matrix. Leaching of DNA from biological material into the soil could result in the exchange of DNA between different specimens and even cross contamination between animal and human bones. Again, this cannot be prevented. The excavation process and post-excavation analysis is where modern DNA is most likely to contaminate archaeological material. During excavation, care must be taken to minimise contamination of the archaeological bones with the excavator's own DNA. This can be done by wearing a face mask and sterile gloves whilst handling the material. Post-excavation DNA analysis should always be carried out in a laboratory (lab) and not in the field. Conditions should be as sterile as possible in order to prevent modern DNA, and even other

ancient DNA, from contaminating the archaeological sample. In order to prevent this form of contamination, dedicated labs need to be set up to process aDNA material, researchers should wear appropriate clothing that reduces contamination – gloves, face mask, goggles, lab coat etc – and the surface of the material should be treated with bleach or irradiated with UV light to try to remove any surface contaminants.

Another way to help combat contamination and contribute to authenticity is to clone PCR products and also sequence overlapping clones in order to reconstruct the original sequence. If a single homogenous sequence has been obtained rather than a mixture of sequences it may be tempting to interpret this as an uncontaminated sample, but this may not be the case as any modern DNA, being more complete and present in greater quantity, would be replicated in preference to any ancient DNA present. Cloning will help to determine the extent of contamination and, because it allows a sequence to be determined by consensus of several clones, means that a level of confidence can be applied to the resulting sequence (Bower *et al.* 2005). The discovery that many of the results of the early ancient DNA studies were not authentic sequences but modern contaminants, made it clear that stringent criteria were needed for aDNA analysis. Cooper and Poinar (2000) reiterated the need for these stringent criteria, including cloning, in a response to several studies being published that did not meet the recommended criteria or adhere to the necessary controls. Their key criteria were:

- A physically isolated work area (to prevent cross-contamination)
- Control amplifications (multiple extractions and PCR controls)
- Appropriate molecular behaviour (large base-pair products are unusual; sequences should make phylogenetic sense)
- Reproducibility (results should be repeatable)
- Cloning (sequences must be verified by multiple clones)
- Independent replication (replication in another lab)
- Biochemical preservation (can indirectly assess DNA survival)

They felt that without such criteria, dubious claims, such as the replicated dinosaur DNA in the early 1990s, would bring the discipline into disrepute.

6.5 Archaeological Issues

In terms of analysing genetic material from ancient human remains, there are also issues specific to archaeology. Firstly, the storage of archaeological human remains is not consistent. Many institutions have created their own policies and guidelines for storage and these range from large industrial warehouses (occasionally subject to flooding and often subject to seasonal temperature fluctuations) stacked high with boxes, with some in contact with the ground and others on shelves, to temperature and humidity controlled rooms with mobile storage shelving and anything and everything in between. As discussed above, burial conditions are very important for DNA preservation but the same applies to storage conditions. Burger *et al.* (1999) carried out experiments on some archaeological material to determine what effects storage would have on aDNA retrieval at a later date. They had specimens in three different storage situations – some were immediately frozen after excavation (at -20°C), some were stored at room temperature for three years, and some were stored at room temperature for 16 years. Those that were stored at room temperature for three years yielded more DNA than the frozen samples, and were as likely to produce reproducible results, as long as a large amount of tooth powder was used (0.3g). As soon as this was optimised to 0.1g of tooth powder, the deep-frozen samples yielded more reproducible results. Those samples stored at room temperature for 16 years did yield a decent amount of aDNA (68% of samples for 0.3g tooth powder, 53% for 0.1g), but the results were not reliable in terms of reproducibility; a maximum of 40% could be reproduced. This demonstrates that frozen samples are much more stable and will produce consistent results no matter what sample size is taken, whereas for samples stored at room temperature the material will eventually deteriorate and the amount of intact aDNA molecules will decrease. The authors recommended that if samples could not be stored long term in a

freezer then they should be stored in an environment at least as cold as they were found.

Even with good preservation of DNA, the amount that is yielded for analysis depends upon the extraction methods used. In 2002, Bouwman and Brown performed a comparison of five different extraction methods for aDNA. These methods were each tested on five different bones from 18th to mid-19th century sites in London. It was found that two of the methods stood out from the others (Rapid QIAquick and Qiagen QIAamp) because they produced better yields of aDNA and more reproducible results. However, of the two methods, the Rapid QIAquick method was deemed the better overall because it contained fewer steps and therefore provided less chance for contamination to occur. The authors concluded that, although this was the best method for the bones they used in their study, ultimately, the best choice of extraction method would depend upon the individual burial and preservation circumstances of the bone or tooth being analysed.

The second issue stemming from archaeology is that the techniques used in genetic analysis are destructive. Often the amount of human bone, especially intact or complete remains, recovered from a site is limited and therefore valuable in terms of research potential. Understandably, curators and other researchers are sometimes hesitant to allow a destructive technique to be carried out on osteological material as this may mean that it is no longer available for research. Situations whereby a 2-3cm section of long bone may be needed for genetic analysis could mean the possibility of a key diagnostic feature or trait being lost, or even that standard measurements may no longer be possible. If the bones are being displayed in a museum then the curator may not be happy with sections of bone being cut away as it takes away from the aesthetics of the collection. Of course, one questions why the material is curated in the first place if it is not to be used for research. So long as the research questions being asked are valid, and aDNA analysis is the only way in which to further advance the field and such research will answer the questions, then the analysis should be carried out. One solution to this problem is to take a tooth sample, which would be less destructive and less noticeable in terms of the appearance of the skeleton,

as long as the tooth is fully recorded first. Regardless of what part of the skeleton is sampled, the documentation and recording of the remains, including photographs, is very important for future research when taking bone or tooth samples for DNA analysis.

The third issue when dealing with archaeological remains is that they have often been studied by many different researchers. It is not standard protocol for bioarchaeologists or archaeologists to use latex gloves when handling human bone; many prefer to be able to 'feel' the bone for its texture, condition, and pathology, and so there is potential for a lot of contamination by modern DNA. However, most of this contamination will be surface contamination which can be removed with the use of acid or irradiation with UV light (Bouwman and Brown 2002).

Finally, there is the issue of being able to carry out the genetic analysis itself. Although bioarchaeologists employ a variety of techniques from a range of disciplines in their study of human remains, a great deal of expertise or training is needed in order to carry out and interpret the genetic analysis of ancient material. It is also an expensive undertaking as dedicated facilities are needed in order to safeguard against the contamination of ancient samples with modern human samples, and it is also costly as specialised equipment and consumables are needed. Many researchers prefer to send their samples to specialised labs for analysis. This way, the task of actually obtaining clear results is given to somebody else, freeing up both time and money for the researcher. However, it is still expensive for the aDNA samples to be processed and, as there are only a few dedicated labs in the United Kingdom, the wait for results can be quite long.

6.6 Uses of Ancient DNA

Despite the obstacles discussed above, the information that can be gained from ancient DNA analysis is highly valuable. DNA analysis is commonly used to

identify skeletal remains, whether this is at the level of species or individual. In situations where there are commingled, cremated, or fragmented remains this is very useful. It can be determined whether a small fragment of bone is human or animal, whether there is more than one individual present in a burial, and which bones belong to the same individual (Hummel 2003:167). The origins of different people and migration patterns are also areas of investigation with aDNA. Sequences from the hypervariable regions of mitochondrial DNA can be examined and compared for similarities and differences and haplotypes determined. With these techniques, the questions of which group of people may have founded a population or which route they may have taken in their migration to another area can be answered (Stone 2000:357-9). Researchers have also been able to answer questions about cemetery organization - whether maternally related individuals are buried close to each other, whether those graves containing multiple individuals are members of the same family, and whether those buried in a particular way share the same mitochrondrial sequences (Stone 2000:358).

In recent years, the archaeological literature on ancient DNA has ranged from reviews of extraction and analytical techniques to case studies, whereby these techniques are put to use establishing relationships between individuals or identifying them. Prior to the refinement of genetic analysis for ancient remains, relationships between individuals in burial grounds had to be established through the examination of skeletal and dental measurements and non-metric traits. Alt *et al.* (Alt *et al.* 1997) carried out such a study on a triple burial of two men and a woman from Dolni Vestonice. They had already developed a catalogue of 137 odontological kinship traits for this population though more than 1000 possible traits were available for study. The researchers examined over 900 of these traits along with skeletal non-metric traits and compared them to the reference population to determine if the traits they found in the three individuals could indicate a relationship or were common traits in the population being studied. Alt *et al.* eventually determined that these three individuals were related and, since they were close in age (16yrs, 17yrs, 20yrs), it was suggested that they might be siblings.

If Alt *et al.* (1997) had used genetic analysis they may have been able to determine the exact nature of the relationship of their three individuals as Adachi *et al.* (2003) were able to do in their study of a double burial from Japan. Adachi *et al.* (2003) combined mtDNA analysis with dental crown measurements to examine the relationship between two juvenile skeletons from a 2000yr old burial from the Usu-Moshiri site in Hokkaido, Japan. They calculated correlation coefficients for the tooth crown diameters, which were shown to be both positively correlated and have a high correlation coefficient; they compared them both to the data from the archaeological population they were studying and to the modern Japanese population to see if the level of correlation was significant or not. The mitochondrial DNA analysis demonstrated that the sequences from the two individuals were identical and, when compared to DNA data from the modern population, were also found to be distinctive, indicating that not only were they were related but that these sequences were not commonly found in the modern population as a whole. However, as they did not have a comparative mtDNA database for the archaeological population, it was possible that the sequence they identified was common during that time period so, to strengthen their hypothesis, they used the odontological data. By comparing the results from the two individuals to related and unrelated individuals in the tooth size proportion database they were able to determine that the two burials were most likely first-degree relatives – siblings.

Mitochondrial DNA has also been used independently as a tool to assess kinship. Dudar *et al.* (2003) and Mekel-Bobrov and Lahn (2004) both used the hypervariable regions of mtDNA to determine the degree of relatedness between individuals buried in the cemeteries they were studying. They first determined the haplotypes present in their populations, and then looked for clusters of burials sharing the same haplotype that could have been interred during the same time period or burial phase. Once these were identified they were able to calculate the sequence and nucleotide diversity between haplotypes and determine how similar these sequences were and whether they were related. Dudar *et al.* (2003) also calculated kinship probabilities of the clustered individuals sharing a haplotype using equations borrowed from forensic science that can determine the

probability of these groupings occurring by chance. Based on these probabilities they were able to determine how likely it was that these groups were families.

Another method of determining relationships between individuals is through short tandem repeat (STR) analysis. STRs are sequences of two to twelve bases that are tandemly repeated, sometimes hundreds of times (Stone 2000:362). Hummel and Schultes (2000) reviewed the development of STR typing, also known as DNA typing or DNA profiling. They discuss how this technique changed the discipline because it allowed basic scientific questions to be answered, such as the sex ratio of infants at burial sites (sex identification is often included in an STR profile), and the reconstruction of kinship through the examination of either autosomal STRs or Y-chromosomal STRs.

Hummel *et al.* (1999) demonstrated the application of STR analysis with the simultaneous amplification of nine STRs and the amelogenin gene (megaplex STR typing) in an attempt to establish the relationship between the Saxon warrior Widukind and others buried at the Convent Church, Enger, in the North Rhine, Germany. Tooth roots were used as the source of aDNA so that damage to the skeleton was minimal and, as the megaplex STR technique meant all the STRs were amplified simultaneously, the amount of material required for analysis was also reduced to a minimum. Whilst conducting this research, the authors decided to also investigate the relationship between DNA preservation and the ability to use megaplex amplifications. They tested a variety of bones and teeth from a variety of ages from fresh to 3000 years old. They found that STR typing was possible regardless of burial age, and that the quality of the profile was not a function of age either. Hummel *et al.* (1999) also highlighted the fact that they could authenticate their results through comparison of the archaeological profiles obtained with those of all the investigators involved in the research.

A similar study on the relationship of church burials was carried out by Gerstenberger *et al.* (1999) but using autosomal STR and Y-specific STR analysis. These techniques were used to determine the relationship of eight skeletons thought to be the Earls of Konigsfeld at St. Margaretha's Church, Reichersdorf, Germany. The names of these individuals were known as they

were inscribed on the tombstones, but these tombstones were mounted on the walls of the church and not on the tombs of the individuals so identification of the skeletons had to be carried out by other methods, in this case, genetic analysis. The tombstone inscriptions and historical documentation were used to reconstruct a family tree that could be compared with the results from the DNA typing. As the Earls were meant to be connected patrilineally, they used Y-STRs for the DNA profiling. The authors were able to confirm a patrilineal relationship for five skeletons; three others were not attempted, one because there was not enough DNA preserved, and the other two because they were identified as female. Although one of these females had been morphologically assessed as female the other was expected to be male; it was concluded that this individual could have been one of the sisters of the Earl as they were known to have also been buried in the church. The other female was proposed to be the wife of one of the Earls, Georg Josef. Through the Y-STR typing the authors were also able to correct a portion of the family tree that identified two individuals as father and son, whereas the DNA evidence showed that these positions should be reversed so that the individual named as 'father' became 'son' and the individual named as 'son' became 'father'.

As seen in the study above, identification of sex is very important for those remains that cannot be easily identified morphologically. Although sex can usually be determined with relative certainty in adult skeletal remains through examination of the morphology of the cranium, pelvis and other bones, it cannot be determined in children (subadults) as they do not develop the key characteristics for determining sex until puberty. The use of aDNA allows not only the sex of the individuals to be determined but also gives answers to questions such as the male/female infant mortality ratios and preferential burial locations for each sex (Hummel 2003:162).

Faerman *et al.* (1995) helped to develop the technique of sex identification based on the amelogenin gene and, in 1998, applied this method to the question of selective infanticide at a Roman bathhouse in 4^{th} - 6^{th} century Ashkelon. More than one hundred infants were recovered from the sewer of the bathhouse and determined to be full-term, but only one or two days old, on the basis of

osteological analysis. As there was documented evidence of selective female mortality during this period, the authors tested the sex ratio of the infants using aDNA analysis of the left femurs. The results were the inverse of what they expected on the basis of the documentary evidence, yielding a sex ratio skewed towards males rather than females. The authors concluded that this selective male mortality was due to the bathhouse being used as a brothel (a well documented practice during this time period), with the female prostitutes deliberately choosing to keep female babies to raise as prostitutes (Faerman *et al.* 1998). Without the DNA evidence it might not have been possible to make such an interpretation and, conversely, without the documentary evidence that brothels were in existence it might not have been possible to interpret the results of such analysis.

6.6.1 Application of aDNA Analysis to Palaeopathology

Another application of aDNA is to use it to diagnose disease in past populations. This is difficult to do as any viral or bacterial DNA present will likely be in very small amounts. However, despite this obstacle, several pathogens have been extracted and diagnosed in archaeological skeletons including plague (*Yersinia pestis*), leprosy (*Mycobacterium leprae*), tuberculosis (*Mycobacterium tuberculosis* and *M. bovis*), and malaria (*Plasmodium falciparum*) (Drancourt *et al.* 1998, Rafi *et al.* 1994, Spigelman and Lemma 1993, Taylor *et al.* 1997). These studies are important because they allow researchers to confirm skeletal diagnoses of disease, to determine which disease is present in a skeleton displaying lesions associated with many different diseases, to determine when diseases first became established in different parts of the world, to study the evolution of a disease, the population frequencies of disease, and to discover how disease may have influenced human lives.

The human form of tuberculosis (*M. tuberculosis*), for example, was first extracted and amplified from bone in 1993 (Spigelman and Lemma 1993). This disease displays identical bone lesions in the skeleton, regardless of whether the

infectious organism is *M. bovis* or *M. tuberculosis,* but the cultural implications of the two different forms of the disease are very different. *M. bovis*, for example, is transmitted through cattle, usually via infected meat or milk, so infection by this organism implies a close relationship with cattle. *M. tuberculosis*, on the other hand, is transmitted through humans and so infection by this organism implies populations large enough to sustain such a disease and, possibly, an endemic disease. With the use of DNA, distinguishing between the two strains of the disease is now possible.

Tuberculosis (TB) has also been used to test the link between disease and the changes that are seen in the skeleton. Mays *et al.* (2002) conducted a study to look at rib lesions in archaeological skeletons with TB, a pathological lesion long suggested as a possible , but non-specific, skeletal marker of this disease (Bennike 1999, Pfeiffer 1991, Roberts 1999, Weiss and Moller-Christensen 1971). Unfortunately, the study that Mays conducted indicated that not all those skeletons who had rib lesions had TB, and vice versa, that not all the skeletons with TB exhibited rib lesions. However, this study was only conducted on seven individuals so it is not necessarily the final word on this link between tuberculosis and rib lesions; recent studies indicate that the possibility of a causal relationship still exists (Santos and Roberts 2006).

In addition to confirming the identity of a disease and its manifestations on the skeleton, bioarchaeologists can also use aDNA to answer questions about disease that are not possible to determine from skeletal remains alone. Population frequencies of disease in the past, particularly those that do not always result in manifestations of skeletal lesions such as TB and leprosy, are impossible to estimate from the skeletal evidence but pathogenic DNA extracted from the population in question can help to answer those questions. Fletcher *et al.* (Fletcher *et al.* 2003) were able to do just this in a Hungarian population affected by tuberculosis. They were able to take DNA samples from 168 individuals in this 18^{th}-19^{th} century population and determine that TB was present, in the most conservative estimate, in 39% of the population.

6.6.1.1 DNA Studies and DISH

Although a genetic aetiology for DISH is often proposed in clinical and bioarchaeological papers, there has been very little discussion about it or investigation into potential links. The general idea about people with DISH being 'bone formers' as cited in Rogers *et al.* (1997) suggests a genetic predisposition to the condition but this concept is one that is difficult to explore. Many of the earlier studies suggested that there might be a genetic link through the HLA (human leukocyte antigen) gene, as this is common in spondyloarthropathies, but this antigen has not been commonly found in association with DISH so an alternative explanation must exist (Gorman *et al.* 2006, Havelka *et al.* 2001).

Julkunen et al. (1971)'s study of over 12,000 Finnish adults is one that is often cited in studies of DISH. It looks at the role of glucose tolerance and obesity in DISH and finds that there is a correlation between males with glucose impairment and DISH, but not females, and that, therefore, glucose impairment and DISH are independent of each other. In general, males were found to have a higher prevalence than females, but there was also a geographic component in that females in the east of Finland had a higher prevalence rate than those in the west of the country; there was no such correlation with glucose intolerance. Julkunen *et al.* offer no explanation for this, but perhaps genetics play a role here.

Crubezy (1996) attempted to explore these genetic links using skeletal material with DISH from differing environments and time periods. Although not a DNA study, Crubezy suggested a genetic link for DISH by using the prevalence rates and demographic profiles of the two populations, who were separated by a period of about 6000 years. Since the two populations had similar prevalence rates and demographic profiles, yet were exposed to differing environments, he proposed that DISH was genetic.

Havelka *et al.* (2001) tried to determine whether a gene which has a strong association with OPLL (ossification of the posterior longitudinal ligament), a

condition also associated with low glucose tolerance and obesity, was present in DISH patients. No significant difference was found in allele frequency between the DISH and non-DISH groups, however. The following year, Havelka *et al.* (2002) published another genetic study on DISH, examining whether a gene responsible for bone mineral density was associated with DISH but found no link between them.

There have also been a few reported family incidences of DISH. Gorman *et al.* (2006) reported cases of DISH in three generations of the same family. A few possible links were explored, such as shared alleles and presence of HLA, but neither yielded any positive relationships.

Population studies of DISH are not uncommon, but they do not often combine with genetic studies. However, many of them still cite, or allude to, genetic factors as playing a role in its aetiology (Kim *et al.* 2004, Pappone *et al.* 1996, Pappone *et al.* 2005, Sarzi-Puttini and Atzeni 2004). As can be seen from the discussion above, the studies that have looked at the possible genetic links are still inconclusive. Although similar bone disorders, such as OPLL and AS, have strong genetic links through their associations with HLA, DISH does not seem to demonstrate this, and yet it has differing prevalence rates in different populations, sex-biased prevalence rates, and evidence of family incidences, all of which point to a potential genetic aetiology.

The objective of this research, in terms of DNA analysis, is to test for genetic links between individuals with DISH. As the bone lesions characteristic of DISH are being used to differentiate between those with the condition and those without, the concept of "hidden heterogeneity" (Wood *et al.*1992:344) must also be considered. This concept postulates that skeletal populations are made up of individuals who varied in their susceptibility to disease, often due to genetic factors. Therefore, those individuals who exhibit skeletal lesions (such as those of DISH) may not represent all who suffered from the condition. It could be that some individuals with DISH died before they exhibited the skeletal changes that are characteristic of DISH. This would certainly impact upon the number of DISH individuals within a population, but should not impact upon the ability to

find related individuals with DISH, although the chances of finding related individuals would certainly increase with a larger sample size.

Occurrences of DISH amongst possible related individuals have not been explored in archaeological populations and, as there is evidence of family incidences of DISH in the clinical literature, it stands that there may also be such evidence amongst people in the past. It is quite likely that family groups would be buried within the same cemeteries, family members often requested to be buried near or next to other members (Daniell 1997:101-103), but within monastic cemeteries family groups would be less likely. This does not mean that finding related individuals within a monastic cemetery is impossible. The Dominicans, for example, had a secular order of friars who were often married and lived in the towns and, as men could become monks at any age, many had wives and children whom they left behind when they joined their respective orders (LaCorte and MacMillan 2004:11, Lawrence 1990:37). As monks, friars and canons were sometimes permitted to bury lay people in the monastic cemetery (Daniell 1997:96), it is possible that these family members would have been buried with the religious in the monastic cemeteries and thus related individuals might be present amongst the monastic samples.

6.7 Conclusion

The value of aDNA analysis in archaeological studies is evident. It has allowed the investigation of questions that could never be answered through macroscopic analysis or documentary evidence alone. The identification of commingled remains, sexing of sub-adults, establishment of relationships between individuals, and the identification of disease, are only a few of the possible applications to bioarchaeology.

However, despite the large body of research into disease in past populations, little has been done on looking at possible genetically inherited diseases in the past. It has been postulated that DISH, the subject of this research, may have a

genetic factor in its development (Rogers *et al.* 1997, Havelka *et al.* 2002), and the group of non-monastic skeletons with DISH in this study offer a unique opportunity to test the theory that DISH may have a genetic link. As demonstrated by the studies presented in this chapter, it should be possible to establish whether genetic relationships exist between the non-monastic individuals buried in the same cemetery to see if there is any support for the theory that DISH may be genetically linked. Using either STR analysis or analysis of the hypervariable regions of mitochondrial DNA, as seen in the studies presented, should yield information on relatedness and aDNA analysis could also confirm (or refute) the sex assessments of these individuals as determined by osteological analysis. As explained above, the monastic individuals are less likely to be related than the lay people, but it is still possible that relatives may have been buried in the monastic sites along with the monks, and the aDNA analysis can be used to explore this idea. Using the techniques discussed here, together with the anti-contamination controls recommended, should yield some interesting findings and open up a new avenue of research for palaeopathology.

Chapter 7: Materials and Methods

7.1 Sites

7.1.1 Site Selection

The sites selected for this research are from the late medieval period in England as this is where the evidence for diffuse idiopathic skeletal hyperostosis (DISH) seems to be most predominant, especially so within the monasteries of this time period (Waldron 1985, Rogers and Waldron 2001, Mays 1991, Janssen and Maat 1999, Verlaan *et al.* 2007). Monastic sites were originally chosen on the basis of their prevalence rates for DISH; those with a higher prevalence of the condition were given a higher priority. However, once the process of trying to track down the sites and where the skeletons were curated began, it became clear that adjustments would have to be made in order to account for the accessibility and availability of some sites. The non-monastic sites were chosen to act as a control for the monastic data, hence they were ideally to be in the same geographical area as the monastic sites and being used during the same time period.

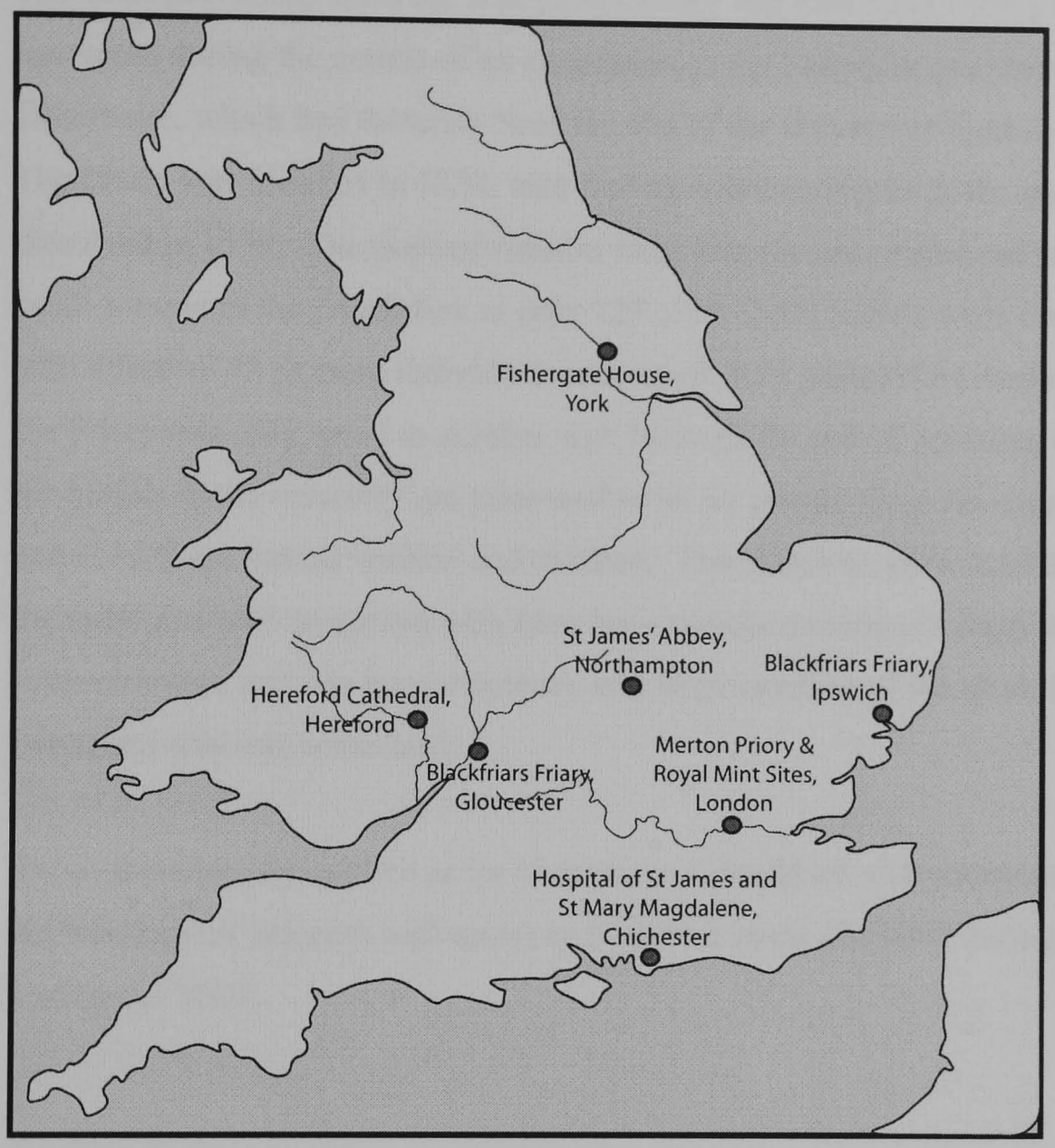
Identifying sites to be used in the research was quite a complex task. There are no comprehensive databases of skeletal material or archaeological sites which means that finding suitable samples to study was difficult. Although a few potential sites had been identified through literature searches prior to starting the project, the site reports and the location of the skeletal material still had to be established. The methods for finding this information and searching for other potential sites was somewhat obtuse and included conducting literature searches for potential sites, sending requests for information via specialist mailing lists, conducting internet searches for archaeological contractors who were known to have worked on the sites and emailing them with requests for information, and contacting different university archaeology departments and county

archaeological units to find out if they were curating any relevant material. This was a very slow process because a) there is no way of knowing what sites exist which reduces the literature search to a 'guess and test' exercise whereby it is believed, or heard, that a site exists in a certain location, and then attempt to find it referred to in the literature, b) many sites are unpublished and the contractors who worked on them have often moved to new jobs, or even passed away, c) people have to be relied upon to respond to enquiries, and d) some material has been reburied or is currently being used for research and is thus inaccessible (see Roberts and Cox 2003:26-30 for a discussion of the difficulties encountered by bioarchaeologists when trying to locate relevant skeletal reports and material).

A list of 71 late medieval monastic and non-monastic sites was compiled and eventually narrowed down to those sites with two or more cases of DISH. For those sites for which reports or skeletal information could not be found, attempts were made to contact the relevant parties to find out if skeletons with DISH existed in the sample. This reduced the number of sites to 43. The locations of the sites with the highest rates of DISH were then explored and preliminary enquiries were made to determine if sampling might be possible. Once the sites that had been re-buried, where there had been a negative response to requests for stable isotope and aDNA analysis, had been un-responsive to the enquiries, for which a contact could not be found, were currently being analysed or researched, and were being re-housed or to which access was denied were ruled out, nine sites remained – four monastic and five non-monastic sites – where permission to be documented and sampled was granted (see Figure 1). These were Blackfriars Friary, Gloucester; St James' Abbey, Northampton; Merton Priory, London; Royal Mint Site (Late Graves), London; Royal Mint Site (Black Death Cemetery), London; Blackfriars Friary, Ipswich; The Hospital of St. James and St. Mary Magdalene, Chichester; Hereford Cathedral, Hereford; Fishergate House, York. Although the original idea was to have non-monastic sites that were located near the monastic sites so that they would be more comparative in terms of local availability of foods, there were not always any suitable sites (or those that were suitable were unavailable for study). Therefore, three of the five non-monastic sites do not 'pair up' with the monastic sites. However, they are all

from the same time period as the monastic sites – the late medieval period (12th to 16th century AD).

Figure 3. Map of Sites



7.1.2 Site Description

The following provides contextual background information for each of the sites from which skeletal samples were derived. For each monastic site, samples were taken from skeletons presumed to be monks on the basis of burial goods or location and style of burial. Of course, there is not definite proof that these individuals were in fact monks. It should be noted that the information available for each site varies in amount and detail, and some are not yet published.

7.1.2.1 Blackfriars Friary, Gloucester

(The following information is summarised from Wiggins *et al.* 1993)

The skeletal remains from the Blackfriars Friary site (site code 19/91) were recovered during the course of an excavation in the Ladybellegate Street car park, Gloucester, which had formerly been the site of the Gloucester Dominican Friary. The Friary was founded in 1239, with burials commencing in 1246, and was dissolved in 1539. The skeletal remains from this site are considered to be a small sample of the population as only 129 of the 2000 burials were excavated, with a further 43 or more individuals recovered from unstratified contexts. As the Friary was only small in number with between 30 and 40 brethren, many of the burials in the cemetery are presumed to be lay people from the community, and several are indeed women and children. The skeletons excavated were for the most part well preserved with little bone surface erosion although some skeletons were missing bone elements, and fragmentation of the skull, long bones and ribs was often seen.

These skeletons are curated at the University of Bradford's Department of Archaeological Sciences and access to them was made available during the summer of 2005.

7.1.2.2 St. James' Abbey, Northampton

(The following information is taken from Chapman and Morris n.d.)

St James (site code EL00) was an Augustinian abbey founded by William Peveral in 1104-5 and dissolved in 1538. Originally the buildings were all wooden constructions until a stone church was built to replace the timber buildings in 1173. This was rebuilt during the reign of Edward I and thought to be completed in 1310 as this is when the two altars were dedicated. At the time of its dissolution, the abbey contained an Abbot, Prior and four Canons. Their lands were granted to Nicholas Giffard of Duston who demolished all the monastic buildings to build his own mansion. It was later, in the 16th and 17th

century, the site of the fair of St James and then much later, in the 20th century, the site of the Express Lift factory.

In 2000-2001 the site was excavated by Northamptonshire Archaeology as part of the redevelopment of the Express Lift factory site by the Lifebuilding Company. The abbey church was found to be built in a basilica formation rather than with transepts, containing both a cloister and a chapter house. The cemetery formed a second cloister to the south-east of the chapter house where a total of 294 burials were excavated. The earlier part of the cemetery was laid out in rows and contained wooden coffins, stone lined graves, graves lined with ceramic roof tiles, and also a single stone coffin which the excavators interpreted to mean that the interred individuals in this area were of high status. The later use of the cemetery was less ordered with a lot of intercutting of graves and disturbance of earlier interments. Most of these burials were in simple shallow graves and the individuals were buried in shrouds rather than coffins so these were determined to be people of lower status. The initial interpretation of the site of St James' Abbey is that the early use of the cemetery, with the neatly ordered rows and coffin burials, was for monastic use and that the later less organised phase of the cemetery was for lay people as there were some clusters of burials that may have indicated family burial plots, especially as some contained women and children.

The analysis of the burials themselves was carried out by the late Trevor Anderson at Canterbury Archaeological Trust who determined that there were a high percentage of elderly individuals with trauma such as leg fractures and other pathological conditions, including fused leg joints and also DISH.

The individuals from the site of St James' Abbey are stored at Northamptonshire Archaeology in Northampton and were accessed in the summer of 2005.

7.1.2.3 Merton Priory, London

(the following information is summarised from data collated by MoLAS n.d.)

Merton Priory, London, (site codes MPY77 and MPY86) was the site of the Augustinian Priory of St Mary Merton from 1117 to 1538. Its 42 acres were excavated between 1976 and 1988 by the Department of Greater London Archaeology (DGLA) and the Museum of London Archaeological Services (MoLAS), uncovering 738 burials. These burials were concentrated into four main groups which were labelled the Northern or 'Lay' cemetery, the Southern or 'Canons' cemetery, the Priory Church, and the Chapter House. Each cemetery was divided into four phases of burial with the exception of the Northern cemetery and Priory Church which were deemed to represent all phases, hence covering the entire period of monastic occupation.

The Northern Cemetery that lies to the north of the Priory Church was utilised throughout the life of the abbey and contained 426 burials. Eighty-four of these burials were from the first phase (1117-1222) with 64 males, six females and 10 unknown adults; eight of which had evidence of wooden coffin burial. Eighty-nine burials were excavated from the second phase (1222-c1300) – 57 males, 12 females, 12 unknown adults. Amongst these there were seven burials with wooden coffins, nine shroud burials, and three cist burials. In the third phase (1300-1400), 143 burials were excavated consisting of 95 males, 12 females, and 13 unknown adults; two of which had significant grave goods – one in a wooden coffin with a paten and chalice,. In phase four (1390-1538) 24 males and two unknown adults were excavated, including a cist burial. In phase five (1117-1538) 54 males, four females and nine unknown adults were excavated.

In the Southern Cemetery, which lies to the south of the Priory Church, a total of 162 burials were recorded. Phase one included nine males, one female, two unknown adults with two stone cists and two wooden coffins. Phase two included 18 males, four unknown adults and four stone cists, two of which were empty, one wooden coffin and one sandstone coffin. Phase three included two distinct groups of burials. The group in the western portion of the cemetery contained 66 males, two females and 10 unknown adults. The group immediately north of the infirmary chapel contained 26 males, and three unknown adults of which 22 were in wooden coffins (only two with lids), a stone cist burial, and a stone coffin burial. There was also one individual buried on a bed of grey ash

and charcoal, a unique finding at this site. No burials were excavated from periods four or five.

The Priory Church has not been fully excavated but seems to have been used for high status burials from both the monastic and lay community, with those of the highest status being buried near the high altar. There were no burials from phase one. From phase two there were three male adults and three of unknown age or sex. From phase three there were 35 males, six females, and two unknown adults. Three were cist burials, two were in stone coffins and one grave contained a chalice, paten and gold thread and was assumed to be a priest. From phase five 26 males, two females and two unknown adults were excavated.

The Chapter House was thought to be the burial place of those of higher status in the priory community as they were all mature males with the exception of one young adult. Thirty-two burials in total were excavated and included stone cists, wooden coffins and graves without linings. Nine were from phase two, two from phase three, and 21 from phase four. There were no burials excavated from phase one.

The individuals from Merton Priory are curated by the Museum of London Archaeological Services (MoLAS) and were accessed during the summer of 2005.

7.1.2.4 Royal Mint Site, London

(The following information is summarised from Grainger *et al.* 1988, except where indicated)

The Royal Mint Site (site code MIN86), which lies to the north east of the Tower of London, actually comprises several sites including a Black Death cemetery, a Cistercian Abbey, and a Royal Navy Victualling Yard, as well as some Roman foundations but only the former two sites were used in this study. This site was excavated by the Department of Greater London Archaeology (DGLA) from the Museum of London between June 1986 and June 1988. The Black Death

cemetery, also known as East Smithfield or the churchyard of the Holy Trinity, was the site of an emergency burial ground for victims of the 1349 Black Death (Grainger *et al.* 1988, Hawkins 1990). Only a small portion of the cemetery was actually used for burial in two distinct areas in the west and east (Hawkins 1990). The western area contained two mass burial trenches, a mass burial pit, and graves in 11 parallel rows – 14 burials may have contained individuals interred in shrouds; the eastern area contained one mass burial trench and graves in four parallel rows some of which were empty due to chemical contamination from the Royal Mint (Hawkins 1990). The excavators believe that the trenches and graves represent different levels of demand for burial rather than being related to status. The remains of 762 individuals were found, 230 of which were interred in coffins; the trench burials were carefully but densely laid out with burial layers up to five people deep, and both sexes and all ages were well represented. There was, however, a lower proportion of 15-25 year olds than expected from a catastrophic cemetery (Magerison and Knusel 2002). Six hundred of the plague pit skeletons were examined and 46 were considered too poorly preserved to assign a sex and 125 too poorly preserved to assign an age more specific than 'adult' (Waldron 1993 §2:2)

In March 1350, the site was granted to the Cistercian order and it became the Abbey of St Mary Graces until it was dissolved in 1538. At the time of the Dissolution the abbey had 10 monks and an abbot and was the third richest Cistercian abbey in England. Its layout deviated from that of the classic Cistercian model which could be due to the fact that it was built 70 years after the last Cistercian foundation had been built in England and also because it was the only one to be built near a city. There were 133 burials in the church and chapels of which 61 were interred in coffins. Several large tombs were uncovered in the choir and presbytery indicating that there was some social stratification, and south of the church are a series of chapels, some of which contained burials of notable individuals. The churchyard overlies part of the earlier Black Death cemetery with two phases of burial. The first phase contained 253 burials of which 109 were buried in coffins; the second phase contained 57 burials of which 22 were buried in coffins. According to the summary from MoLAS, there was no clear evidence for zoning of burials, with

the exception of a concentration of infant and juvenile burials to the south of the church, and a higher ratio of males to females closer to the church, possibly indicating that the monks were buried there. However, it is cautioned that there is no definitive grouping of lay or monastic burials (MoLAS summary of Min86). The skeletons from the abbey cemetery and the church were poorly preserved but a large number of diagnostic elements required for ageing and sexing survived, allowing age and sex to be assigned (Waldron 1993 §3:1, §4:1).

The Royal Mint Black Death individuals and the Abbey of St Mary Graces individuals, also known as the Royal Mint Late Graves, are both curated at the Museum of London Archaeological Services (MoLAS) and were accessed in the summer of 2005.

7.1.2.5 Blackfriars Friary, Ipswich

(The following information is summarised from Mays 1991.)

Blackfriars Friary (site code IAS 4801) on the School Street site in Ipswich was excavated between 1983 and 1985. The burials from within the Friary complex represent both friars and benefactors and were interred between 1263 and 1538. Two hundred and fifty burials were recovered of which there were 148 males, 64 females, 14 unsexed adults, and 24 juveniles. Eighty-nine of these appeared to have been buried in coffins as there was evidence of wood and iron nails found, but there was no association between coffin burial and sex. There was no evidence of spatial patterning in the burials either with respect to age or sex, suggesting that the burial of friars was not segregated from the burial of layfolk. The preservation of the skeletal material varied, with some skeletons only remaining as soil silhouettes with no bone surviving at all, others being nearly complete and well preserved skeletons, and everything in between. Age of the skeletons was assessed by Simon Mays using dental attrition, cranial suture closure and pubic symphyseal degeneration; sex was assessed by examination of the skull and pelvis.

The Blackfriars Friary, Ipswich skeletons are curated in a warehouse belonging to the Suffolk County Council Archaeology Unit and were accessed during the summer of 2005.

7.1.2.6 The Hospital of St James and St Mary Magdalene, Chichester

(The following information is summarised from Lee 2001 except where indicated)

The Hospital of St James and St Mary Magdalene (site code CH86) was one of five hospitals in Chichester and one of the four that was founded for the care of the leprous. It was founded prior to 1118 and housed males with leprosy, a master, a few benefactors and some carers up until the 15th century. Interments at the site began in the early 12th century and continued up to the 17th century. As leprosy was regarded as a "moral and spiritual contagion" (Lee and Magilton 1989:274) rather than a transmittable disease during this time period, the function of the hospital was not to segregate the leprosy brethren from the rest of the community but to pray for them (*ibid.*). They provided care and sheltered accommodation but not necessarily medical help. By 1540 sisters as well as brethren were admitted and the hospital then became an almshouse for the care of the poor and the disabled (Magilton and Lee 1989).

There were two areas of burials – A and B. Area A, with a minimum of 149 individuals, was at the western end of the cemetery and contained groups of graves sharing a common north-south line with many intersections indicating space may have been limited. Area B, with a minimum of 235 individuals, contained the easternmost burials, which were also in north-south rows but the graves rarely intersected. Those burials to the western end of this area were less organised with lots of intersecting graves and little evidence of burial planning. It has been suggested that Area B may represent the Almshouse phase of the cemetery, as it has more balanced ratios of males and females, with the western end a transitional phase or one preferred for burying children. Preservation of these burials varied. Most were reported to be in a 'good' to 'fair' condition with some post-mortem fragmentation and weathering; Area A was reported to have

the best preservation whilst Area B had the most complete burials. None of the skeletons selected for this research exhibited signs of leprosy.

The individuals from the Hospital of St James and St Mary Magdalene are curated by the University of Bradford's Department of Archaeological Sciences and were accessed in the summer of 2005.

7.1.2.7 Hereford Cathedral, Hereford

(The following information is summarised from Stone and Appleton-Fox 1996)

The city of Hereford is located on the north side of the river Wye and to the east of the English-Welsh border. The precise date of the establishment of Hereford Cathedral (site code He93A) is not known but is thought to be some time before 676AD, the date that the bishop of the new diocese of Herefordshire was appointed. The cathedral that remains today, however, is Norman in style as a result of rebuilding that began in the early 12th century. The first reference to the cathedral cemetery was in 1140 though a cemetery certainly existed before this date as, prior to 1108AD, the nearby monastery of St Guthlac claimed burial rights in this area. The cemetery was closed in 1791 as a result of overcrowding.

In total, 1129 individuals were excavated from the site from three main areas – a burial pit, three mass graves, and the main part of the cemetery. The pit, which was 5.5m deep, covered a third of the excavation area and was estimated to contain over 5000 individuals, half of which were reburied without any analysis owing to lack of resources to process the human bone. Many of these burials appeared to have been a result of a secondary interment, with the exception of 23 individuals which were still articulated and thought to be primary interments. The reason for the burial pit is unknown but it has been postulated that it came about as a result of the building of the Norman cathedral which, being located adjacent to the Saxon cathedral, would have disturbed the burials there, although it could also have arisen from a disused charnel house. The three mass graves were all of similar size and dimension and laid out in a row, suggesting they were created at the same time. Approximately 200 individuals were excavated from

the pits although there may have been as many as 400 originally interred; disturbances from later excavations made this difficult to assess. As none of these skeletons exhibited any pathological lesions it has been suggested that these pits arose from an outbreak of the plague which was known to have been present in Hereford in 1349 and again in 1361-62. Despite the large number of burials, the method of interment still adhered to normal practice, for the most part, with individuals being laid in rows in a west-east orientation.

The excavation of the main part of the late medieval cemetery, from which the skeletons used in this research derived, resulted in 1085 individuals. These individuals were all densely packed and consisted of 215 adult females, 187 adult males, and 225 children. The earlier graves were stone-lined – in some cases this was continuous around the grave, and in others concentrated around the upper body and head – whereas the later graves contained iron nails that were taken as evidence of wooden coffins. These individuals were laid out in the typical Christian burial tradition with the head to the west and feet to the east but with a variety of arm positions – arms folded over the stomach in earlier periods, crossed over the chest in later periods, and more random positions in the latest burials probably due to coffin burials.

Many of the inhumations in the Hereford Cathedral cemetery had been disturbed by later burials, resulting in lots of partial skeletons and disarticulated remains. As a result, the preservation of the material was reported as ranging from 'good' to 'very poor' with most of the bones being in a 'fair' condition. Only 13% of the skeletons were complete and only 24% had survived *in situ*. As a result of the poor condition of many of the skeletons, assessments of preservation, age, sex, pathological lesions, and any long bone measurements were made before the skeleton was lifted out of the ground. In addition to DISH, pathological conditions noted included periostitis, degenerative diseases, some trauma, tuberculosis, a few congenital conditions such as fusion of cervical vertebrae, and some dental abscesses, although dental caries were rare.

The skeletons from Hereford Cathedral cemetery are curated at the University of Bradford's Department of Archaeological Sciences and were accessed in the summer of 2005.

7.1.2.8 Fishergate House, York

(The following information is summarised from Holst 2004 [accessed September 2006])

Fishergate House (site code YFH) was excavated between August 2000 and March 2001 by Field Archaeology Specialists Ltd (FAS). The site is located 350m south of York's city walls and south of the Gilbertine Priory of St. Andrew's in the medieval 'suburb' known as Fishergate. It is not known which church the cemetery is associated with but it has been postulated, on the basis of documentary evidence, that it was linked with St. Helen's as the dates for this church fit well with the cemetery inhumations (thought to be late medieval) and the church was also located within Fishergate. However, the exact location of St. Helen's is not known. Despite it's proximity to St Andrew's Priory, Fishergate (Stround and Kemp 1993), the cemetery is not believed to be associated with it as the skeletal populations appear to be very different on the basis of osteological analysis.

Two hundred and forty-four individuals were excavated from the Fishergate House cemetery. The skeletons in the cemetery were densely packed and there was a lot of intercutting of graves. In total, there were four excavated areas, and a high concentration of juveniles and infants although children were found to be buried in all areas of the cemetery. Only 37% of the skeletons were more than 80% complete, but the majority of them were in 'good' or 'excellent' condition and could be aged and sexed. There were found to be 56 males or probable males, 54 females or probable females, 113 subadults and 23 indeterminate burials. Most individuals had been buried in shrouds though there were a few coffin burials and one in a grave lined with stone slabs. Three graves were found to include grave goods consisting of a scallop shell (representing pilgrimage to St. James' shrine in Santiago del Compostella), a buckle, and a ring. As none of

these objects were considered to be high status goods, there was a predominance of shroud burials and the osteological analysis showed there to be poor general health, the population was presumed to represent the lower socio-economic classes of York.

The skeletons from Fishergate House are stored at Durham University, in the Department of Archaeology, and were accessed there in the autumn of 2005.

7.2 Recording methods

Prior to collecting the samples for analysis, only basic data about the skeleton was recorded. There were three reasons for this; one, the purpose of the investigation was to record skeletons with DISH and not create a skeletal inventory of the sites examined; two, skeletal data already existed for the sites being used and so additional information could be located if necessary; and three, time was limited and there were usually only four or five days to sort through and record 25-30 skeletons from each site that had been identified as being affected by DISH. The information that was recorded included the:

- Site code
- Skeletal number
- Condition
- Preservation
- Age at death
- Sex
- Samples taken
- Assessment of DISH ('DISH', 'probable DISH', 'possible DISH' or 'non-DISH')
- Description of the vertebral pathology and extra-spinal pathology so that DISH could be assessed
- Description of any other major pathological findings that may affect the observance of DISH
- Shaded drawing of the skeleton indicating bone presence

Each skeleton examined was laid out in full so that a macroscopic assessment could be made. Age and sex was assessed and recorded where appropriate, as was condition, preservation, and stature. DISH was assessed and recorded

according to the criteria outlined below (see section 7.2.2). Photographs of the vertebral skeletal pathology and any other interesting bones were also taken so as to create a visual record of the data.

7.2.1 Ageing and Sexing Methods

For the purposes of this research, age and sex categories were obtained from the skeletal reports of the curated material at each individual institution. Each of the skeletons was assessed by the original researchers using different combinations of the methods below and, for the most part, these were felt to be reliable estimates of age and sex that could be used. As age was not a component being tested as part of this research, and all skeletons used in the research were 'older adults', the exact age range represented was not felt to have a significant bearing on the outcome of the data. However, sex was always confirmed by the researcher whilst recording the skeletons and, in those few cases where the skeleton had not been aged, or this researcher had any doubt as to the validity of the categorisation, they were reassessed using the methods discussed below.

7.2.1.1 Ageing

Ageing methods for adults are not as accurate or as easy to assess as those for subadults (Molleson and Cox 1993:21, Roberts 1996). In subadults there are chronological changes to the skeleton that occur within specific time frames, for example fusion of the distal epiphysis of the humerus occurs between the ages of approximately nine and 13 years of age in females and between the ages of 11 and 17 years of age in males (Buikstra and Ubelaker 1994). Dental eruption is also a reliable indicator of subadult age as the eruption of certain teeth corresponds with certain age ranges; for example, the first permanent molar usually starts to appear at the age of six years (plus or minus 24 months) (Bass 1995). In adult skeletons, developmental and epiphyseal bone fusion and tooth eruption has already occurred, for the most part, so all the changes to the skeleton that take place, and that are used to assess age, are degenerative. Since this is a

qualitative measurement it makes assessment of age much more difficult. The most commonly used features in ageing adult skeletons are degeneration of the auricular surface, sternal end of the rib, and the pubic symphysis, closure of the cranial sutures, and dental attrition. These are the main ageing techniques that have been used to age the skeletons in this research and are discussed below.

7.2.1.2 Auricular Surface Ageing

Meindl and Lovejoy (1989) published their method of ageing adult skeletons through examination of the auricular surface as a paper in 1985 (Lovejoy *et al.* 1985) and as a book chapter in 1989 (pg.137-168). Both detail the observational technique, with photographs and written description, that correlate changes to five year age ranges from age 24 to 60, the latter two age groupings being a ten year phase (age 50 to 60) and a 60+ age range. Essentially, the researcher is observing changes to the texture and topography of the auricular surface, including the degree of 'billowing' and 'granularity' as well as changes to the retroauricular area.

7.2.1.3 Pubic Symphysis Analysis

Meindl *et al.* (1985) produced a revised method for ageing using the pubic symphyseal face based on Todd's method of 1920. This method includes a series of descriptions of the appearance of the symphyseal face, along with photographs that illustrate the bone changes, corresponding to age ranges in years. The surface of the pubic symphysis changes from a billowed appearance in younger adults to a degenerated and eroded appearance in old age. The researcher compares the unidentified skeletal material to the photographs and descriptions as given by Meindl *et al.* (1985) and assigns an age or age range accordingly. This method was improved upon by Brooks and Suchey (1990) who developed their system of age determination based on analysis of the pubic bone from a collection of 1225 individuals of known age and sex. They provided descriptions of six phases of bone change in the pubic symphysis for males and females,

along with appropriate casts of the early and late stages of bone change for each sex. By comparing the bone of unknown age with the descriptions and casts of the Suchey-Brooks system, a mean age and standard deviation from this mean can be assigned. A combination of both techniques was used to provide an age estimate.

7.2.1.4 Cranial Suture Closure

Meindl and Lovejoy (1985) developed a method of age estimation based on the degree of suture closure on the ectocranium. This method uses a scoring system developed with the use of the Hamman-Todd Collection for seven sites known as the vault system and three sites which, when combined with the pterion and mid-coronal sutures of the vault system, are known as the lateral-anterior system. The suture at each of these sites is scored as 0, 1, 2, or 3, according to the criteria described by Meindl and Lovejoy and a composite score produced. Each possible composite score for the two cranial suture systems correlates to a mean age so two possible ages are produced for the individual, of which the lateral-anterior system is thought to be the more reliable estimate.

7.2.1.5 Dental Wear Analysis

Although several different dental attrition charts exist for ageing adult skeletons, Brothwell (1981) is probably the most relied upon. His charts show tentative age classifications in Neolithic to Medieval British skulls based on wear in the molar teeth. The wear pattern is divided into quadrants and shows the different variations in attrition for each of the molars in each of the four age categories – 17-25 years, 25-35 years, 35-45 years, and 45+ years. As long as there is at least one molar (tooth) present in the skeleton being examined, it should be possible to obtain an age estimate.

7.2.1.6 Rib Phase Analysis

Iscan *et al.* (1984) developed a system of age identification using the sternal end of the fourth rib from a collection of 118 autopsied males. Only those individuals over 17 years of age were included in the analysis; the oldest individual was 85 years old. This system of ageing also used a phase method, similar to the pubic symphysis method. Nine phases are described accompanied by pictures illustrating changes to the rim of the sternal end of the rib and the transition from a V-shaped indentation in the medial articular surface to a U-shaped one. Each phase corresponds to an age range that can be assigned to the bone, the earlier phases having narrower age ranges and hence more useful age assignments, than the later phases which have very broad age ranges. The rib phase analysis for female skeletons was developed later, in 1985.

7.2.1.7 Sexing

Unlike with ageing, assessing the sex of a skeleton is much easier in adults than it is in subadults. This is due to the fact that the secondary sex characteristics, that is, those that manifest themselves physically, develop at puberty and alter the morphology of some key bones which bioarchaeologists can then use to assess biological sex. The two key areas are the skull and pelvis. More specifically, the general shape and size of the cranium including the orbits, nuchal crest, frontal bossing, and mastoid process; the gonial angle and degree of gonial eversion of the mandible; and on the pelvic bones the size and shape of the greater sciatic notch, the shape of the obturator foramen, the subpubic angle, and the presence or absence of the ventral arc and pre-auricular sulcus. The aforementioned features are the major characteristics that were used to sex the skeletons in this research and, as such, are discussed below.

7.2.1.8 Skull and Pelvis

Acsadi and Nemeskeri (1970) compiled a list of secondary sex characteristics in the cranium, mandible, and innominates that could be used to determine the sex of the skeleton in question. These secondary sex characteristics included such traits as the size of the mastoid process, the size and shape of the orbit, the prominence of the brow ridge, the shape of the greater sciatic notch, and the angle of ramus of the mandible. They provided a series of examples for each of the traits that demonstrated the range of variation for that particular trait and labelled each variant as 'hypermasculine', 'masculine', 'indifferent', 'feminine', and 'hyperfeminine' (Acsadi and Nemeskeri 1970:87). By scoring each of the traits in this way, a general consensus can be reached with regard to the sex of the individual.

7.2.1.9 Os Pubis of the Innominate

In 1969, Phenice published a new method of sex determination through examination of the pubis of the innominate. This technique allowed a sex to be assigned even when the innominate was not complete, provided the pubic portion of the bone remained, and with a high degree of accuracy - over 96% if two of the criteria concur. The three criteria used in this sexing method are the presence or absence of a ventral arc, the presence or absence of a subpubic concavity, and the appearance of the ridge on the medial aspect of the ischio-pubic ramus. All three features are present, or more developed, in females and absent, or less developed, in males.

7.2.2 Identifying Diffuse Idiopathic Skeletal Hyperostosis

The criteria used in identifying cases of diffuse idiopathic skeletal hyperostosis (DISH) were adapted from Resnick and Niwayama (1976) and Rogers and Waldron (2001). It was decided to combine what were felt to be the most

pertinent aspects of these radiological and archaeological approaches to come up with the following criteria:

- minimum of four contiguous fused vertebrae
- extra-spinal ossification/enthesophytes
- fusion predominance in the right-side of the thoracic vertebrae
- maintained disk space
- no apophyseal joint ankylosis

If the skeleton met all of these criteria then a diagnosis of 'DISH' was applied and, if none of the criteria were met, then the skeleton was recorded as 'non-DISH'. However, as not all the skeletons were in perfect condition or had all the vertebrae or extra-spinal bones present, and some exhibited varying degrees of spinal fusion, some adjustments had to be made. In those cases where there were three contiguous fused vertebrae but the suggestion that there were more fused vertebrae before being broken post-mortem, evidence of prolific osteophyte growth on the remaining vertebrae, or another set of contiguous fused vertebrae and/or extra-spinal enthesophytes, then they were considered a case of 'probable DISH'. In those cases where there was fusion of only two contiguous vertebrae, but evidence that others could have been affected ante-mortem, or very large osteophytes throughout the spine that extended to meet but were not fused and/or had evidence of extra-spinal enthesophytes, they were designated as 'possible DISH' (see Table 1 for a summary). These DISH/probable DISH/possible DISH categories were created so that future reassessments and research could take place if desired; for the purposes of the scientific analysis, the three categories were combined to represent skeletons with DISH. Identifying those individuals that were definitely not DISH required a complete or near complete set of vertebrae and, if possible, extra-spinal elements so that all potential for bone fusion and significant osteophyte or enthesophyte development could be ruled out.

Table 9. Summary of Key Criteria Used to Diagnose DISH

DISH Category	Key Criteria
Definite DISH	Minimum 4 contiguous fused vertebrae
Probable DISH	Minimum 3 contiguous fused vertebrae
Possible DISH	Minimum 2 contiguous fused vertebrae

Problems were encountered when reviewing the skeletons in various assemblages that had been identified in the site reports as having DISH; often they had been overinterpreted. As sites were chosen based on their reported prevalence of DISH it was often disappointing to find that many of those skeletons that had been recorded as having DISH actually were only cases of what would have been categorised in this study as 'possible DISH'; sometimes there were not even enough vertebrae remaining to make a definitive diagnosis. However, the fact that these skeletons had been categorised as 'DISH' on the curators' databases does at least give the researcher an opportunity to assess them on an individual basis. Had they not been categorised in this way, the skeletons would probably never have been considered despite the presence of some bone fusion.

There were also some problems with access to particular skeletons and sites. There were some skeletons known to have excellent examples of DISH, with good preservation, that were unattainable either because they had not been returned by a previous researcher or because they simply could not be located. There were a couple of sites in addition to those used in this research that may have yielded some good information and samples for analysis, but one of these could not be found within the institution's store room and the other was so poorly labelled that it was impossible to locate the relevant boxes.

7.3 Sampling

The sampling method was dictated by the methods of analysis that were to be used. Two osteological samples were needed, one for the ancient DNA analysis and one for the stable isotope analysis, and the most appropriate samples were determined in accordance with the needs of the analytical techniques. The key consideration for the ancient DNA analysis was contamination; contamination from other archaeological material, from soil bacteria, and from other individuals who handled the material post-excavation. Therefore, teeth were considered to be the most appropriate sample to take as they would be least susceptible to these

contaminants and also easier to clean (A Bouwman, pers. comm., February 2005). Using teeth was also a much more discreet way of sampling as the alternative would be to take a large piece of long bone which would be more destructive, and would possibly make obtaining permission for sampling more difficult. The teeth had to be free from caries and deep cracks that would permit soil (and soil bacteria) to enter; they were to be ideally still rooted in the mandible or maxilla as this would prevent any contaminants from entering the tooth via the root apex. However, loose teeth were taken as samples when none were present in the mandible or maxilla. The techniques involved in DNA analysis meant that the DNA would be obtained from the dental pulp and therefore molars and pre-molars were chosen as the best types of teeth to sample as these would provide the largest pulp cavities. The actual method of extracting the teeth from the mandible or maxilla varied according to the condition of the bone and tooth. Sometimes, it was possible to simply pull the tooth out by hand, exerting steady but gentle force, otherwise it was necessary to use a pair of bent long-nose pliers being careful not to damage the tooth or the bone. Latex gloves were worn when handling the teeth, a new pair for each sample, so as not to contaminate the teeth with the researcher's own DNA or spread contamination from one sample to another, and the pliers were cleaned after each extraction with a small paintbrush. Once the teeth were extracted they were placed in small plastic ziplock bags and stored long-term in a refrigerator (fridge) until ready to be analysed. The methods of sample analysis, as described below (section 7.3.1.1), apply to mtDNA analysis of the samples. As explained in the introduction (Chapter 1) and section 9.4, it was decided that mtDNA analysis, rather than STRs, would yield the best results.

The requirements for the stable isotope analysis were 1-2 grams of bone from each analysed skeleton. As the type of bone sampled was not important, disarticulated ribs were deemed suitable for collection. If the ribs were intact or nearly complete then a small piece, approximately 1-2g, would be sawn off the bone using a small handsaw and a wooden block on which to rest the bone. If the ribs were fragmentary then a suitable rib fragment would be taken for analysis. Sometimes this would mean that it was greater than the required amount, but if a fragmented piece was available it made more sense to collect

that than to cut a new piece from a complete bone. Gloves were not worn to collect the rib samples as contamination of the archaeological samples by the researcher was not a concern. However, the handsaw and wooden block were cleaned with a paintbrush in between samples in order to minimise potential cross-contamination between bone samples. Once the rib fragment or sawn off piece of rib was collected, its weight was recorded and it was placed in a plastic ziplock bag and stored in the fridge long-term until ready for analysis.

Although skeletons were chosen primarily on the basis of good preservation, there were a few problems with the rib and tooth sampling in that not every skeleton examined had both teeth and ribs suitable for analysis. If this was the case then it was noted in the recording form for the skeleton.

7.3.1 Sample Preparation and Analysis

7.3.1.1 Ancient DNA

The teeth were processed and analysed at the University of Manchester in Professor Terry Brown's biomolecular archaeology laboratories (labs). As these labs are used for human DNA analysis, both ancient and modern, there were strict rules in place to help guard against contamination. Three labs existed: one for extraction, one for PCR work, and one for post-PCR work and general lab use. Movement of people and materials was only allowed in a certain direction – extraction lab → PCR lab → post-PCR lab – to prevent cross-contamination of modern human DNA with ancient samples. Therefore, work had to be carefully planned out so that movement between labs was reduced and, whenever it was necessary to go back to an earlier stage lab, there would have to be a day's wait until clothes had been changed and hair and skin washed. There were other anti-contamination procedures in place as well. Worktop surfaces and electrical equipment had to be wiped down with 5% bleach or DNA Away (Molecular BioProducts) before use each time, and smaller equipment and utensils such as pipettes, eppendorf tubes, beakers, marker pens etc (of which there were separate

supplies for each lab), had to be exposed to UV light in a Stratalinker at 120,000μjoules/cm^2 for 10-20 minutes before use. Ideally, there was only one person allowed in the extraction and PCR labs at a time to minimize contamination risks. Each of these labs had its own set of lab coats, gloves, face masks, hair nets, and lab glasses and, before beginning any work, the researcher had to put these on, making sure that they put on a double set of gloves – nitrile and latex - with the first pair taped to the lab coat so that there was no skin exposure (and potential contamination of the sample with modern skin cells), and ensure that the hairnet covered all the hair and the eyebrows so that no hair could accidentally fall and contaminate the ancient DNA (aDNA) sample.

During the extraction process, dentine from the teeth was extracted for analysis. All materials and samples were UV-irradiated for 20 minutes before starting any work, and all surfaces and electrical equipment were wiped down with 5% bleach and/or DNA Away. All work was carried out on a foil-covered tray that had been treated with DNA Away in order to minimise sample contact with other surfaces and minimise contamination. The surfaces of the teeth were carefully cleaned with undiluted bleach and also 37% phosphoric acid gel (Tower Dental Production Ltd) to remove any surface contaminants. A series of small dental hand drills (ProTaper from Maillefer Dentsply) were used to drill a hole through the root apex into the root canal in order to extract a minimum of 0.01g of dentine from inside the pulp cavity. Once this was acquired, the dentine was transferred to a labelled eppendorf tube. The remainder of the extraction protocol was adapted from Yang *et al.* (1998) using the QIAquick ® PCR Purification Kit from Qiagen. A 1mL extraction solution was made up of 0.5M EDTA at pH 8.0, 0.5% sodium dodecyl sulphate and 100μg/mL proteinase K and added to each dentine extraction, plus a blank of milliQ H_20, vortexed briefly and then placed on a shaking incubator at 55°C for 24hrs.

Following the incubation period, the samples were centrifuged at 2000rpm for 5 minutes then 0.5mL of each supernatant was transferred to 15mL falcon tubes (the remainder was stored in the freezer) along with 2.5mL of PB buffer from the Qiagen kit and mixed. Qiagen spin columns were used to centrifuge 0.75mL of this sample/PB buffer at 14,000rpm for one minute, after which the flow-through

was discarded. This process of transferring 0.75mL to the spin column, centrifugation and discard of the flow-through was repeated until all the extract had been passed through the column. The DNA was then eluted from the silica column. First, it was washed by adding 0.75mL of PE buffer (Qiagen), centrifuged at 14,000rpm for one minute and the flow-through discarded. The columns were centrifuged again to ensure all ethanol had been removed and then they were transferred to new 1.5mL centrifuge tubes, 50μL of EB (Qiagen) was added to each column to elute the DNA, and it was centrifuged at 14,000rpm for one minute, after a minute incubation at room temperature. This step was repeated to give a total of 96μL for each purified DNA extraction and blank, of which 20μL was taken to the PCR lab for further analysis, the remainder being stored in the fridge in the extraction room.

In the PCR room, the purified aDNA extracts were amplified by polymerase chain reaction (PCR). As in the extraction room, all surfaces and equipment were cleaned with 5% bleach and/or DNA Away before use. New blanks of milliQ H_20 were created during the PCR so that any contamination that occurred during this stage of the analysis could be identified. A master mix of all the reagents needed for the PCR was made up so as to reduce contamination that can occur with opening and closing of the eppendorf tubes multiple times. Each reaction always contained a total volume of 100μL so the quantities in the master mix were adjusted accordingly for n+1 samples (enough for the DNA samples, blanks and some extra). For a single sample, the master mix contained:

	Taq buffer	(1x)	10μL
	$MgCl_2$	(2mM)	8μL
	Forward primer mtC*	(2μg/mL)	4μL
	Reverse primer mtC	(2μg/mL)	4μL
	Each dNTP	(200μM)	2μL x 4 = 8μL
	BSA	(1%)	1μL
	milliQ H_20		59.5μL
	Taq DNA polymerase	(2.5U)	0.5μL
Plus	DNA sample/blank		5μL

			100μL total volume

*mtC forward sequence: 5'-CCA CCT GTA GTA CAT AAA AAC CCA-3'
mtC reverse sequence: 5'-GTG GGT AGG TTT GTT GGT ATC CTA-3'

All the materials, including pipettes, tips, marker pens etc were UV irradiated for 20 minutes – 10 minutes on each side – and solutions, empty microtubes for 10 minutes. The master mix was prepared for *n*+1 samples without *Taq* polymerase and UV-irradiated for 5 minutes. During this time, 5μL of the aDNA samples and blanks were aliquoted into labelled microtubes then the *Taq* was added and mixed into the master mix and 95μL of this was added to each sample or blank. The PCR samples were quickly transferred to the thermocycler in the post-PCR lab and run on the following programme:

4 mins at 94°C

44 cycles of {
1 min at 55°C
1 min at 72°C
1 min at 94°C
}

1 min at 55°C
8 mins at 72°C
cooling period to 4°C

The PCR products were then stored in a fridge in the post-PCR lab until needed for the next stage of analysis.

In the post-PCR lab, the PCR products were run on an agarose gel to check that DNA was present and that there was no evidence of contamination, i.e. DNA present in the extraction blanks. A 3% agarose gel was prepared and 15μL of PCR product and 3μL of loading dye for each aDNA sample and blank was dry loaded onto the gel using λ DNA restricted with *Hin*d III and *Pst* I as markers. The gel was then covered with 1xTBE and run at 67V. Once the dye was nearing the bottom of the gel the electrophoresis was stopped and the gel was immersed in 1mg/mL ethidium bromide (EtBr) and covered with foil for 15 minutes (EtBr is light sensitive). It was then immersed in dH_20 for 10 minutes to remove excess EtBr, rinsed with dH_20, and taken to the transilluminator so that the DNA bands could be seen (EtBr fluoresces under UV light) and a photograph obtained. If DNA was present in the samples and there was no contamination of the extraction blanks the gel was taken to the photographic room and the bands of DNA were cut out with the aid of a UV lightbox and razor blades – one for each sample – and placed in eppendorf tubes. The bands of DNA were then

isopropanol precipitated to get rid of buffers and reagents from the PCR reaction and gel electrophoresis to ensure that only DNA was present in the sample. Using a Qiagen kit, QG buffer was added to each sample in a 6:1 ratio of buffer to gel band. The tubes were placed in a 50°C water bath for 10 minutes until the agarose had dissolved and then vortexed gently. Refrigerated 100% isopropanol was added, 1μL for every milligram of sample, transferred to spin columns and then centrifuged for one minute at maximum speed. The flow-through was discarded, 0.75mL of PE buffer was added, centrifuged for one minute and flow through discarded. Samples were spun again at 13,000 rpm for one minute to ensure all the buffer had run through the column and the flow-through discarded. The samples were placed in clean eppendorf tubes with 60μL ethanol (EtOH) and 3μL sodium acetate (NaAOc); 30μL EB buffer was added to the center of the column and, after resting at room temperature for a minute, they were centrifuged for one minute at 14,000 rpm. The purified samples were then stored in the freezer at -20°C overnight. Samples were ethanol precipitated to prepare them for cloning. The samples were taken from the freezer and centrifuged at 4°C for 30 minutes. The supernatant was removed, 250μL of 70% ethanol added, and the tubes were inverted gently to mix. The samples were centrifuged for 15 minutes at room temperature and the supernatant was again removed. Samples were centrifuged for a further 3 minutes and any remaining liquid removed before being dried in a dessicator. The pellet was then resuspended in 5μL of milliQ H_2O and put in the freezer until needed for cloning.

Cloning was also carried out in the post-PCR lab. New plates of LBA + ampicillin were usually poured for each cloning attempt unless refrigerated plates less than two days old were available from a previous cloning exercise. Four hundred microlitres of ampicillin was added to 400mL of LBA to pour approximately 15 plates. Forty microlitres each of XGal and IPTG were added to each plate with the use of a sterile glass spreader and left to dry. At room temperature, 0.25μL salt solution and 0.25μL vector (Amersham) was added to 1μL of purified DNA and left for 30 minutes. On ice, 10μL of *E. coli* cells were added and gently mixed and left on ice for 30 minutes. The samples were placed in a 42°C water bath for exactly 30 seconds and then placed back on ice for a few minutes. Seventy-five microlitres of SOC medium was added to each tube and

they were placed in a shaking incubator at 37°C for an hour (along with the agar plates) to allow the cells to grow. Once the plates and samples had been removed from the incubator, 76.5μL of each of the cells was plated onto each warmed petri dish and incubated at 37°C overnight. Those colonies that were white or contained a blue insert were carefully picked using a pipette tip and placed in eppendorfs so that another PCR reaction could be performed and the products run on 2% agarose gels to see if the colonies contained the correct sized DNA inserts. Those clones that did contain the correct size bands were purified then ethanol precipitated, sequenced, and isopropanol precipitated. Purification was carried out using the Qiagen kits. Seventy-five microlitres of PB buffer was added to 15μL of cloning product (5:1 ratio), transferred by pipette to a spin column and centrifuged for one minute at 14,000rpm. The flow-through was discarded and the column washed with 750μL of PE buffer then centrifuged for one minute. The flow-through was discarded and then centrifuged one final time to remove any remaining traces of ethanol. The spin column was placed in a 2mL eppendorf tube containing 5μL NaAOc and 100μL EtOH and 50μL of elution buffer was added to the center of the membrane. After resting at room temperature for one minute, the tubes were centrifuged at 14,000rpm for one minute, the spin column was discarded and the eppendorf was stored in the freezer. The ethanol precipitation was carried out as described above in the pre-cloning step. The sequencing reaction involved adding 2μL of sequencing mix, 1μL of a forward primer (M13), and 1μL of distilled water (dH_2O) to 1μL of each purified clone DNA sample and putting it in the thermocycler on the following sequencing programme:

10 mins at 95°C

25 cycles of {
1 min at 95°C
1 min at 55°C
1 min at 72°C

10 mins at 72°C

The M13 Forward primer sequence was: 5'-GTAAAACGACGGCCAG-3'

To carry out the isopropanol precipitation, 20μL of 75% isopropanol was added to the sequenced DNA and then the tubes were wrapped in aluminium foil and

left at room temperature for 45 minutes. They were then centrifuged for 20 minutes at full speed with the tube hinges facing out. The fluid was pipetted off and 250μL of 75% isopropanol was added and mixed gently by inverting the tubes a few times. The tubes were centrifuged for 8 minutes and the fluid pipetted off. The tubes were centrifuged for 2 minutes and fluid pipetted off using a 2μL pipette. This step was repeated if necessary. Tubes were then placed in a vacuum pump for 30 minutes to remove any remaining traces of fluid and then stored in the freezer if necessary.

Once the isopropanol precipitation had been carried out, the sequenced clones were sent off to the DNA Sequencing Facility in the Department of Biochemistry at the University of Oxford lab for dideoxy sequencing. Electropherograms – visual representations of the sequence - were sent back for analysis in return.

In order to check for contamination within the sample sequences, this researcher's DNA was also sequenced. A similar procedure was followed for this modern DNA as the ancient except the initial step involved a Chelex extraction (Walsh *et al.* 1991) using saliva. The saliva was centrifuged for ten minutes (in a microtube) and the supernatant decanted, then 0.5mL of 10% Chelex solution (pre-mixed) was added and the tube vortexed. The solution was boiled for ten minutes to release the DNA from the cells, put on ice until cool enough to handle and then centrifuged for five minutes. The supernatant was transferred to a new microtube and this then underwent a PCR, PCR purification, and sequencing the same as the aDNA samples.

As would be expected, there were problems encountered at various stages of the ancient DNA analysis. As these techniques are not normally in the repertoire of an bioarchaeologist, they had to be learned 'on the job' and, as there were only 10 weeks to carry out the analysis at the University of Manchester, this was a steep learning curve. During the extraction stage, the biggest problem encountered was with trying to get a sufficient amount of dentine out of the teeth. The hand drills were very small and fragile and they were prone to breaking inside the root canal if twisted slightly the wrong way. The idea with using the hand drills was to start with the smallest drill and progress up to the largest but,

after several failed attempts, it was discovered that starting with one of the middle drills was best as they were a bit more sturdy and could withstand the pressure of making the initial hole into the root canal better than the smaller drills. If the drill did break inside the root canal there was no way to retrieve it, and if the tooth had only one root then it was not possible to make another attempt to obtain more dentine from that tooth. This problem only occurred with a few samples (less than ten). Sometimes it was just not possible to make progress with drilling into the root canal because the tooth root was too dense. Some of the teeth seemed to have a cement-like substance obscuring the root canal which made it impossible to drill a hole with the fragile hand drills. Dentine was not obtained from these teeth.

There were no specific problems encountered with the PCR process. However, this procedure involved very precise timings and reagent amounts in order to work optimally and deviations from this may have affected the results. The procedure became easier with practice and so, not surprisingly, the first few sessions in the PCR lab were less fluid and often took longer to complete. Contamination is an issue with any ancient human DNA work, and it is very easy to contaminate samples at any stage. Thankfully, not too many of the samples became contaminated during PCR but when it did happen it was quite frustrating to try and obtain clean products.

Problems encountered with the post-PCR process were the types of problems that were expected of this kind of lab work – having to repeat experiments, cloning not working properly etc. There were some issues with equipment: the freezers defrosted on a couple of occasions which meant ordering new primers and enzymes but it is not thought that this would have affected the samples used in this research.

The actual sequencing analysis was carried out by the University of Oxford's DNA Sequencing Facility and the sequences were sent back in digital form to be interpreted in the lab in Manchester. Each peak in the electropherogram represented a nucleotide base and each of the four bases was represented by a different colour. The sequences were read by comparing that of the DNA sample

with that of the Cambridge Reference Sequence for hypervariable region 1 (HVRI - the region that was being investigated in these experiments), taking care to read the sequence of bases from the peaks of the graph rather than the written bases above the peaks that the computer had interpreted as these were sometimes incorrect.

The Cambridge Reference Sequence (CRS) is as follows:
5'-CCA CCT GTA GTA CAT AAA AAC CCA ATC CAC ATC AAA ACC CCC TCC CCA TGC TTA CAA GCA AGT ACA GCA ATC AAC CCT CAA CTA TCA CAC ATC AAC TGC AAC TCC AAA GCC ACC CCT CAC CCA CTA GGA TAC CAA CAA ACC TAC CAC-3'

Analysis of the results included editing these sequences manually so that any incorrectly labelled peaks were corrected. These were then imported into a sequence analysis and editing program, BioEdit (Hall 1999), and each aligned with the CRS and each other. The final result is that all the sequences are presented as a single alignment which can then be used to determine where mutations occur and whether there is any consensus amongst clones of the same sample. The samples were considered to have a consensus mutation(s) if the majority of the clones (>50%) shared the same set of mutations. These mutations were then translated into haplogroups, if possible, with the use of a mitochondrial haplotype tree (Macaulay and Richards 2008, accessed February 2008; Richards and Macaulay 2000:142).

7.3.1.2 Stable Isotopes

Preparing the rib samples for stable isotope analysis involved several stages as outlined in Richards and Hedges (1999), with an added filtration step (Brown *et al.* 1988), and were refined by Dr. Andrew Millard at Durham University. The aim of this protocol was to recover and purify collagen from the rib samples so that they could be analysed for carbon and nitrogen isotopes in a gas-source mass spectrometer. The initial stage requires 90-200mg of bone to be sawn off from each rib sample using the handsaw and wooden block and then to demineralise the bone. To do this, the bone is crushed in a pestle and mortar (which is cleaned with purified water after use with each sample), then the pieces of bone are put in

a 15mL glass test-tube to which 10mL of refrigerated 0.5M HCl is added. These test tubes are then covered with parafilm, pierced so as to release any gases that may build up, and refrigerated for several days. During this refrigeration period, the test tubes are shaken once or twice a day to ensure that all bone surfaces are exposed to the acid and the acid is changed every two days in order to ensure that the correct pH level is maintained. Once the bone sample was soft or floating it was considered to be demineralised – a process that took anywhere between five and ten days – and the supernatant was then decanted using Ezee filters and the sample rinsed in purified water several times until the sample was approximately pH 3.0.

The next stage was to gelatinise the sample. This involved heating the collagen pellets in pH 3.0 water at 75°C for two to four days to dissolve the collagen. Once dissolved, the samples are then transferred to ultrafilters and centrifuged at 4000 rpm for 15 to 20 minutes. The highly concentrated supernatant is transferred from the filter to empty labelled and weighed centrifuge tubes using a pipette and, in order to improve yield, the filter is rinsed with purified water and this diluted supernatant is also transferred to the centrifuge tubes. The tubes are then covered with parafilm, pierced, and freeze-dried for up to 48 hours to produce fluffy, purified collagen.

The final stage of preparation is to use tweezers to weigh out between 250μg and 350μg (0.25-0.35mg) of collagen into 3.5mm x 3.75mm tin capsules ready to be analysed in the mass spectrometer. In order to weigh out the correct amount of freeze-dried collagen, the tin capsules had to be rested on a penny coin in which a hole had been drilled the same size as the tin capsule. The coin and the tin capsule were then placed on the balance so that the collagen could be weighed directly. Small pieces of the collagen were removed using tweezers and added to the capsule until the weight was within the desired range. To seal the capsule, the top of the tin foil was pressed shut and then folded over using tweezers so that none of the collagen sample could be lost. The samples were then placed in labelled eppendorf tubes ready to be transferred to the mass spectrometer.

During this process, several problems were encountered. The first set of samples took a long time to demineralise, about ten days, which is about double the average for the remaining samples. This same set of samples also took twice as long as the other samples to gelatinise, being heated for 4 days. In fact, the samples were not completely dissolved when taken off the heating block but were centrifuged regardless and freeze-dried to see what the result would be. Despite the problems with this first set of samples they still appeared to give good yields. It is possible that the problems encountered were a result of inexperience with the stable isotope preparation protocol as these difficulties only occurred with the first set of samples prepared. There are also several other possible contributing factors that also coincide with the processing of the first set of samples. The acid used to demineralise the first batch of samples was taken from a bottle that had been stored in the fridge from previous experiments some months ago and was not freshly made up, as was the case for the other samples. Some of the supplies to be used during the sample preparation had not yet arrived when the experiments were started. The Ezee filters that were supposed to be used to decant the samples were not available and so the supernatant was simply decanted, which meant that not all the supernatant could be removed from the test tubes for fear of also losing the sample. Additionally, the ultrafilters that the samples were centrifuged in, before being freeze-dried, were not available for the first set of samples either. These samples were centrifuged in regular centrifuge tubes and then the supernatant carefully decanted by hand into pre-weighed tubes to be freeze-dried. Any of these situations may have contributed to the difficulties encountered with preparing the first set of samples. There was also separation of some of the collagen samples that occurred upon freeze-drying, resulting in a band of darker, brown, collagen on the bottom of the sample, and a lighter, yellow-white collagen on the top. It was not known why this happened but it is not thought to have impacted upon the stable isotope results.

The analysis of the collagen samples was carried out with the assistance of continuous-flow isotope ratio mass spectrometry in the Department of Earth Sciences at Durham University and also in the Alaska Stable Isotope Facility at the University of Alaska Fairbanks' Water & Environmental Research Center. The samples, in their tin capsules, were combusted in the chamber of the mass

spectrometer and the amounts of nitrogen and carbon gases released as a result were measured and the data stored. The data was presented in numerical form for interpretation by the researcher.

Once the raw isotope data was obtained, the duplicate results are averaged together so that a single result is produced for each sample. Any samples that have poor C/N ratios falling outside the accepted range for collagen of 2.4-3.6 (the combined range of DeNiro 1985 and Schoeninger *et al.* 1989) are thought to be compromised by diagenetic processes and discarded. Scatterplots are created with different groups of data and statistical analysis is used to determine if any differences are significant. Mann-Whitney tests are used to test for differences between groups of samples. These are non-parametric tests which make no assumptions about the distribution of the data and whether it has a normal distribution or a homogeneous variance, so they can be used for data sets with extreme values (Dytham 2003). As it makes no assumptions about the distribution of the data, the Mann-Whitney test is less powerful than the t-test but it is also less likely to find significant results when no differences exist (type 1 error) (*ibid.*).

Chapter 8: Results

8.1 Skeletal Data for DISH

Every skeleton was assessed macroscopically according to the criteria presented in the materials and methods chapter and assigned to a category of "DISH", "Probable DISH", "Possible DISH" or "No DISH". For the purposes of the scientific analysis, all probable and possible cases of DISH were included in the DISH category. In total, there were 34 skeletons with DISH, seven skeletons with probable DISH, six skeletons with possible DISH, and 46 without DISH from the nine monastic and non-monastic sites. These are summarised in the tables below along with brief descriptions of the vertebral and extra-spinal fusion.

Table 10: Summary of Skeletons from Blackfriars, Ipswich

Skeletal Number	DISH Category	Description of Fusion
IAS4801 2591	DISH	T7-T11
IAS4801 2654	DISH	T4-L1
IAS4801 2005	DISH	T9-11, T6-7, possible fusion of T4-12 but broken post-mortem
IAS4801 1834	DISH	T5-T9
IAS4801 1799	DISH	4 mid- or lower thoracics fused
IAS4801 1757	DISH	4 upper thoracics fused, T10-L1, L2-3
IAS4801 1417	DISH	T3-10
IAS4801 2496	Probable DISH	Fragment of 3 fused vertebrae
IAS4801 1457	Probable DISH	Fragment of 3 thoracic? vertebrae with osteophytes extending superiorly (sup.) and inferiorly (inf.)
IAS4801 2508	No	
IAS4801 1933	No	
IAS4801 1872	No	
IAS4801 1919	No	
IAS4801 1762	No	
IAS4801 2642	*No (*not all vertebrae present)	
IAS4801 1391	*No	
IAS4801 1451	*No	
IAS4801 1415	*No	
IAS4801 2640	*No	

Table 11. Summary of Skeletons from Blackfriars, Gloucester

Skeletal Number	DISH Category	Description of Fusion
19/91 56	DISH	T6-9,
19/91 420	DISH	T6-10
19/91 302	DISH	T4-10, T11-L1
19/91 257	DISH	T6-T11
19/91 122	poss. DISH	T9-12 large osteophytes but not fused
19/91 104	No	
19/91 212	No	
19/91 92	No	
19/91 233	No	
19/91 143	No	

Table 12. Summary of Skeletons from the Hospital of St. James and St. Mary Magdalene, Chichester

Skeletal Number	DISH Category	Description of Fusion
CH86 123	DISH	T1-12
CH86 357	DISH	T2-12
CH86 18	prob. DISH	T12-L1 but L3 may have been fused and broken post-mortem
CH86 45	No	
CH86 175	No	
CH86 23	No	

Table 13. Summary of Skeletons from Hereford Cathedral, Hereford

Skeletal Number	DISH Category	Description of Fusion
He93A 1997	prob. DISH	T8-10? fused, osteophytes T9-10 extend to meet, as do L2-3, L3-4, L4-5
He93A 1664	poss. DISH	2 fragments of fused thoracic, with large thick osteophytes extending superiorly and inferiorly
He93A 2073	No	
He93A 606	No	

Table 14. Summary of Skeletons from St. James' Abbey, Northampton

Skeletal Number	DISH Category	Description of Fusion
EL00 3039	DISH	T9-12
EL00 3027	DISH	T3-12
EL00 3096	DISH	T4-5, T8-12
EL00 3099	DISH	T3-10
EL00 3121	DISH	T3-12
EL00 3090	DISH	T4-12
EL00 3271	DISH	T4-5, T6-7, T9-L2
EL00 3127	DISH	T4-10
EL00 3292	DISH	T5-L1, L6 body fused to sacrum
EL00 3116	prob. DISH	T5-6, T7-9
EL00 3266	prob. DISH	T6-8, T10-12 though possibly fused T6-12 and broken post-mortem
EL00 3285	poss. DISH	T8-10 have large osteophytes extending to meet but not fused
EL00 3100	No	
EL00 3014	No	
EL00 3095	No	
EL00 3005	No	
EL00 3145	No	
EL00 3073	No	
EL00 3079	No	
EL00 3094	No	
EL00 3141	No	
EL00 3019	No	
EL00 3068	No	

Table 15. Summary of Skeletons from the Royal Mint Black Death Cemetery, London

Skeletal Number	DISH Category	Description of Fusion
MIN86 BD 6412	DISH	T5-11, T12-L1
MIN86 BD 11944	poss. DISH	large osteophytes extending to meet T7-12, possible fusion of some lumbar vertebrae but intermediate fragments lost
MIN86 BD 8341	No	
MIN86 BD 5960	No	

Table 16. Summary of Skeletons from the Royal Mint Late Graves, London

Skeletal Number	DISH Category	Description of Fusion
MIN86 LG 13518	DISH	T7-11, growth approaching fusion on T1-6
MIN86 LG 16344	DISH	fragments (frags) of 4 fused vertebrae (verts), two other frags of 2 fused verts
MIN86 LG 16098	DISH	4 fused thoracics,
MIN86 LG 12480	prob. DISH	3 contiguous fused verts, two verts above and 3 below with large (lg) osteophytes, possibly were fused and broken post-mortem
MIN86 LG 13663	poss. DISH	2 fused thoracics, verts above and below have lg osteophytes growing to meet but not fused
MIN86 LG 16322	poss. DISH	Several frags of paired fused verts
MIN86 LG 16311	*poss. DISH	2 fused thoracics, remaining 7 thoracics have osteophytes extending to meet but not yet fused - very poor preservation
MIN86 LG 10420	No	
MIN86 LG 12356	No	
MIN86 LG 10348	No	
MIN86 LG 10177	No	
MIN86 LG 12687	No	
MIN86 LG 12520	No	
MIN86 LG 12349	No	

*Not all vertebrae present

Table 17. Summary of Skeletons from Merton Priory, Surrey

Skeletal Number	DISH Category	Description of Fusion
MPY86 4851	DISH	6 fused thoracics
MPY86 4282	DISH	7 fused thoracics
MPY86 3667	DISH	4 fused vertebrae (verts) but 2 broken off post-mortem (p-m), 4 other verts either fused and broken p-m or were nearly fused
MPY86 2748	DISH	6 fused thoracics
MPY86 2905	DISH	8 fused thoracics (possibly T5-12)
MPY86 716	No	
MPY86 3447	No	
MPY86 2376	No	
MPY86 754	No	
MPY86 799	No	
MPY86 623	No	

Table 18. Summary of Skeletons from Fishergate House, York

Skeletal Number	DISH Category	Description of Fusion
28	DISH	T7-11, L1-3,
308	DISH	T6-7, T8-11, (T7-8 probably fused and broken post-mortem)
310	No	
159	No	

8.2 Stable Isotope Data

The stable isotope analysis involves several sets of results obtained from Durham University, IsoAnalytical, and the University of Alaska. Multiple sets of results were obtained owing to problems with the consistency of the results obtained from Durham University which deemed some of them to be unusable. The results section will therefore consider each set of results in turn and then offer an explanation as to which data set was deemed valid and why.

8.2.1 Determining a Valid Data Set

The Durham Earth Sciences results originally consisted of two sets of results – a small group of samples run in August 2006 in order to test the efficacy of the samples, and a large group consisting of all the samples, including those from August, which were run in December 2006. When the $\delta^{13}C$ and $\delta^{15}N$ values for the small set of results from August 2006 were compared to those for the same samples in the December 2006 run, they appeared to be quite different, especially for carbon. The August 2006 samples had a mean of -17.72‰ for $\delta^{13}C$ and 12.41‰ for $\delta^{15}N$, whilst the same samples run in December 2006 had a mean of -18.98‰ for $\delta^{13}C$ and 13.15‰ for $\delta^{15}N$. In order to determine which set of samples should be used, the same samples from August 2006 were run again in May 2007.

After obtaining the set of results from Earth Sciences and comparing the intra-sample results (results for the same samples) of those from August 2006, December 2006, and May 2007, it became clear that the differences between the $\delta^{13}C$ and $\delta^{15}N$ results for the three sets of data were not within an acceptable error range. When looking at the three sets of samples, the means for $\delta^{13}C$ and $\delta^{15}N$ were different and, when looking at them more closely, it became clear that the mean differences of the $\delta^{13}C$ values did not fall within two standard errors of the mean for August-December and August-May comparisons and that the mean differences of the $\delta^{15}N$ values didn't fall within two standard errors of the mean

for the December-May comparisons. There was also an issue with the calibration of the mass spectrometer that produced the December 2006 results which meant that the majority of the samples appeared to fall outside the acceptable C/N ratio range of 2.9-3.6. Although this calibration error was later corrected with a 'correction factor' it still left doubt as to whether the results from December 2006 were reliable. In order to test this, a small set of samples were sent to an independent laboratory (lab), IsoAnalytical, in June 2007 so that the results could be compared to all the previous sets of results for those samples in order that a decision could be made regarding which could be considered valid data.

When comparing the independently-run samples to those obtained in August 2006 and December 2006, the mean differences were found to be more than two standard errors from zero in both $\delta^{13}C$ and $\delta^{15}N$, meaning that they were very different from both of these sets of Durham results. Differences between the IsoAnalytical results and those from May 2007 were within two standard errors of zero for both $\delta^{13}C$ and $\delta^{15}N$ so these results were considered similar. Unfortunately, the May 2007 results were derived from only a small group of samples for one site so the rest of the samples still had to be analysed again. After some re-prep of samples that no longer had sufficient collagen remaining, all of the collagen samples were sent to Earth Sciences at Durham to be run under the same conditions as the May 2007 samples. Frustratingly, the elemental analyser in Earth Sciences broke down in August of 2007 and so another lab that could run the samples was sought. Eventually, all the samples were sent to the Alaska Stable Isotope Facility at the University of Alaska, Fairbanks to be run in December 2007.

Of course, due to the differences found between the earlier sets of Durham University Earth Sciences samples, it was thought wise to test the consistency of the Alaska results against the other independent lab, and against the Durham data. When comparing the mean differences of $\delta^{13}C$ and $\delta^{15}N$ to that for the August 2006 samples, the mean difference of the $\delta^{13}C$ values were found to be more than two standard errors from zero and, when comparing them to data from December 2006, the mean differences in both $\delta^{13}C$ and $\delta^{15}N$ were found to be more than two standard errors from zero. There was also a difference in $\delta^{15}N$ between the

Alaska and May 2007 results but only just (the $\delta^{15}N$ mean difference was 2.3 standard errors from zero) when comparing Alaska with the other independent lab, IsoAnalytical. Therefore, it was deemed that the set of results from Alaska run in December 2007 were reliable and similar to those from IsoAnalytical in June 2007. The sets of data from all five runs were also plotted against each other for both carbon and nitrogen in order to make a visual comparison of the results (see Figures 1 to 8, below). Examination of these visual comparisons makes it easy to see that the June 2007 and December 2006 runs were quite dissimilar, that the June 2007 and May 2007 runs were more similar, and that the June 2007 and December 2007 runs were even more similar still. The December 2006 and December 2007 runs appear to be very dissimilar. This pattern is confirmed when standard collagen results are added, which as a standard should produce identical results every time. For the June 2007 and December 2007 runs it falls on the 1:1 plot line for both carbon and nitrogen, meaning that these sets of results are very similar.

In summary, the data from May 2007 were deemed to be the most reliable of the three sets of results from Earth Sciences, as confirmed by the data from the independent lab (IsoAnalytical) in June 2007. As these were only a small subset of the results, the entire batch of samples was sent to be analysed in Alaska in December 2007. When compared to previous sets of results, the December 2007 results were found to be very similar to those of June 2007 from IsoAnalytical and were therefore deemed to be valid results.

Lab collagen is represented by an X shaped marker in all plots

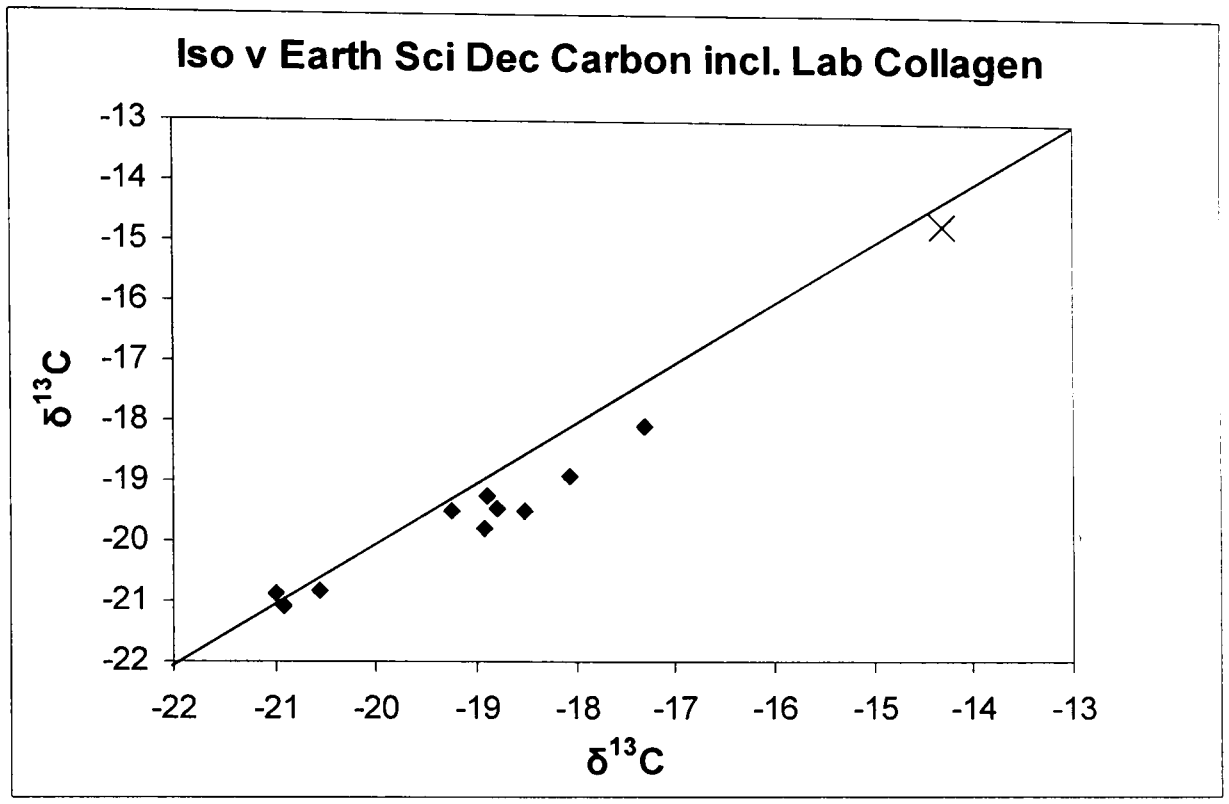


Figure 4. 1:1 Plot of IsoAnalytical and Earth Sciences December Data for Carbon

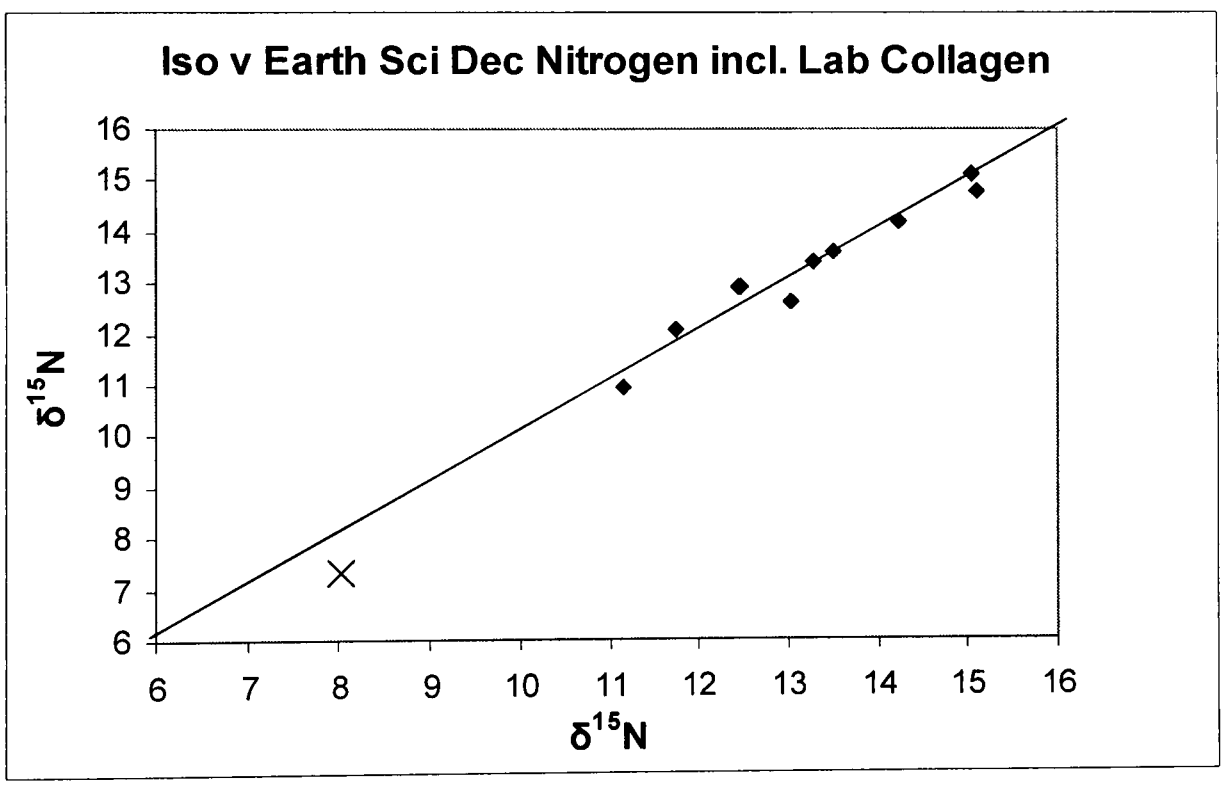


Figure 5. 1:1 Plot of IsoAnalytical and Earth Sciences December Data for Nitrogen

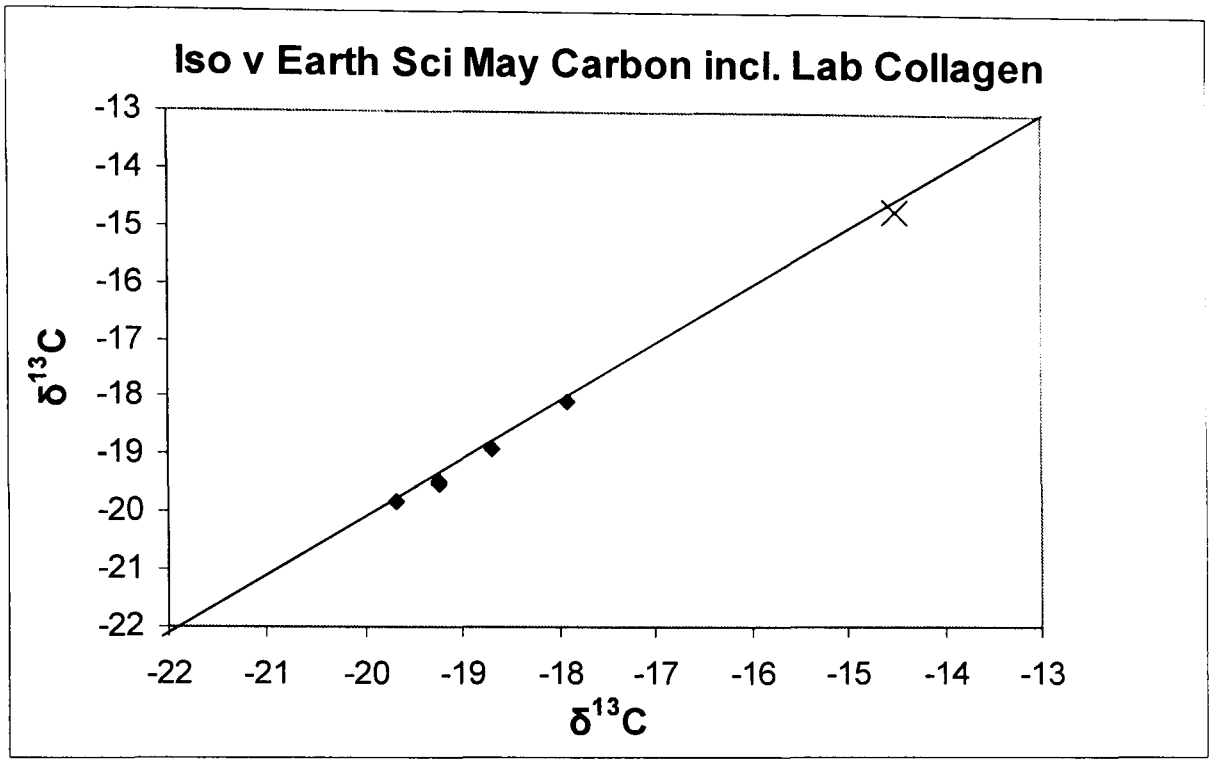


Figure 6. 1:1 Plot of Iso-Analytical and Earth Sciences May Data for Carbon

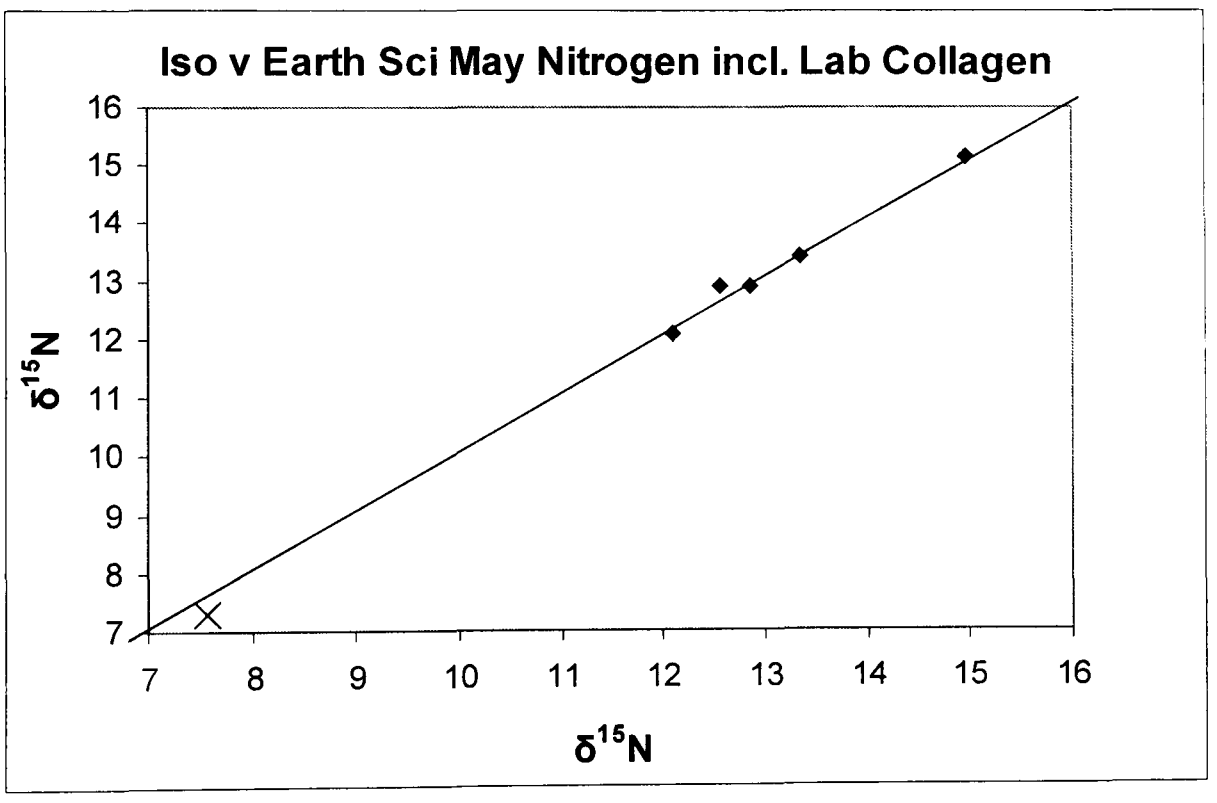


Figure 7. 1:1 Plot of IsoAnalytical and Earth Sciences May Data for Nitrogen

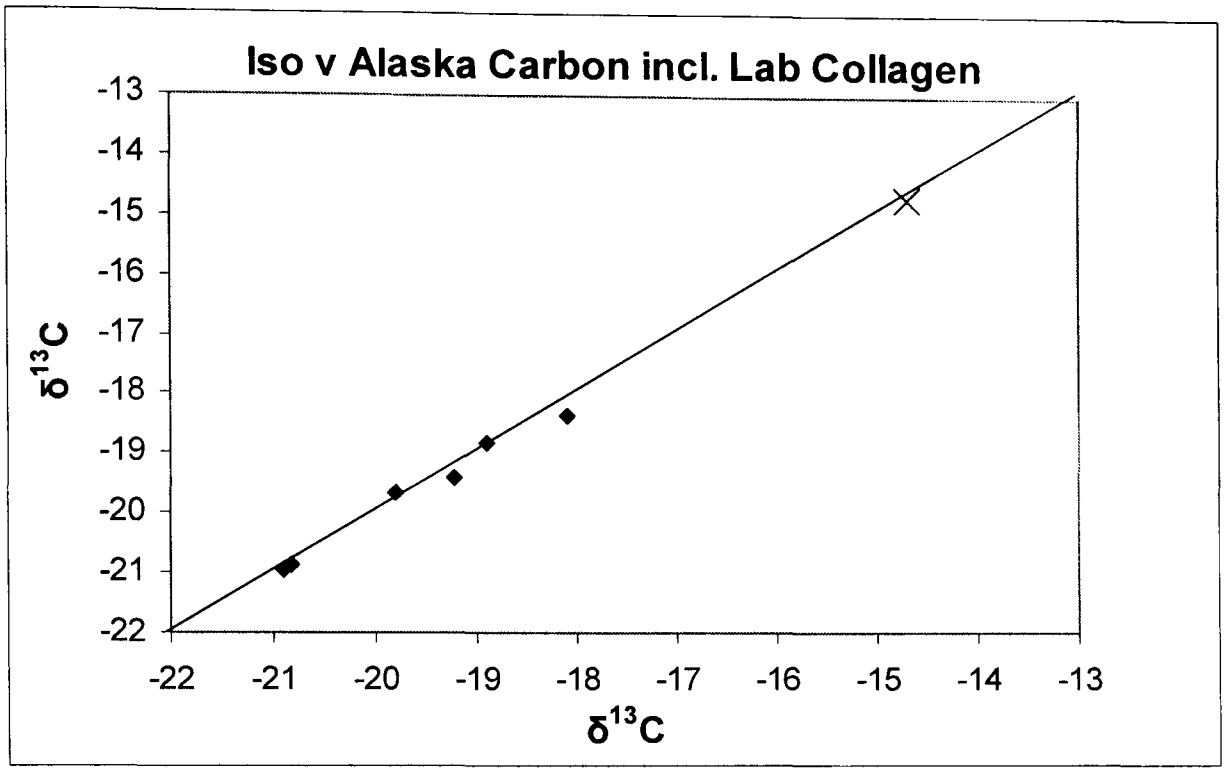


Figure 8. 1:1 Plot of IsoAnalytical and Alaska Data for Carbon

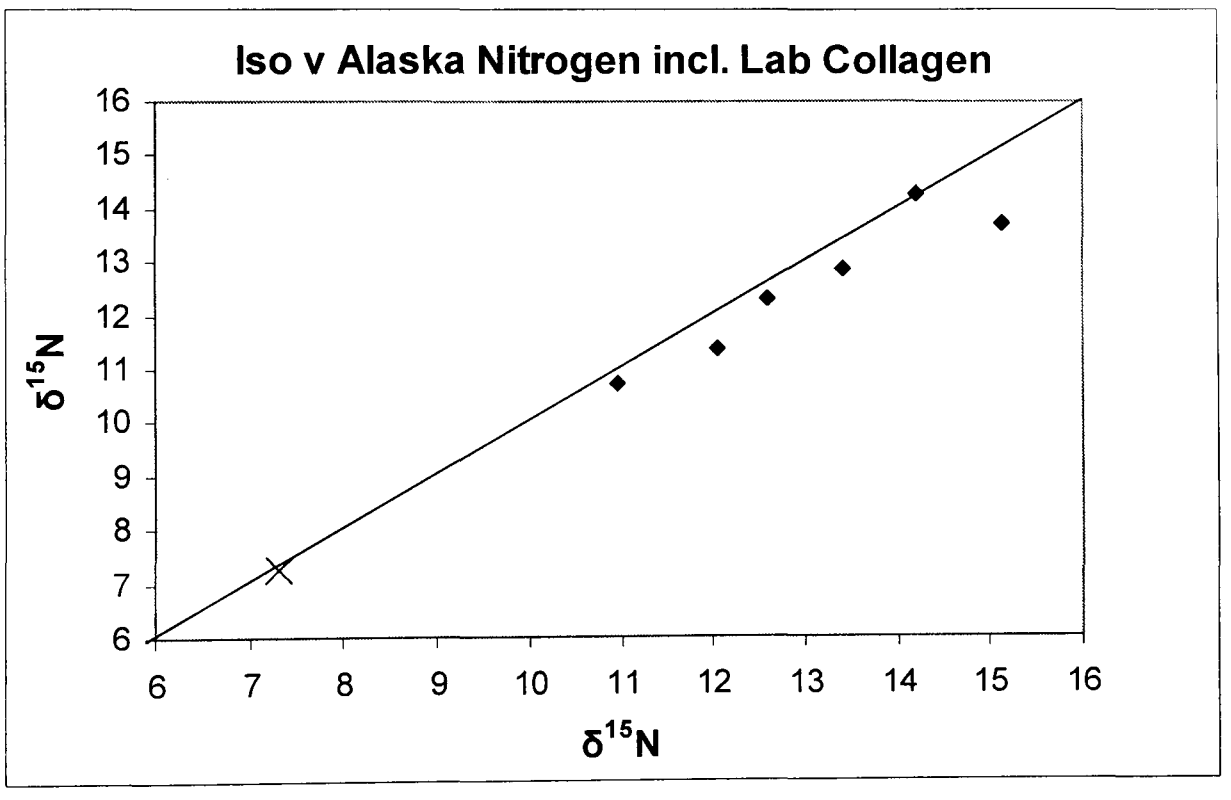


Figure 9. 1:1 Plot of IsoAnalytical and Alaska Data for Nitrogen

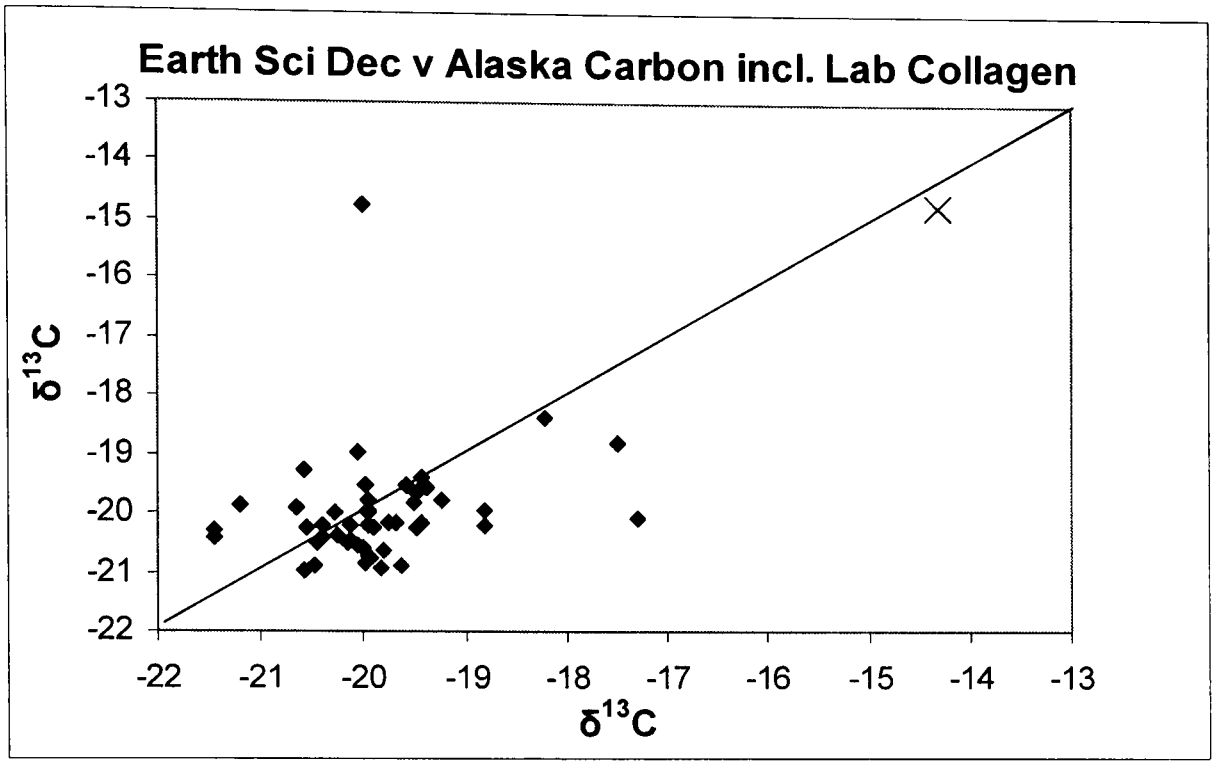


Figure 10. 1:1 Plot of Earth Sciences December and Alaska Data for Carbon

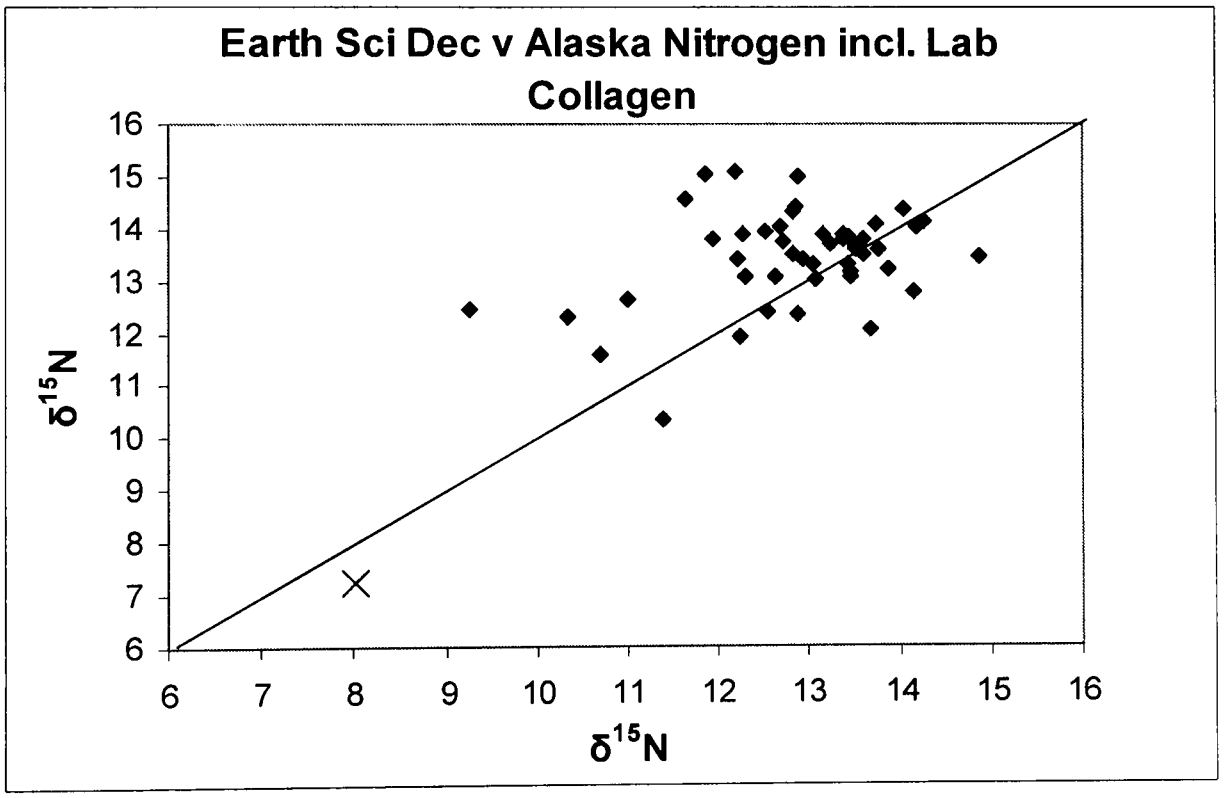


Figure 11. 1:1 Plot of Earth Sciences December and Alaska Data for Nitrogen

8.2.2 University of Alaska Stable Isotope Results

The carbon and nitrogen stable isotope analysis was carried out at the University of Alaska, Fairbanks in the Alaska Stable Isotope Facility in December of 2007. The preparation for the carbon and nitrogen analysis was originally carried out in Durham in January to April 2006, with some re-prep in July 2007 of 33 samples that had no collagen left, or that had C/N ratios outside the acceptable range of 2.9-3.6 (see below for discussion of C/N ratios). In total, 93 rib samples were available for collagen preparation and, of these, 83 samples were sent to Alaska for analysis. The collagen yields ranged from 0.72% to 18.3% with the poorer collagen samples appearing very brittle and dark brown in colour. Several samples did not produce enough collagen. Samples 19/91 122, IAS4801 1834, MIN86 LG 12349, MIN86 LG 12480, MPY86 623, MPY86 2376, MPY86 2748, MPY 86 4282, and YFH 159 did not produce enough collagen, and sample MPY86 3667 did not have enough remaining bone to re-attempt collagen extraction. All the prepared collagen samples were analysed using continuous-flow isotope ratio mass spectrometry (CFIRMS) in the Alaska Stable Isotope Facility at the University of Alaska Fairbanks's Water & Environmental Research Center. Each sample was placed in a tin capsule and weighed between 0.25mg and 0.36mg, the average sample weight being 0.33mg. Controls of pure nylon and collagen were also analysed with each sample run to ensure consistency in the results of the isotope analysis. To determine consistency amongst the samples, two collagen samples were weighed out and run for each skeleton, but in separate batches, so that the results could be compared between samples and also between batches. The error between measurements (standard error) is 0.09‰ for $\delta^{13}C$ and 0.15‰ for $\delta^{15}N$ and machine precision is <0.2‰.

The two key parts of information gleaned from the isotopic results were the $\delta^{13}C$ and $\delta^{15}N$ values, and the C/N ratios. The carbon and nitrogen isotope results from each pair of samples were averaged to obtain a single isotopic value for carbon and nitrogen for each skeleton. The C/N ratios were converted from mass ratios into atomic ratios and examined to see if they fell within the accepted range for collagen, which is between 2.9 and 3.6, according to DeNiro (1985) or

between 2.6 and 3.4, according to Schoeninger *et al.* (1989). The larger combined range of 2.6 to 3.6 was chosen for these samples and any data that fell outside of this range were discarded. Ultimately, only the data for 46 samples could be used, the rest being discarded because the average of their C/N ratios fell outside the accepted range, because the samples produced no collagen, or because there was not enough bone remaining to attempt a collagen re-prep. There were equal numbers of samples affected/unaffected by DISH (23 in each category); 40 males and 6 females; 25 monastic samples and 21 non-monastic. The mean for all samples was -20.0‰ for carbon (+/-0.61‰ 1σ) and 13.1‰ for nitrogen (+/-1.00‰ 1σ).

In order to help interpret the data, the results of the isotope analysis were plotted on a series of graphs comparing data both within and between sites. These values were also compared between different groups using Mann-Whitney statistical tests to confirm whether or not the results found were significant. Female samples were included in the individual sites analyses.

8.2.2.1 Individual Sites

8.2.2.1.1 Monastic Sites:

19/91 Blackfriars Friary, Gloucester Samples

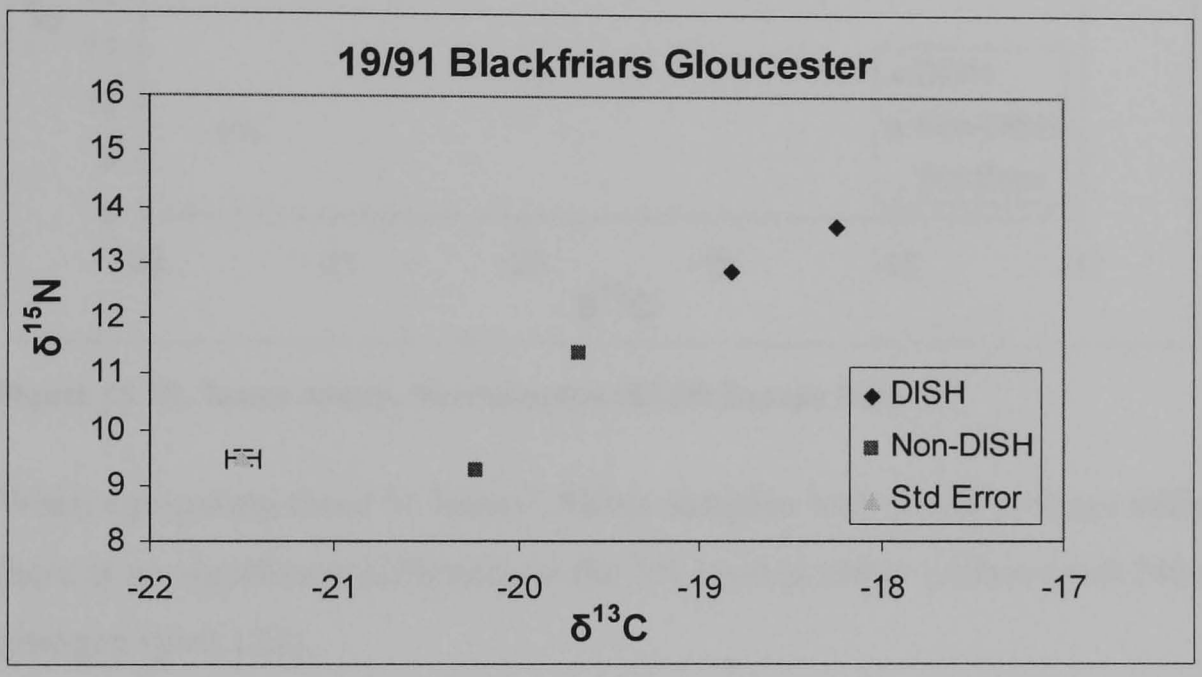


Figure 12. Blackfriars Friary, Gloucester (19/91) Isotope Data.

Even though there appear to be two separate groups in the scatterplot, when comparing those Gloucester Blackfriars samples with DISH to those without, there is no significant difference at the 5% level in either carbon (p=0.333) or nitrogen (p=0.333).

EL00 St James' Abbey Samples

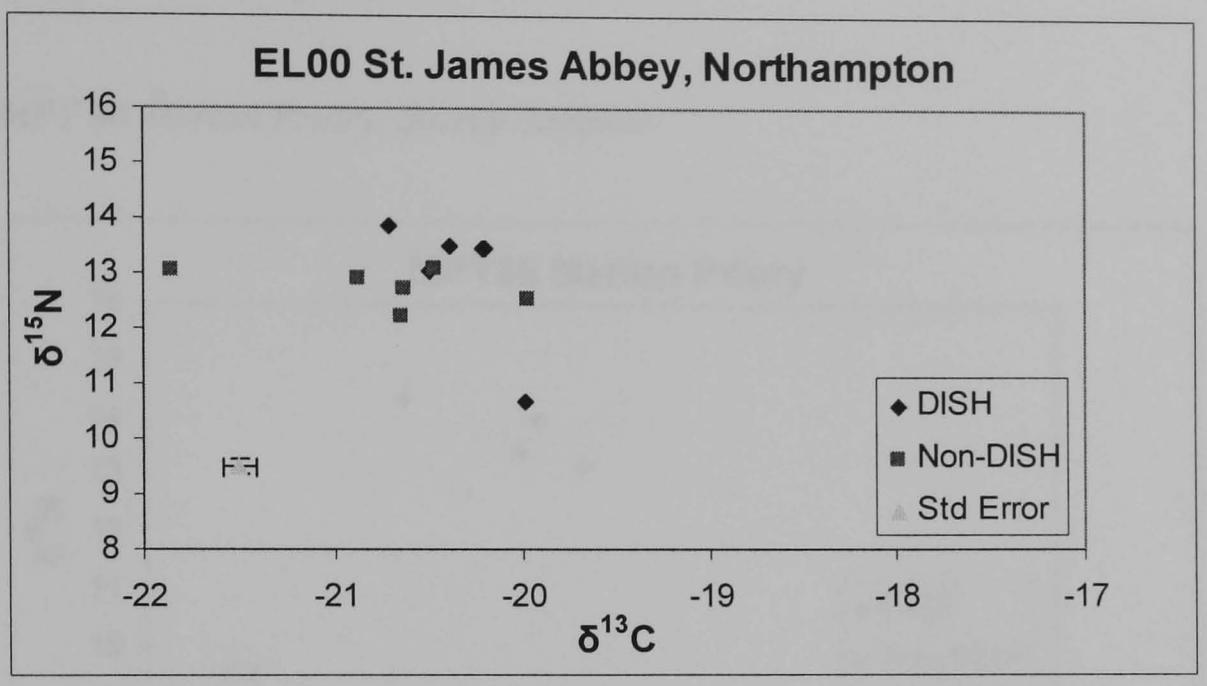


Figure 13. St. James Abbey, Northampton (EL00) Isotope Data

When comparing those St James' Abbey samples with DISH to those without, there is no significant difference at the 5% level in either carbon (p=0.240) or nitrogen (p=0.132).

MIN 86 LG Royal Mint Late Graves, London Samples

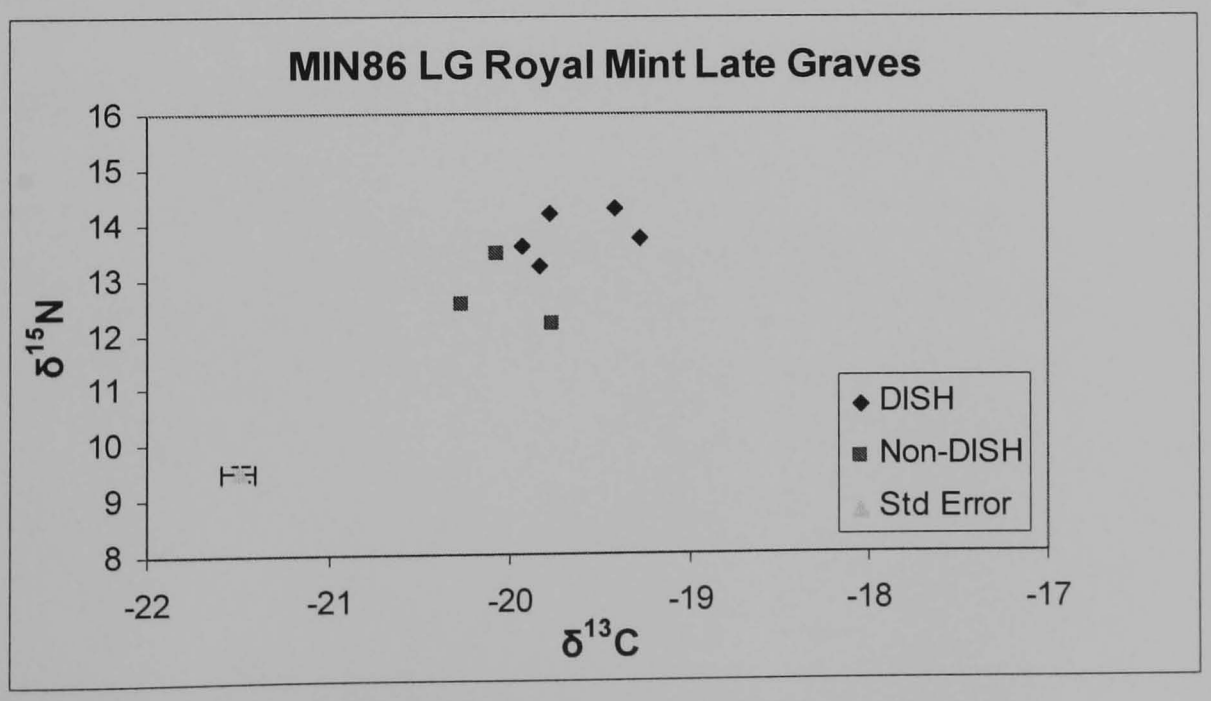


Figure 14. Royal Mint Late Graves, London (MIN86 LG) Isotope Data.

When comparing those Royal Mint Late Graves samples with DISH to those without, there is no significant difference at the 5% level in either carbon (p=0.250) or nitrogen (p=0.071).

MPY 86 Merton Priory, Surrey Samples

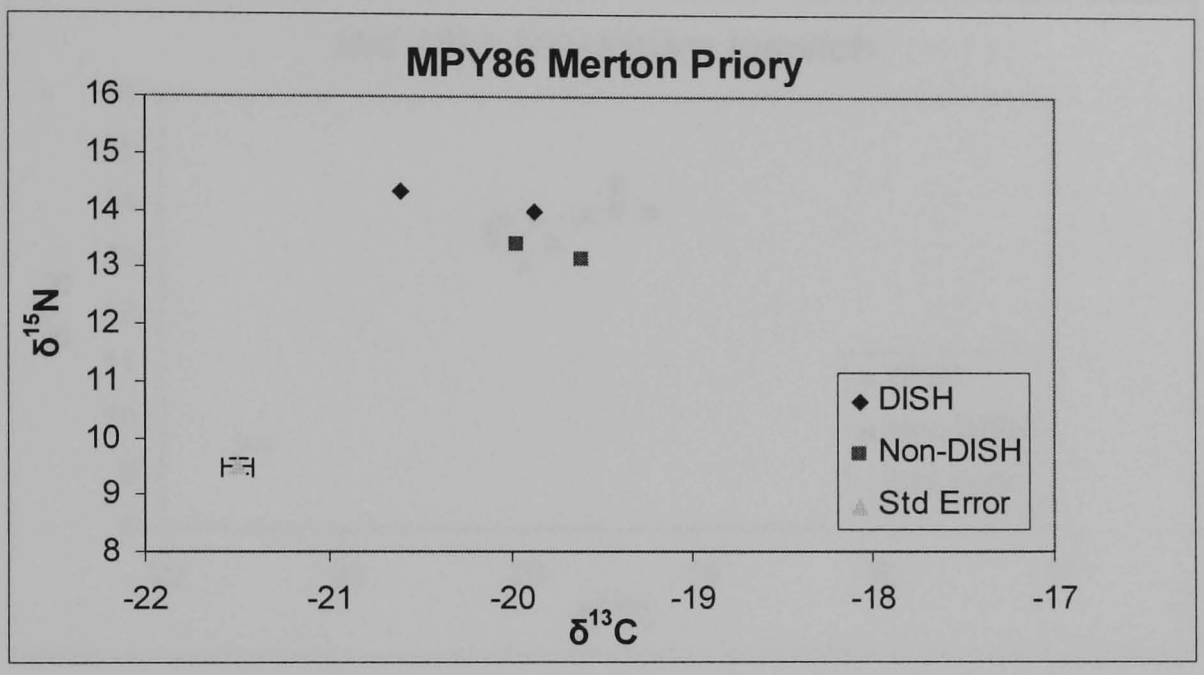


Figure 15. Merton Priory, Surrey (MPY86) Isotope Data.

When comparing those Merton Priory samples with DISH to those without, there is no significant difference at the 5% level in either carbon (p=0.667) or nitrogen (p=0.333).

8.2.2.1.2 Non-Monastic Sites:

IAS 4801 Blackfriars Friary, Ipswich Samples

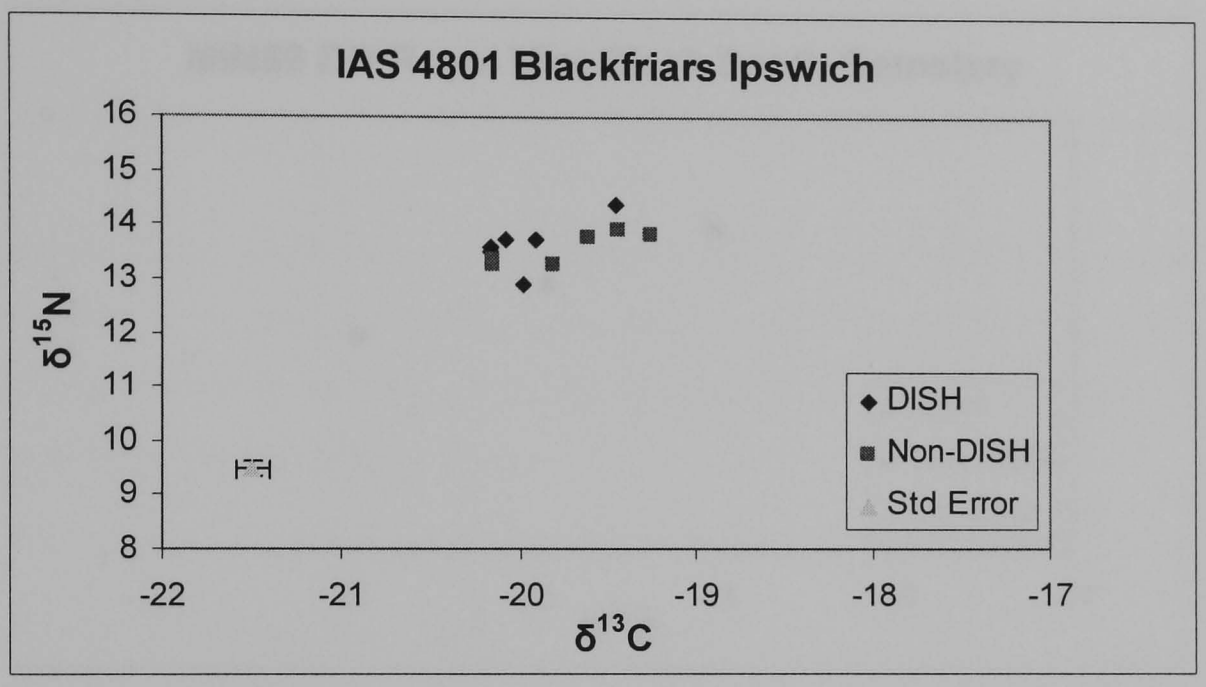


Figure 16. Blackfriars Friary, Ipswich (IAS 4801) Isotope Data.

When comparing those Ipswich Blackfriars samples with DISH to those without, there is no significant difference at the 5% level in either carbon (p=0.240) or nitrogen (p=1.00).

He93A Hereford Cathedral, Hereford Samples

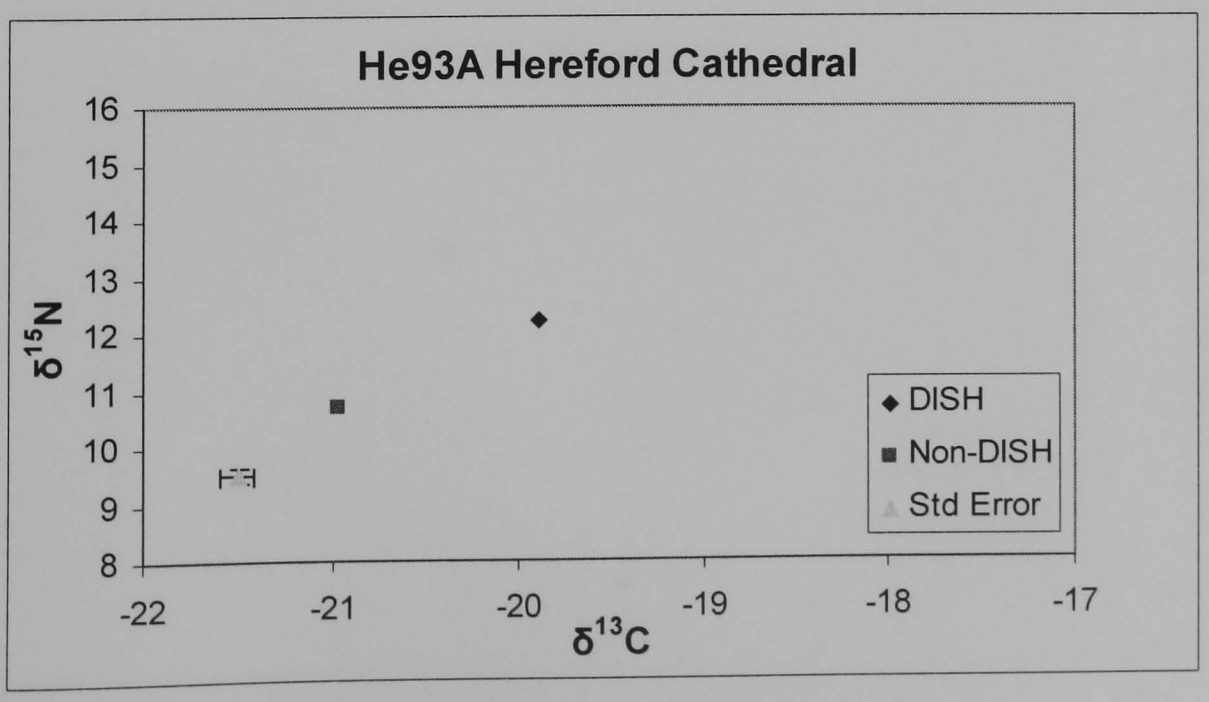


Figure 17. Hereford Cathedral, Hereford (He93A) Isotope Data

As there are only two samples from Hereford Cathedral, statistical analysis cannot be applied.

MIN 86 BD Royal Mint Black Death Cemetery, London Samples

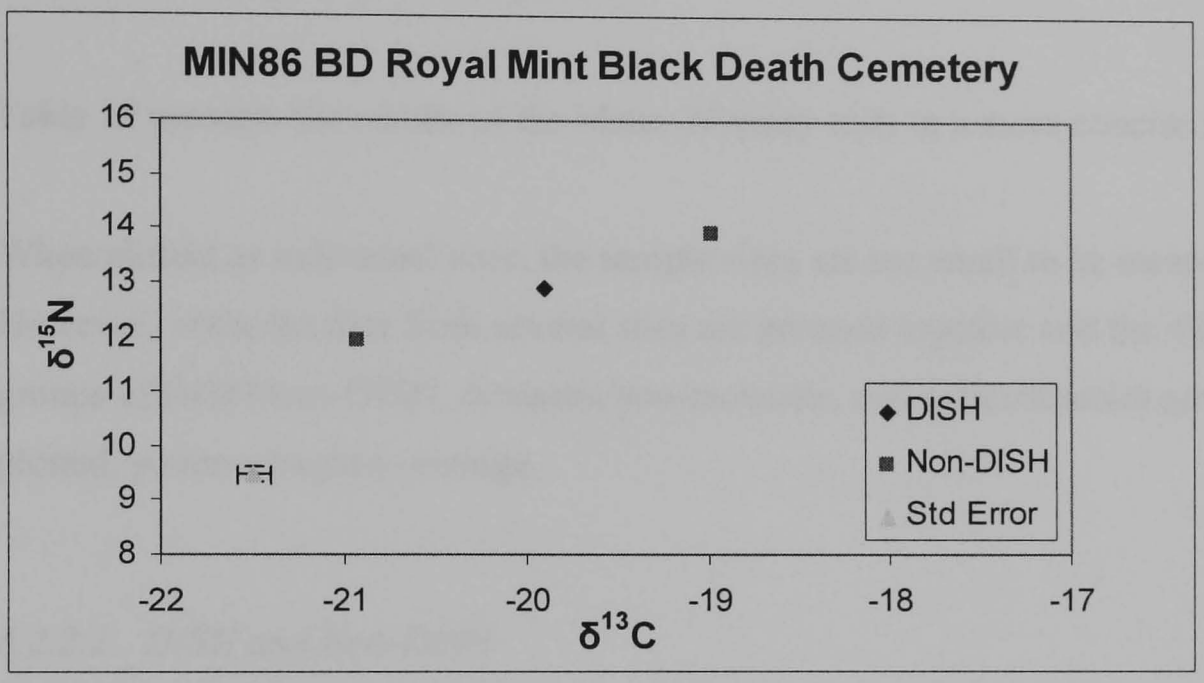


Figure 18. Royal Mint Black Death Cemetery, London (MIN86 BD) Isotope Data

When comparing the Royal Mint Black Death sample with DISH to those without, there is no statistically significant difference at the 5% level in either carbon (p=1.00) or nitrogen (p=1.00).

YFH Fishergate House, York Samples

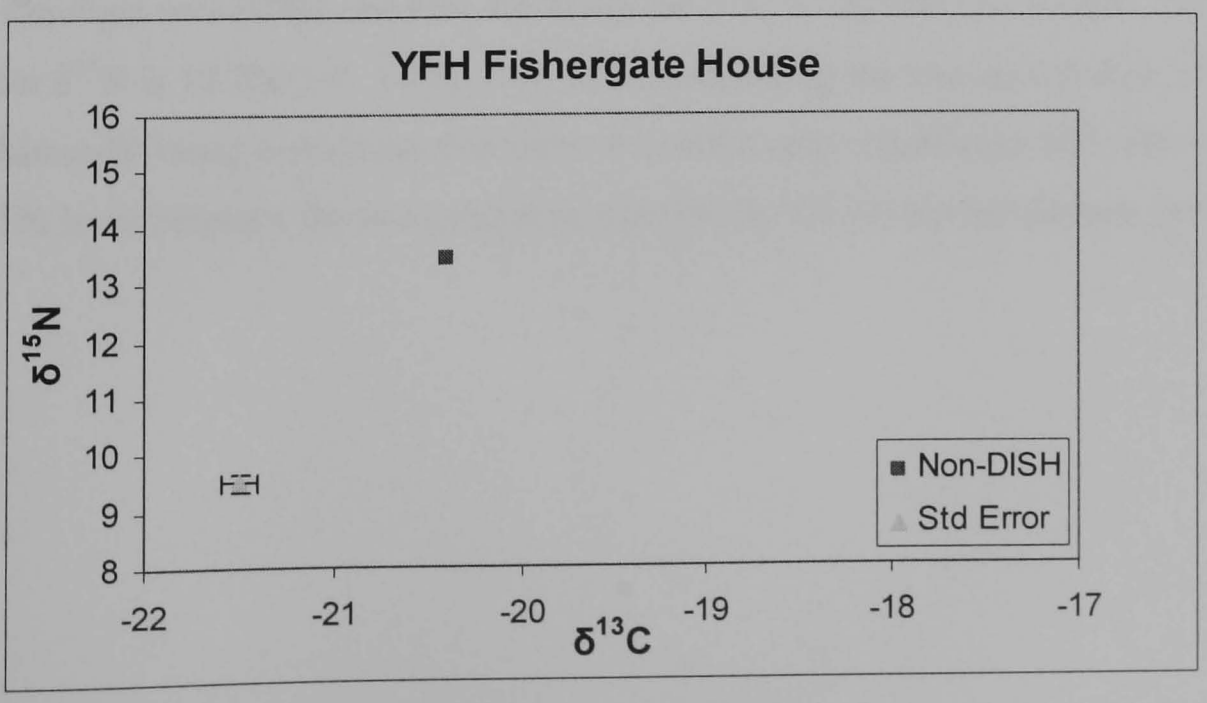


Figure 19. Fishergate House, York (YFH) Isotope Data

As there is only one sample from Fishergate House, statistical analysis cannot be applied.

The Hospital of St. James and Mary Magdalene did not produce any usable carbon and nitrogen stable isotope data.

Table 19 presents the results of the Mann-Whitney tests in a more concise form.

When plotted as individual sites, the sample sizes are too small to be meaningful. However, when the data from several sites are grouped together and the different groups of DISH/non-DISH, monastic/non-monastic, and males/females are plotted, patterns begin to emerge.

8.2.2.2 DISH and Non-DISH

There were 23 measurements for those individuals with DISH and 23 for those without. For DISH samples, these data range from approximately -20.7‰ to -18.2‰ for carbon and from 10.7‰ to 14.4‰ for nitrogen. For non-DISH samples, these data range from approximately -21.8‰ to -19.0‰ for carbon and 9.3‰ to 13.9‰ for nitrogen. Amongst DISH samples, the mean for $\delta^{13}C$ is -19.9‰ (+/- 0.56‰, 1σ) and the mean for $\delta^{15}N$ is 13.4‰ (+/- 0.79‰, 1σ). Amongst non-DISH samples, the mean for $\delta^{13}C$ is -20.1‰ (+/- 0.64‰, 1σ) and for $\delta^{15}N$ is 12.7‰ (+/- 1.1‰, 1σ). When comparing the two sets of data, the Mann-Whitney test shows that there is a statistically significant difference at the 5% level between the two groups in nitrogen ($p=0.012$) but not carbon ($p=0.258$).

Skeletons with and without DISH

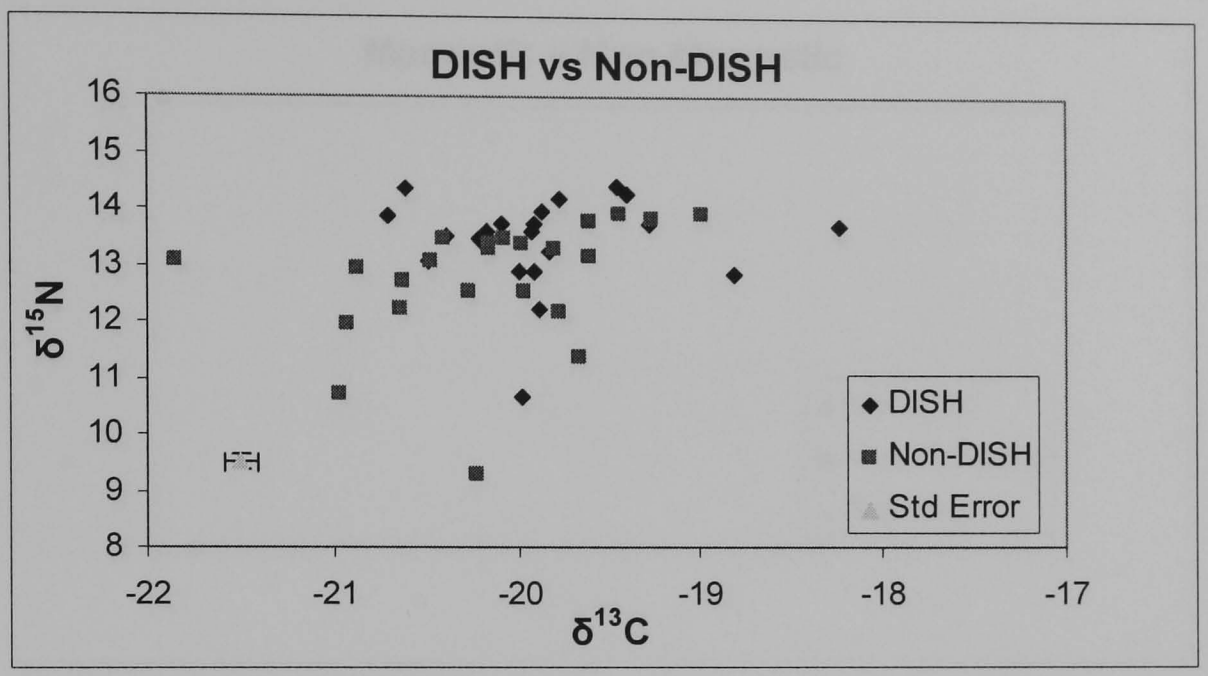


Figure 20. DISH and Non-DISH Samples.

8.2.2.3 Monastic and Non-Monastic Groups

There are carbon and nitrogen data from 25 monastic and 21 non-monastic samples. When plotted individually, the monastic data range from -21.8‰ to -18.2‰ for carbon and 9.2‰ to 14.3‰ for nitrogen, with means of -20.4‰ (+/- 0.62‰, 1σ) and 13.2‰ (+/- 0.96‰, 1σ), respectively. The non-monastic data range from -20.9‰ to -18.9‰ for carbon and 10.7‰ to 14.4‰ for nitrogen, with means of -20.0‰ (+/- 0.59‰, 1σ) and 12.6‰ (+/- 1.1‰, 1σ), respectively. When plotted together the two groups overlap but the small sample sizes of both groups make it difficult to determine if this is meaningful. The results of the Mann-Whitney test show that there is no significant difference at the 5% level between the two groups in either carbon ($p=0.385$) or nitrogen ($p=0.474$).

Monastic and Non-monastic Skeletons

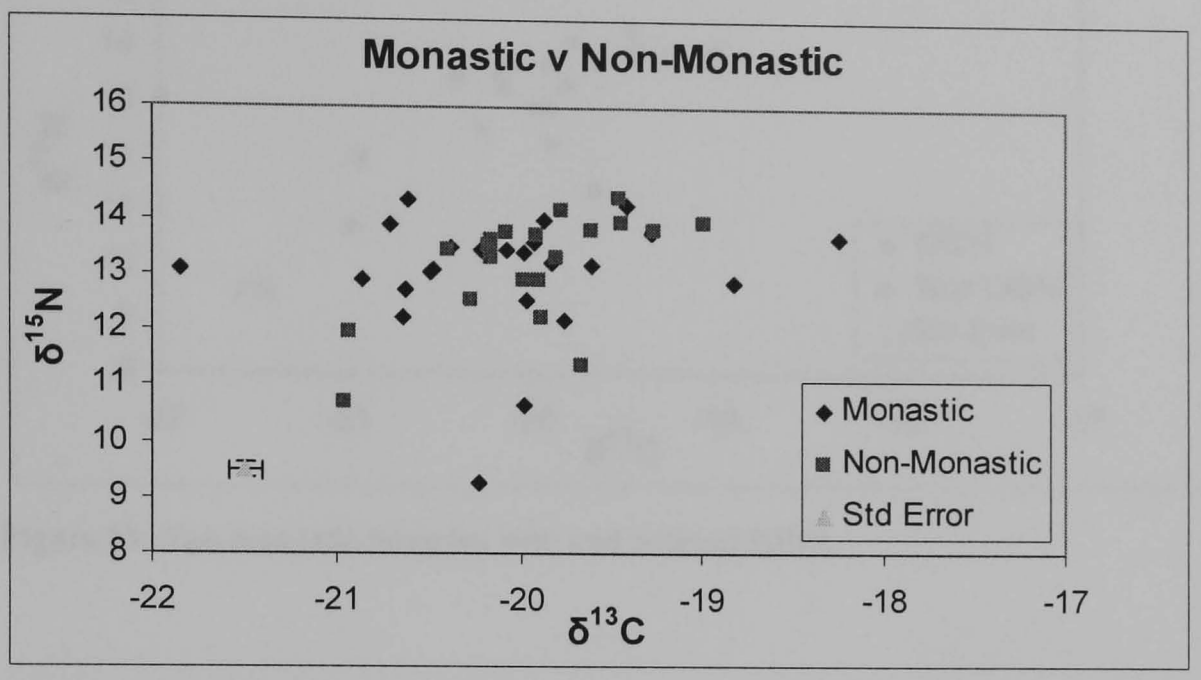


Figure 21. Monastic and Non-monastic Samples.

When comparing monastic samples with DISH to monastic samples without DISH there is a statistically significant difference at the 5% level in nitrogen ($p=0.003$) but not carbon ($p=0.134$).

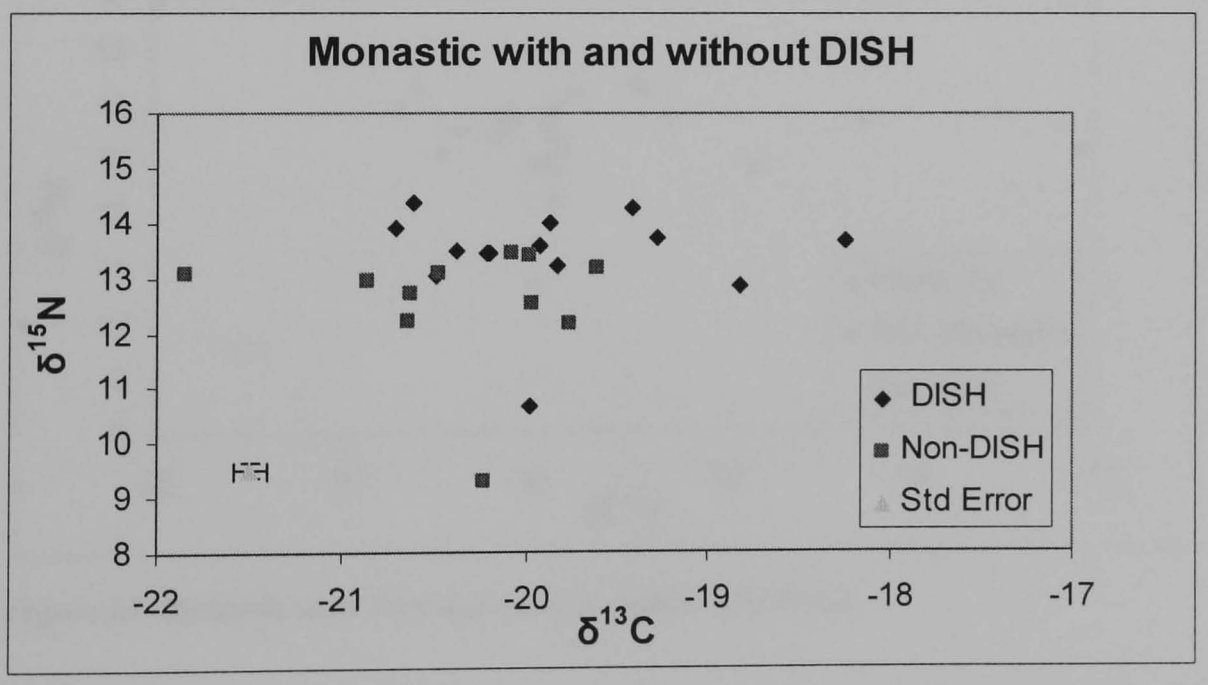


Figure 22. Monastic Samples with and without DISH.

When comparing non-monastic samples with DISH to non-monastic samples without DISH there is no significant difference at the 5% level in either carbon ($p=1.000$) or nitrogen ($p=0.382$).

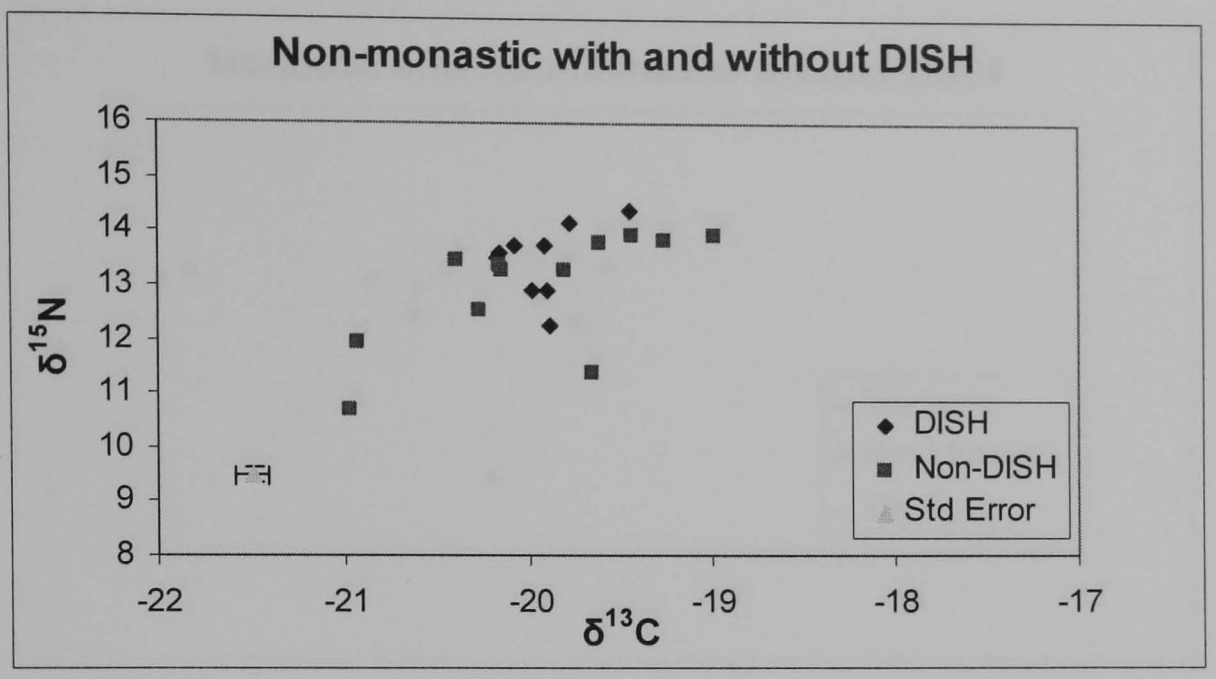


Figure 23. Non-monastic Samples with and without DISH.

When comparing monastic skeletons with DISH to non-monastic skeleton with DISH, there is no significant difference at the 5% level in either carbon (p=0.781) or nitrogen (p=0.975).

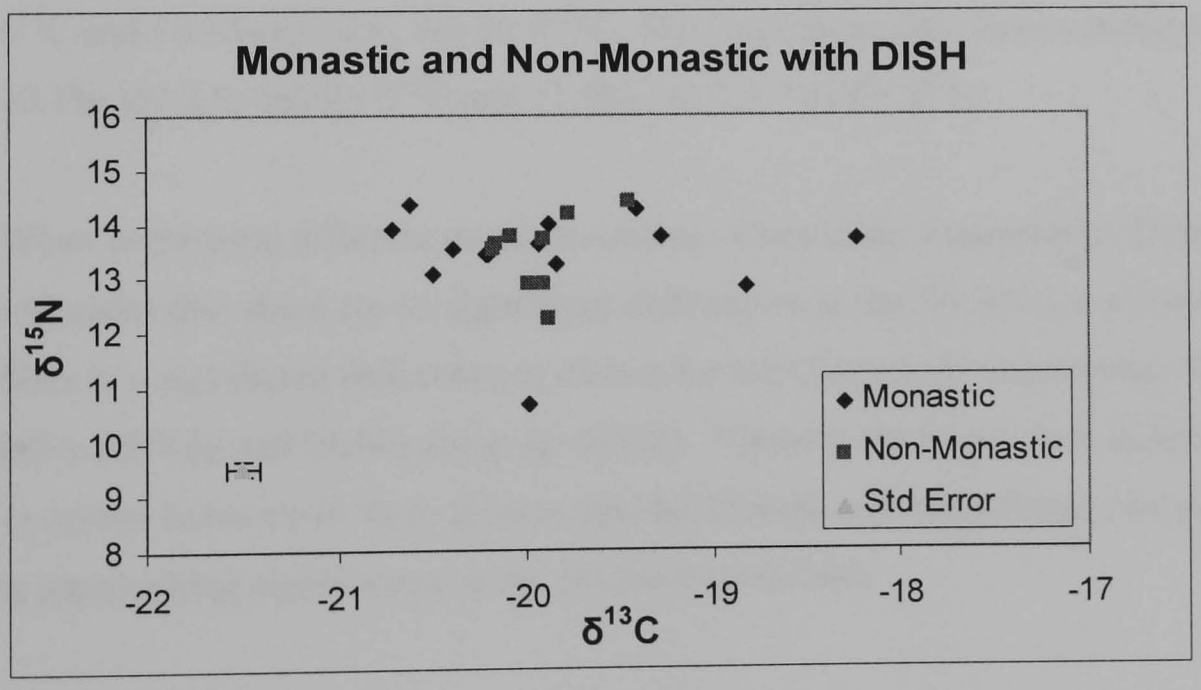


Figure 24. Monastic and Non-monastic Samples with DISH.

When comparing monastic skeletons without DISH to non-monastic skeletons without DISH, there is no significant difference at the 5% level in either carbon (p=0.235) or nitrogen (p=0.190).

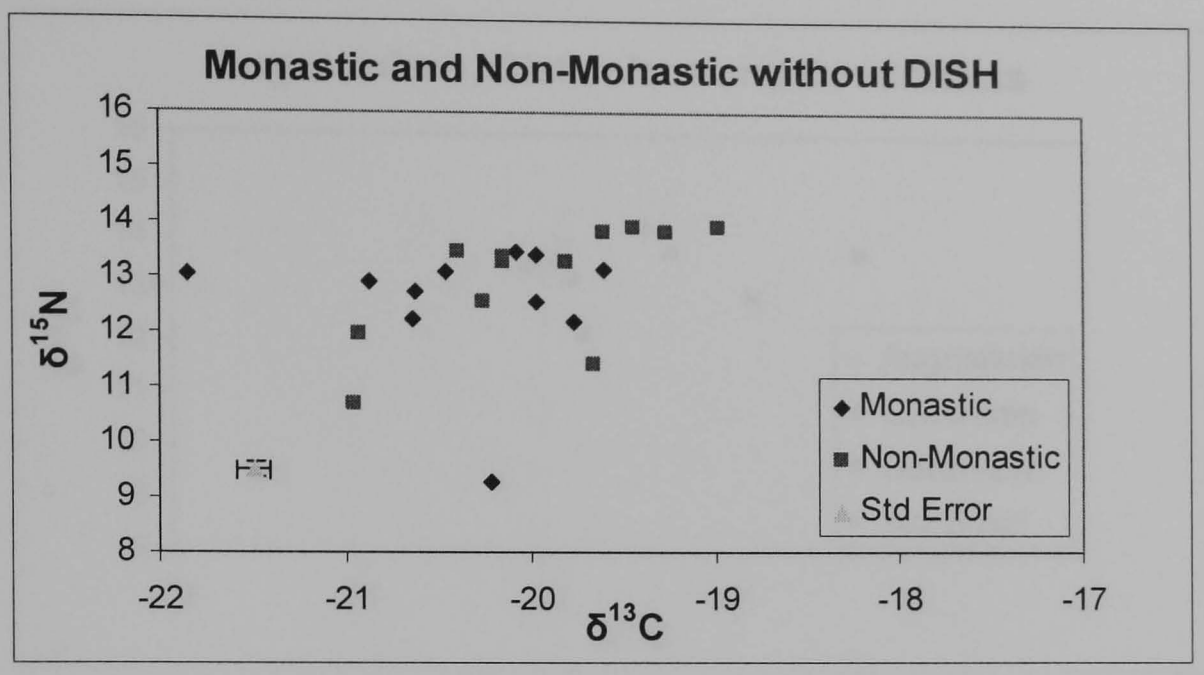


Figure 25. Monastic and Non-monastic without DISH.

8.2.2.4 Monastic Orders

The Cistercian data have a mean of -19.7‰ (+/- 0.3, 1σ) for $\delta^{13}C$ and 13.4‰ (+/- 0.6, 1σ) for $\delta^{15}N$. The Augustinian data have a mean of -20.4‰ (+/-0.5, 1σ) for $\delta^{13}C$ and 13.1‰ (+/-0.8, 1σ) for $\delta^{15}N$. The Dominican data have a mean of -19.1‰ (+/-1.0, 1σ) for $\delta^{13}C$ and 11.9‰ (+/-2.3, 1σ) for $\delta^{15}N$.

When comparing different monastic orders - Cistercian, Augustinian, Dominican - to each other there are no significant differences at the 5% level in nitrogen but there is a significant difference in carbon for the Cistercian/Augustinian (MIN86 LG v MPY86 and EL00) group (p=0.002). Visually, there are clear differences in carbon between all three groups, and the Dominican/Augustinian comparison is approaching significance at the 5% level (p=0.064).

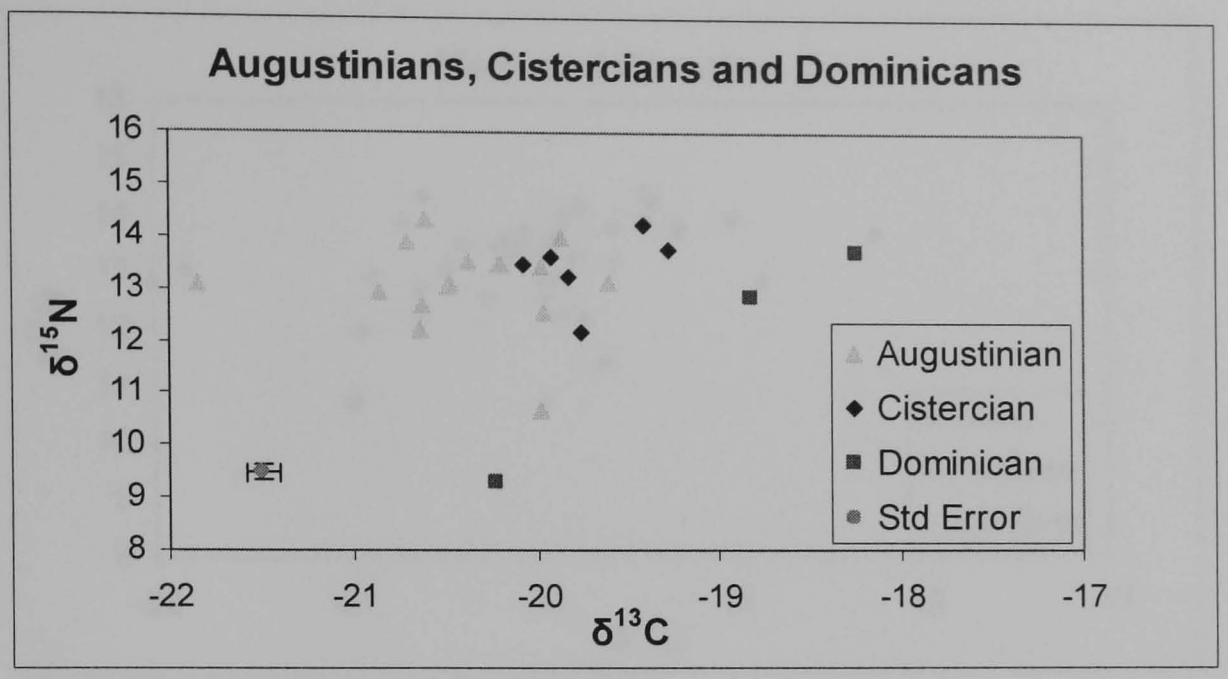


Figure 26. Augustinian, Cistercian and Dominican Isotope Data.

8.2.2.5 Males and Females

There are carbon and nitrogen isotope data for 40 males and 6 females. For males, these data range from -21.0‰ to -18.2‰ for carbon with a mean of -19.9‰ (+/-0.6‰, 1σ) and 9.2‰ to 14.3‰ for nitrogen with a mean of 13.2‰ (+/-0.9‰, 1σ). For females, these data range from -20.9‰ to -19.6‰ for carbon with a mean of -20.0‰ (+/-0.5‰, 1σ) and 10.7‰ to 14.1‰ for nitrogen with a mean of 12.6‰ (+/-1.3‰, 1σ). When comparing the two sets of results visually, there does not appear to be any difference between the two groups but the sample size for the female group is small. The Mann-Whitney test shows no significant difference at the 5% level in either carbon (p=0.379) or nitrogen (p=0.453).

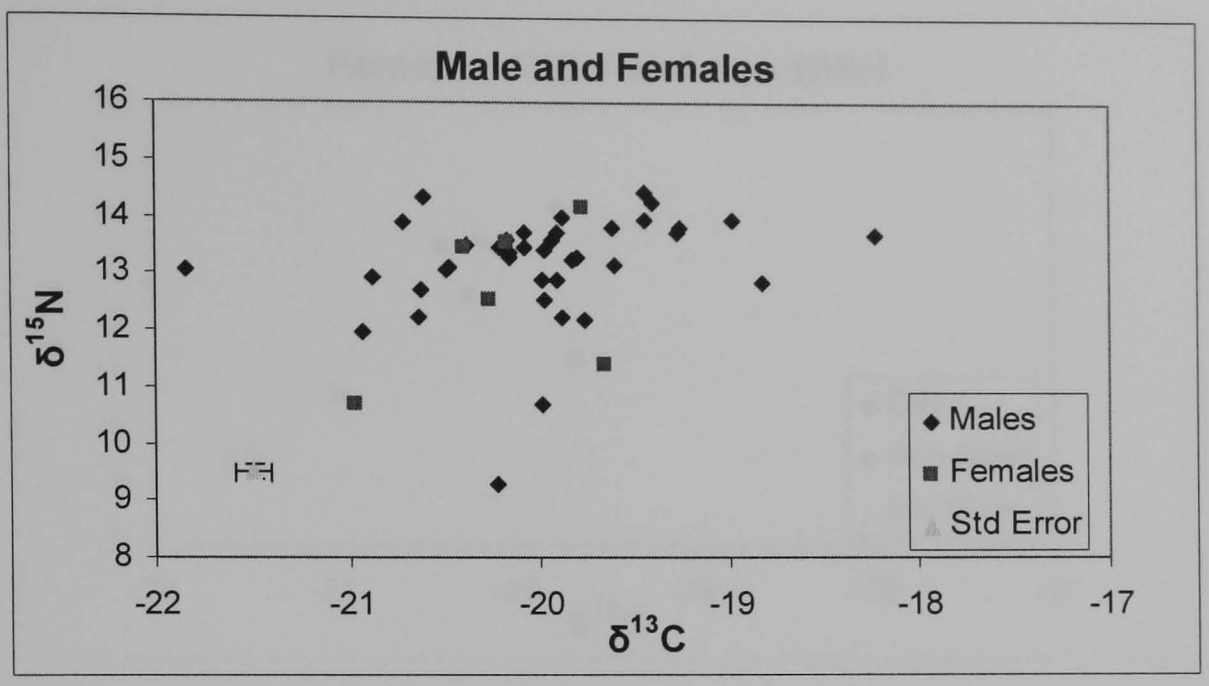


Figure 27. Male and Female Isotope Data.

When comparing males with DISH to males without DISH there is no significant difference at the 5% level in either carbon (p=0.436) or nitrogen (p=0.074).

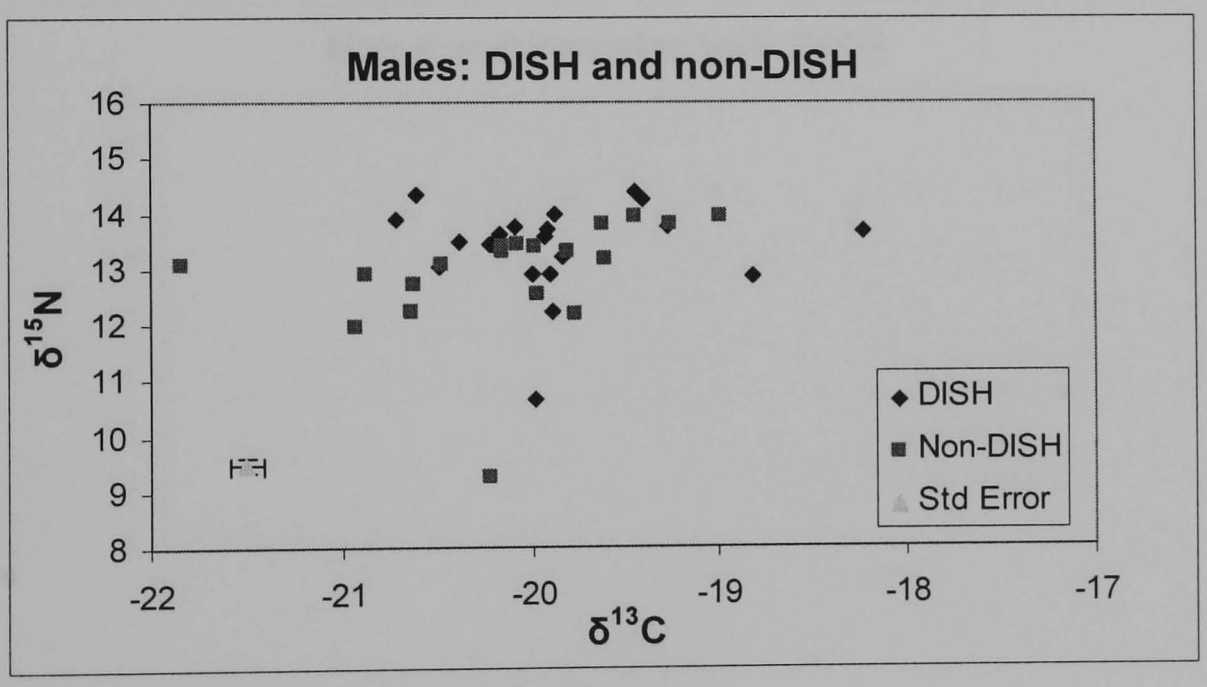


Figure 28. Males with and without DISH.

When comparing females with DISH to females without DISH, there is no significant difference at the 5% level in either carbon (p=0.533) or nitrogen (p=0.133).

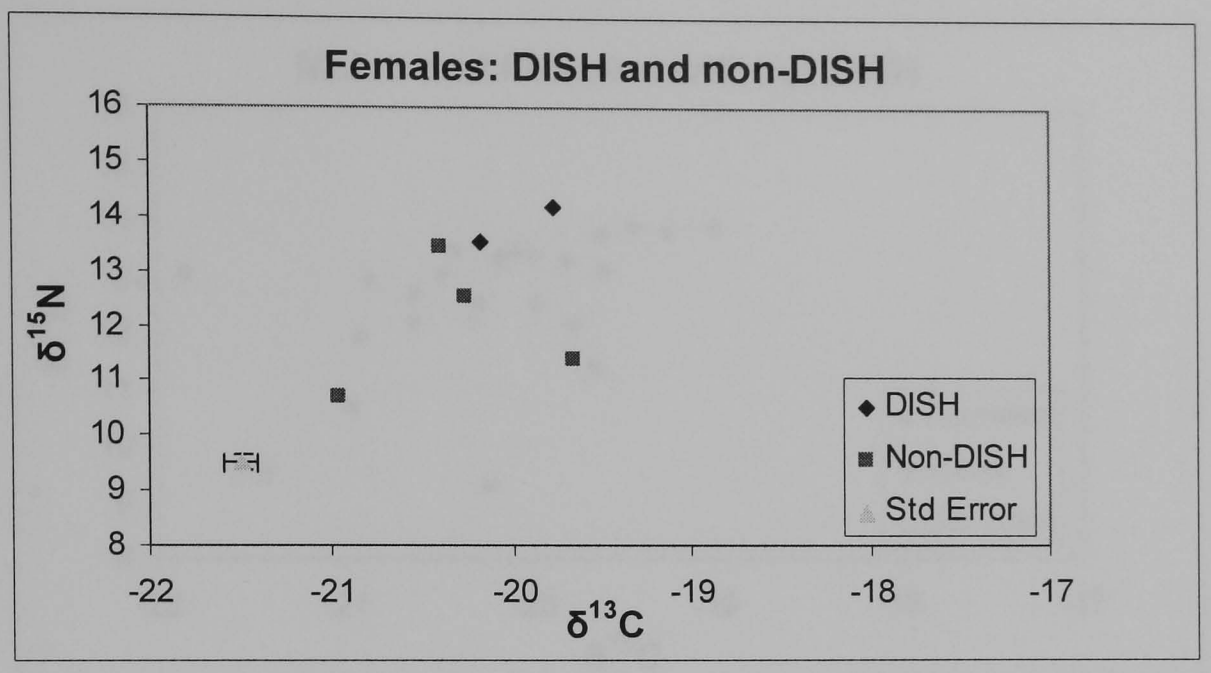


Figure 29. Females with and without DISH.

When comparing males with DISH to females with DISH, there is no significant difference at the 5% level in either carbon (p=0.957) or nitrogen (p=0.506).

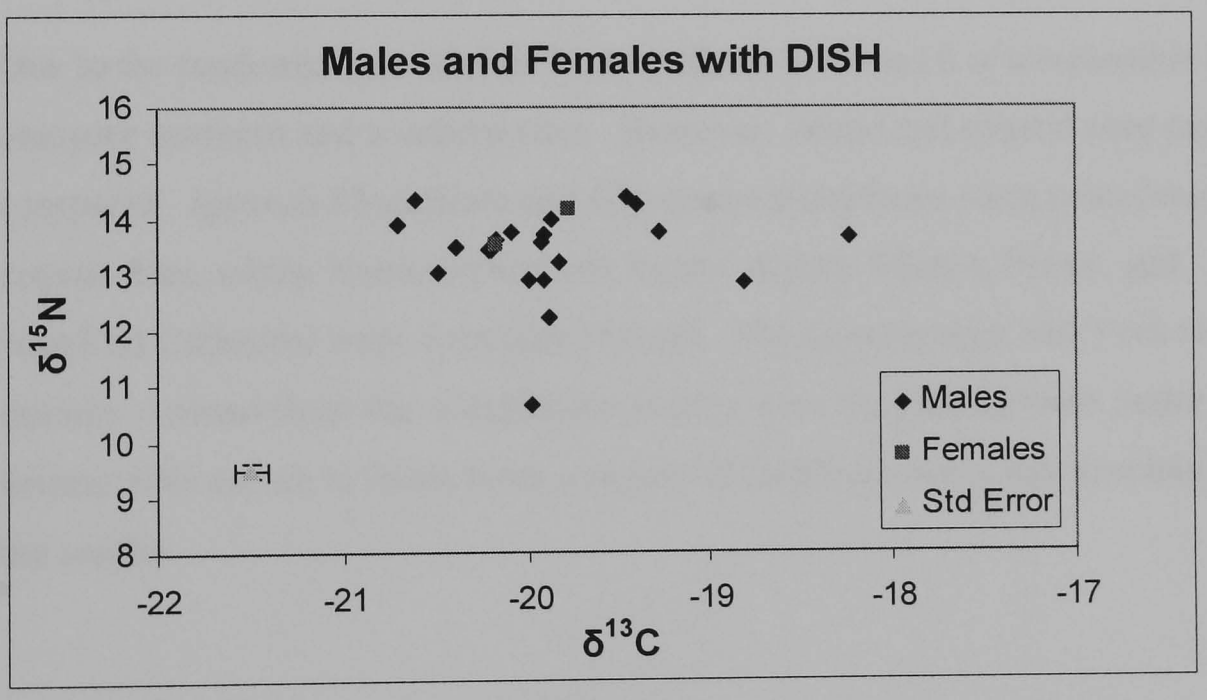


Figure 30. Males and Females with DISH.

When comparing males without DISH to females without DISH, there is no significant difference at the 5% level in either carbon (p=0.409) or nitrogen (p=0.188).

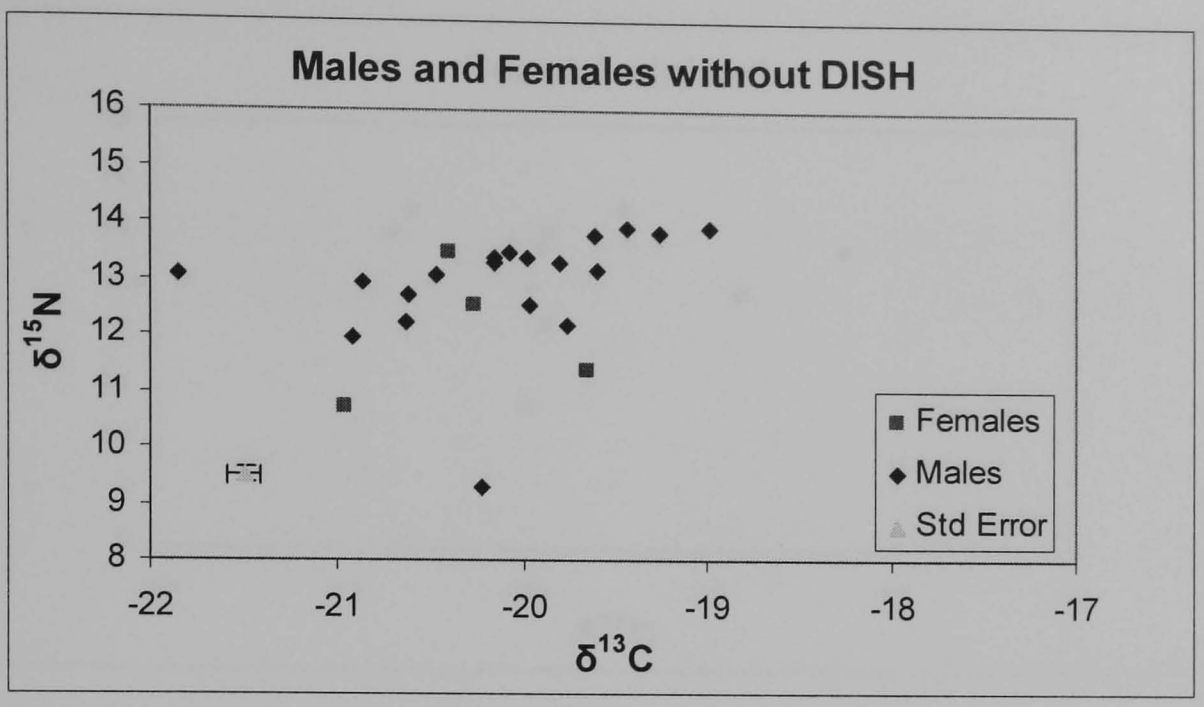


Figure 31. Males and Females without DISH.

8.2.2.6 Geographic Comparisons

Due to the predominance of sites from southern locations it is not possible to compare northern and southern sites. However, inland and coastal sites can be compared. Ipswich Blackfriars and Gloucester Blackfriars were considered coastal sites, whilst Northampton's St James' Abbey, Merton Priory, and Hereford Cathedral were considered inland. The London sites and York were initially omitted from this comparison as they were considered to be major urban centres with access to foods from a variety of locations that could possibly skew the results.

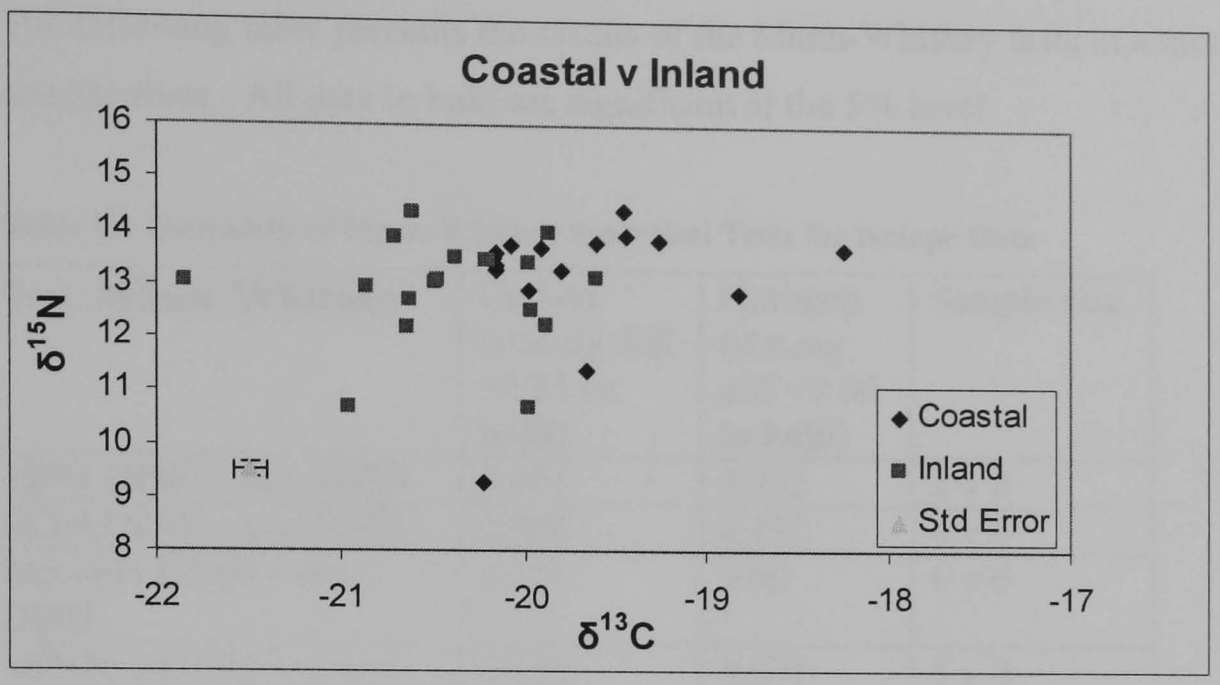


Figure 32. Coastal and Inland Isotope Data.

When comparing coastal and inland sites, there is a significant difference at the 5% level in carbon (p=0.001) but not nitrogen (p=0.154).

If the London and York sites are added, most of them fit in with the coastal data resulting in a significant difference in carbon at the 5% level between inland and urban sites (for C, p=0.010; for N, p=0.285) but not between coastal and urban sites (for C, p=0.698; for N, p=0.909), or when combining coastal and inland and comparing them with urban (for C, p=0.202, for N, p=0.565).

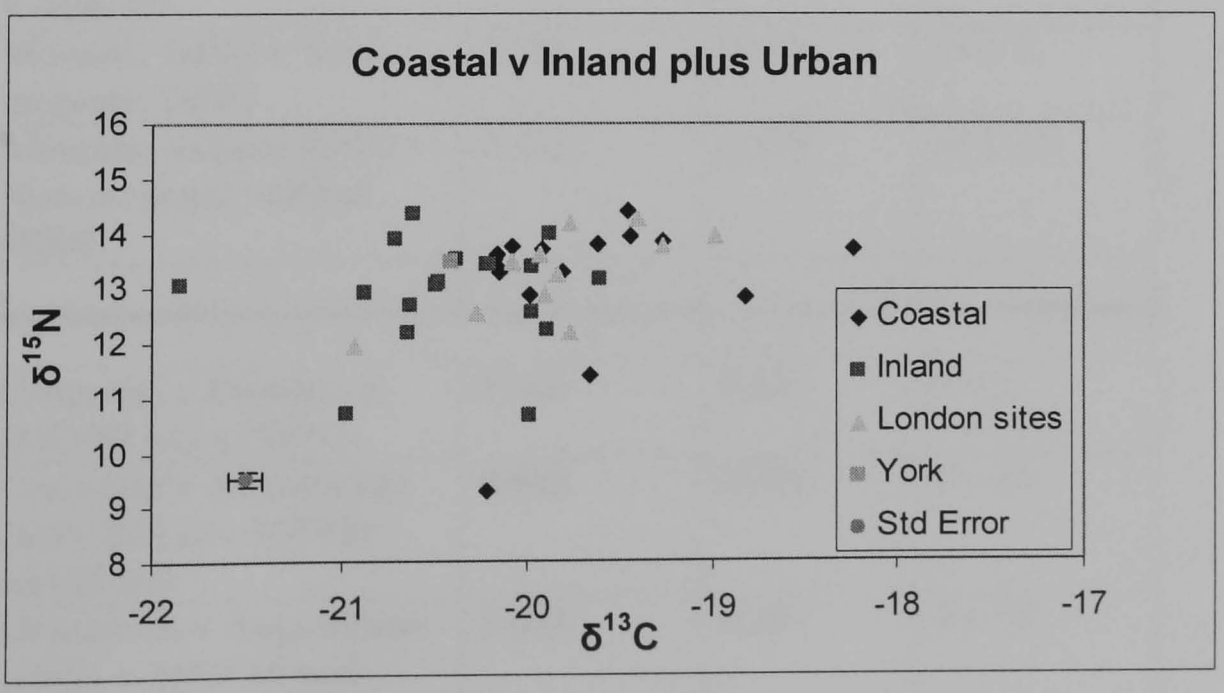


Figure 33. Coastal, Inland and Urban Isotope Data.

The following table presents the results of the Mann-Whitney tests in a more concise form. All data in bold are significant at the 5% level

Table 19. Summary of Mann-Whitney Statistical Tests for Isotope Data

Test: **Mann-Whitney**	Carbon (stat.sig diff <0.05 in **bold**)	Nitrogen (stat.sig diff <0.05 in **bold**)	Sample size
19/91 DISH v non-DISH	0.333	0.333	2 v 2
EL00 DISH v non-DISH	0.240	0.132	6 v 6
IAS4801 DISH v non-DISH	0.240	1.00	6 v 6
MIN86 LG DISH v non-DISH	0.250	0.071	5 v 3
MPY86 DISH v non-DISH	0.667	0.333	2 v 2
He93A DISH v Non-DISH	1.00	1.00	1 v 1
MIN86 BD DISH v Non-DISH	1.00	1.00	1 v 2
DISH v Non-DISH	0.258	**0.012**	23 v 23
Monastic v Non-Monastic	0.305	0.474	25 v 21
Monastic DISH v Non-DISH	0.134	**0.003**	14 v 11
Non-Monastic with DISH v without	1.000	0.382	9 v 12
Monastic DISH v Non-monastic DISH	0.781	0.975	14 v 9
Monastic without DISH v Non-monastic without DISH	0.235	0.190	11 v 12
Cistercian v Dominican (MIN86 LG v 19/91)	0.548	0.381	6 v 3
Cistercian v Augustinian (MIN 86 LG v MPY86 and EL00)	**0.002**	0.294	6 v 16
Dominican v Augustinian (19/91 v MPY 86 and EL00)	0.064	0.487	3 v 16
Male v Female	0.379	0.453	40 v 6

Males with DISH v without	0.436	0.074	21 v 19
Females with DISH v without	0.533	0.133	2 v 4
DISH males v DISH females	0.957	0.506	21 v 2
Non-DISH males v Non-DISH females	0.409	0.188	19 v 4
Coastal v Inland	**0.001**	0.154	16 v 18
Inland v Urban	**0.010**	0.285	18 v 12
Coastal v Urban	0.698	0.909	16 v 12
Coastal & Inland v Urban	0.202	0.565	34 v 12

8.3 Ancient DNA (aDNA) Data

The results of the ancient DNA (aDNA) research were obtained as a consequence of work carried out at the University of Manchester in October to mid-December of 2005 and mid-January to mid-March 2007. All the teeth sampled from the various monastic and non-monastic sites were brought to Manchester to be analysed and remain there now so that other avenues of the research can be explored at a later date. From a possible 74 samples, 51 extractions of ancient DNA were obtained and sequences were produced for 31 of them. The data for these samples is presented below. The sequence obtained for this researcher's DNA is presented in the alignment in Appendix A.

Obtaining the minimum amount of 0.01g of dentine for each tooth extraction was difficult. In total, there were 23 teeth for which sufficient dentine was not extracted, this may be because the dental file broke inside the tooth root, or because the tooth root was difficult to drill into, or simply because it did not produce enough dentine to carry out the analysis.

8.3.1 Individual Sites

8.3.1.1 EL00 St James' Abbey Samples

Sufficient dentine was extracted from thirteen tooth samples from St. James' Abbey in Northampton – DISH samples EL00 3096, 3116, 3121, 3127, 3266, and 3285 and non-DISH samples EL00 3005, 3073, 3079, 3095, 3100, 3141, and 3145. The above samples were amplified by polymerase chain reaction (PCR) using mtC primers and the non-contaminated results when run on a 3% agarose gel are shown in Figures 34 & 35.

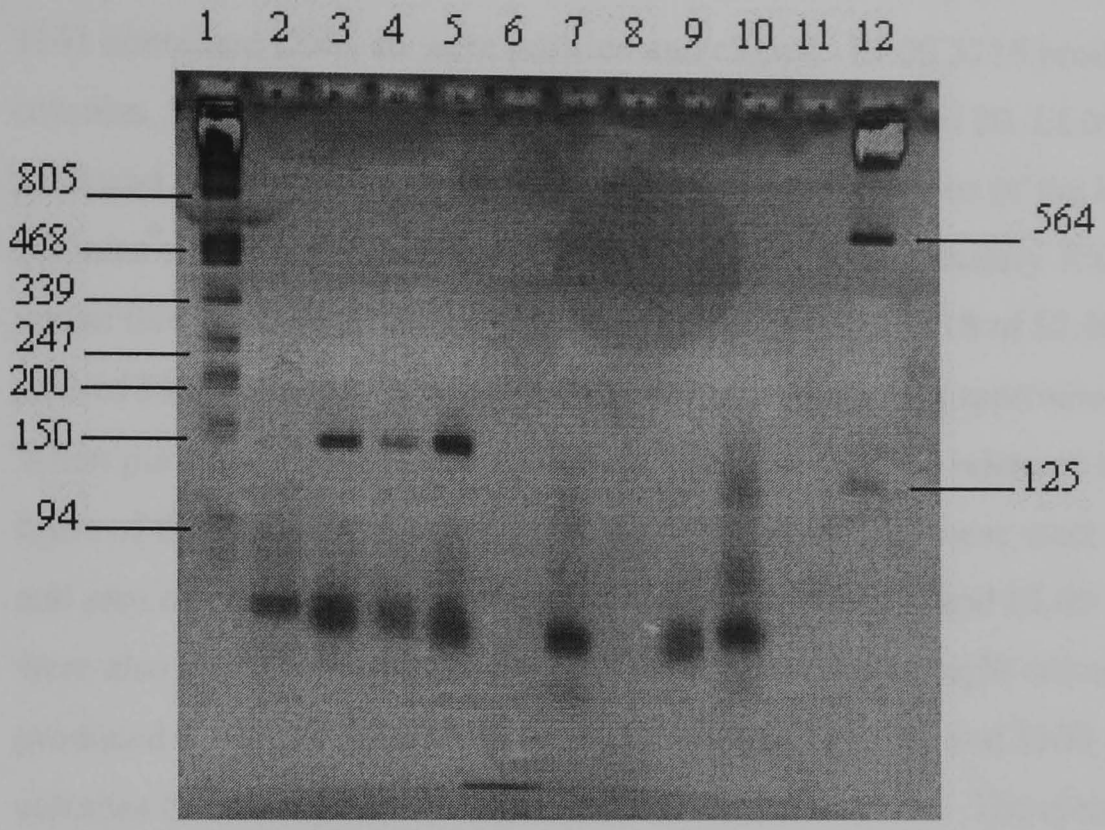


Figure 34. EL00 DISH samples: Lane 1=λ*Pst*I, 2=3096, 3=3121, 4=3127, 5=3266, 6=empty, 7=extr.blank, 8=empty, 9=PCR blank 1, 10=PCR blank 2, 11=empty, 12=λ*Hin*dIII

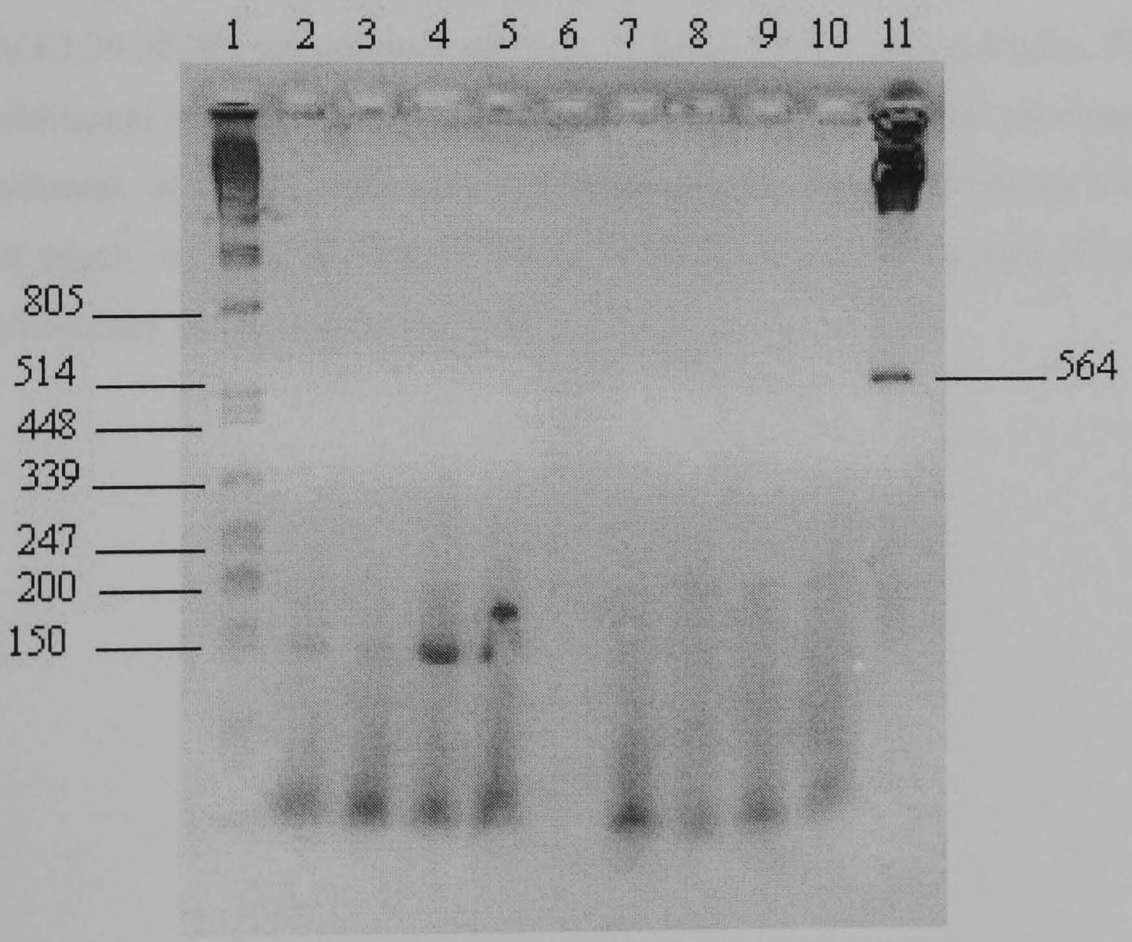


Figure 35. EL00 Non-DISH samples: Lane 1=λ *Pst* I, 2=3005, 3=3073, 4=3079, 5=3100, 6=empty, 7=extr.blank 1, 8=extr. blank 2, 9=PCR blank 1, 10=PCR blank 2,11=λ *Hin*d III

Samples EL00 3116, 3121, 3127, 3266, 3285, 3005, 3073, 3079, 3095, 3100, and 3141 contained DNA so were purified and cloned. EL00 3116 produced six colonies, EL00 3121 produced three, EL00 3127 produced 20, EL00 3266 produced 20, and EL00 3285 produced ten. However, none of the EL00 3121 colonies contained the correct sized DNA insert (approximately 350ng of DNA) whilst five of EL00 3116's colonies, all of EL00 3127's, 18 of EL00 3266's, and nine of EL00 3285's did contain the correct sized inserts (approximately 350ng). When purifying these cloning products, the centrifuge lid released itself and some of the EL00 3266 samples were destroyed. Six of these were unaffected and sent off for sequencing; the EL00 3116, EL00 3127, and EL00 3285 samples were also sent off for sequencing. EL00 3005 produced eight colonies, 3073 produced eight colonies, 3079 produced twelve colonies, and 3100 produced two colonies but none provided any inserts of the correct size. The cloning step was repeated using the same purified DNA products and produced the correct sized inserts for two of 3005's three colonies, two of EL00 3073's three colonies, six of EL00 3079's six colonies and four of EL00 3100's nine colonies. There were additional cloning steps for EL00 3095 and 3141. EL00 3095 produced eight colonies, of which, eight were successful, and EL00 3141 produced six colonies, of which, six were successful. These were all sequenced and sent off to the University of Oxford for analysis.

Table 20. Summary of EL00 Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	3027	×				
DISH	3039	×				
DISH	3090	×				
DISH	3096	✓	×			
DISH	3099	×				
DISH	3121	✓	✓	3	0	×
DISH	3127	✓	✓	20	20	✓
DISH	*3271	×				
DISH	*3292	×				
Probable	3116	✓	✓	6	5	✓
Probable	3266	✓	✓	20	18**	✓
Possible	3285	✓	✓	10	9	✓
No?	3068	×				
No	3005	✓	✓	3	2	✓
No	3014	×				
No	*3019	×				
No	3073	✓	✓	3	2	✓
No	3079	✓	✓	6	6	✓
No	*3094	×				
No	3095	✓	✓	8	8	✓
No	3100	✓	✓	9	4	✓
No	3141	✓	✓	6	6	✓
No	3145	✓	×			

*No teeth from which to extract DNA, **18 clones were successful but lost 12 during centrifugation

As the EL00 3096, 3121, 3127, and 3266 samples were the first to produce any aDNA, they were also amplified with the amelogenin primer to see if there was enough nuclear DNA to produce a sex identification. The products were run on a polyacrylamide (PAGE) gel and 3127 and 3266 were found to have enough DNA present. These two samples were purified, sequenced and sent off for analysis but as the results were unclear no further attempts were made to sex any future samples or carry out any nuclear DNA analysis.

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.2 MIN86 Royal Mint Site, Black Death and Late Graves Samples

Sufficient dentine was extracted from nine Royal Mint site tooth samples – Black Death cemetery DISH samples MIN86 6412 and 11944, Late Graves (abbey) cemetery DISH samples MIN86 12480 and 16344, and Black Death Cemetery non-DISH sample 5960 and Late Graves non-DISH samples MIN86 10348, 12356, 12520, and 12687 (see Figures 36&37). These samples were amplified by PCR using mtC primers and produced non-contaminated results:

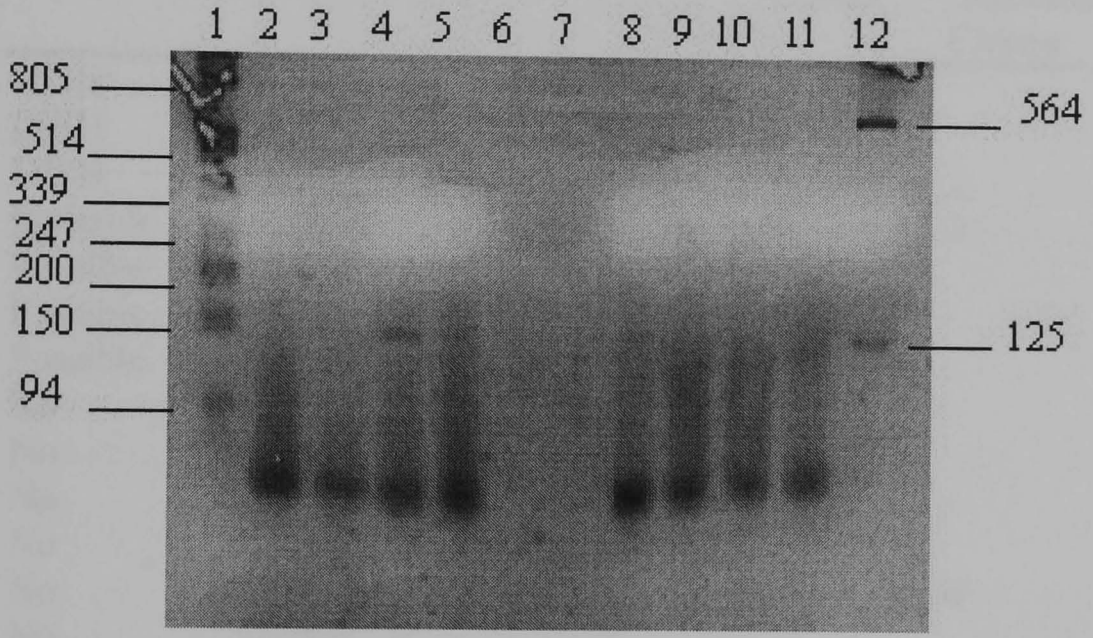


Figure 36. MIN 86 DISH samples: Lane 1=λ *Pst* I, 2=6412, 3=11944, 4=12480, 5=16344, 6=empty, 7=empty, 8=extr. Blank 1, 9=extr. Blank 2, 10=PCR blank 1, 11=PCR blank 2, 12=λ *Hind* III

Samples MIN86 5960, 12356, 12480, 12687, and 16344 contained DNA so they were purified and cloned. MIN86 5960 produced eight colonies but only one was successful, 12356 produced six colonies, six of which were successful, 12480 produced two colonies, neither of which contained the correct size DNA insert, 12687 produced three successful clones from eight colonies and 16344 produced nine colonies of which two produced the correct size insert. These were purified, sequenced, and sent off to the University of Oxford for analysis.

Table 21. Summary of MIN86 BD Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	6412	✓	×			
Possible	11944	✓	×			
No	5960	✓	✓	8	1	✓
No	8341	×				

Table 22. Summary of MIN86 LG Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	13518	×				
DISH	*16098	×				
DISH	16344	✓	✓	9	2	✓
Probable	12480	✓	✓	2	0	×
Possible	*13663	×				
Possible	*16311	×				
Possible	*16322	×				
No	10177	×				
No	10348	✓	×			
No	10420	×				
No	*12349	×				
No	12356	✓	✓	6	6	✓
No	12520	✓	×			
No	12687	✓	✓	8	3	✓

*No teeth to extract DNA from

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.3 IAS4801 Blackfriars Friary, Ipswich Samples

Sufficient dentine was extracted from eight tooth samples from Blackfriars, Ipswich – DISH samples IAS4801 1457, 1834 and 2005, and Non-DISH samples IAS4801 1451, 1762, 2508, 2640 and 2642. These were amplified via PCR with primer mtC but no results were obtained the first time that the IAS 4801, 1457, 1834, 2005, 1762, 2508, and 2640 products were run by electrophoresis. The PCR was repeated and there was found to be some possible contamination in one of the extraction blanks but in the third, uncontaminated attempt, samples

IAS4801 2005 and 1762 were found to contain DNA. IAS 1451 and 2642 produced uncontaminated results on the first attempt.

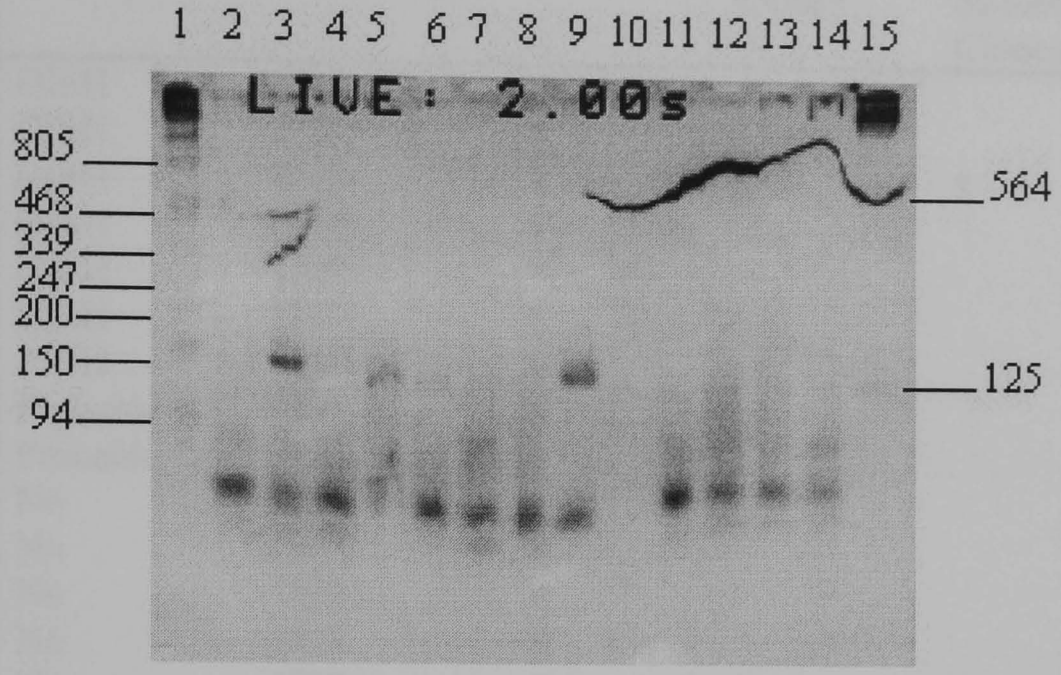


Figure 37. Lane 1=λ *Pst* I, L2=IAS4801-1457, L3=IAS4801-1762, L4=IAS 4801-1854, L5=IAS 4801-2005, L6=IAS 4801-2508, L7=IAS 4801-2640, L8=MIN86-12520, L9=CH86-45, L10=empty, L11=extr. blank 1, L12=extr. blank 2, L13=PCR blank 1, L14=PCR blank 2, L15=λ *Hin*d III

IAS4801 2005, 1451,1762, and 2642 were purified and cloned. IAS 2005 and 1762 produced nine colonies each of which were run on a gel to check if they were the correct sized inserts. All nine of IAS4801 1762's colonies and eight of IAS4801 2005's were the correct size. IAS 1451 and 2642 produced six colonies each resulting in two successful clones for 1451 and six for 2642. These were all purified and sequenced and sent off to the University of Oxford for analysis.

Table 23. Summary of IAS4801 Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	2591	×				
DISH	**2654	×				
DISH	2005	✓	✓	9	8	✓
DISH	1834	✓	×			
DISH	**1799	×				
DISH	1757	×				
DISH	**1417	×				
Probable	2496	×				
Probable	1457	✓	×			
No	2508	✓	×			
No	**1933	×				
No	1872	×				
No	1919	×				
No	1762	✓	✓	9	9	✓
No*	2642	✓	✓	6	6	✓
No*	**1391	×				
No*	1451	✓	✓	6	2	✓
No*	**1415	×				
No*	2640	✓	×			

*Not all vertebrae present, **No teeth from which to extract DNA

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.4 CH86 Hospital of St. James and Mary Magdalene, Chichester Samples

Only one of the three possible Chichester tooth samples provided sufficient dentine to analyse. CH86 45 was amplified at the same time as the IAS4801 samples and so the first attempt produced no data. When repeated, the second PCR was shown to contain possible contamination in one of the extraction blanks but on the third attempt CH86 45 was shown to contain sufficient uncontaminated DNA (see Figure 37) that was then purified and cloned.

The cloning results produced ten colonies which were then run on 2% agarose gels to check the size of the inserts. Eight of CH86 45's colonies were successful and so were purified, sequenced and sent off to the University of Oxford for analysis.

Table 24. Summary of CH86 Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	*123	×				
DISH	*357	×				
Probable	*18	×				
No	45	✓	✓	10	8	✓
No	175	×				
No	23	×				

*No teeth from which to extract DNA

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.5 He93A Hereford Cathedral Samples

All four Hereford Cathedral tooth samples provided sufficient quantities of dentine when extracted – DISH samples He93A 1664 and 1997 and Non-DISH samples He93A 606 and 2073. These samples were amplified by PCR and run on a 3% agarose gel to determine if any DNA was present. Uncontaminated results were obtained on the first attempt and DNA was found to be present in all four samples, although the band for He93A 1664 was very faint. All four samples were purified and cloned.

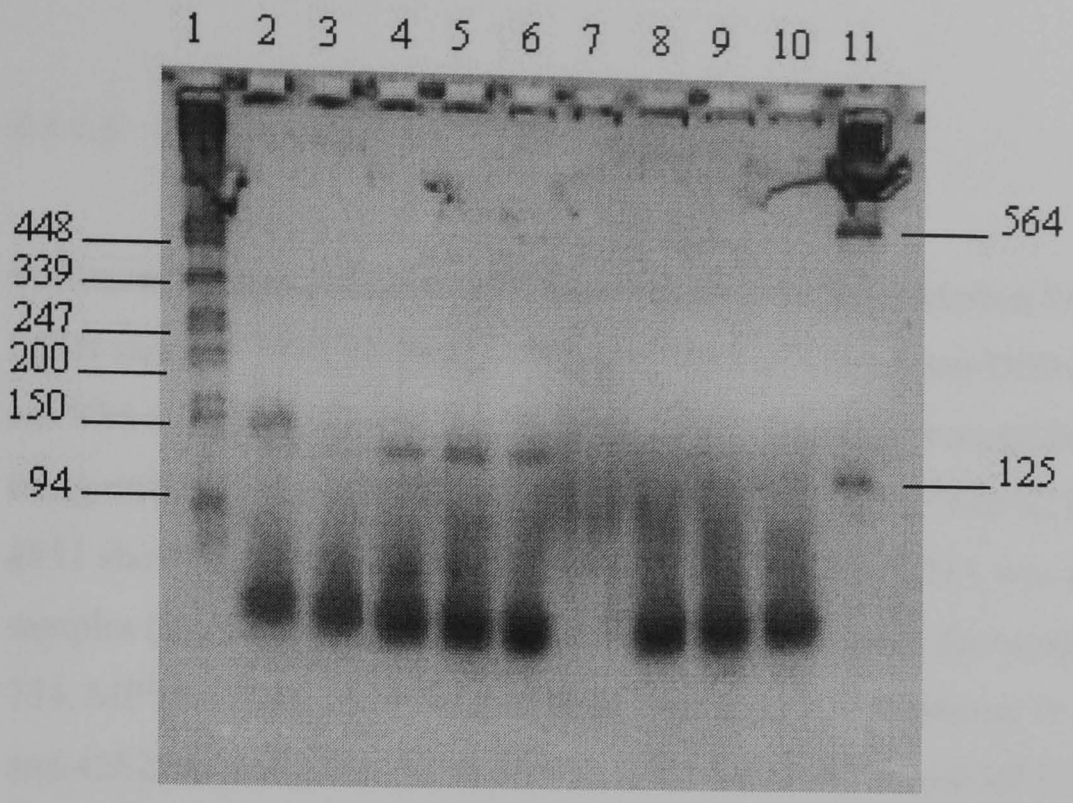


Figure 38. Lane 1=λ *Pst* I, L2=He93A-606, L3=He93A-1664, L4=He93A-2073, L5=MPY86-2905, L6=MPY86-4851, L7=empty, L8=extr. blank 1, L9=PCR blank 1, L10=PCR blank 2, L11=λ *Hind* III

After cloning, He93A 606 produced three colonies, He93A 1664 produced one colony, He93A 1997 produced six colonies and He93A 2073 produced three colonies but, when the sizes of the DNA were verified, only two of the clones from He93A 606 and five of the clones from He93A 1997 were found to be successful. These were purified, sequenced, and sent off to the University of Oxford for analysis.

Table 25. Summary of He93A Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
Probable	1997	✓	✓	6	5	✓
Possible	1664	✓	✓	1	0	×
No	606	✓	✓	3	2	✓
No	2073	✓	✓	3	0	×

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.6 MPY86 and MPY77 Merton Priory Samples

Sufficient dentine was extracted from six samples from Merton Priory – three DISH samples MPY86 2905, 4282, and 4851 and three non-DISH samples MPY86 623, MPY77 754 and MPY86 2376. These were amplified by PCR using mtC primers and the first attempt at amplifying MPY86 623, 2905 and 4851 showed that there was no contamination and that DNA was present in these samples (see Figures 38&39). PCR was attempted twice for samples MPY77 754, MPY86 2376 and 4282 and DNA was found to be present in samples 754 and 4282 but not 2376. MPY86 623, 2905, 4851, 4282 and MPY77 754 were purified and cloned.

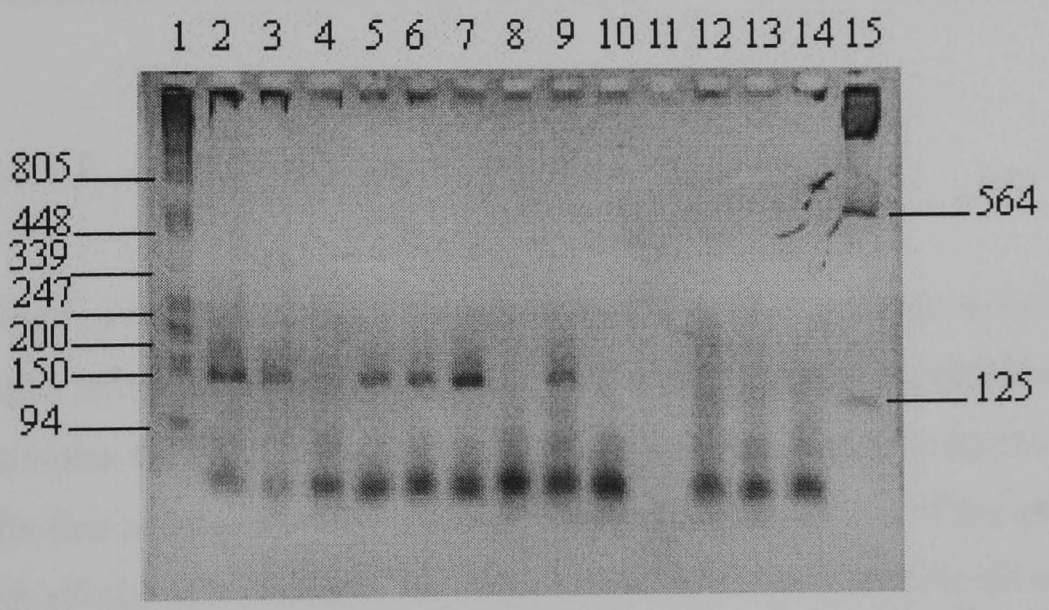


Figure 39. Lane 1=λ *Pst* I, L2=19/91-92, L3=19/91-104, L4=19/91-122, L5=19/91-233, L6=19/91-257, L7=YFH-308, L8=YFH=310, L9=MPY77-754, L10=MPY86-2376, L11=empty, L12=extr.blank, L13=PCR blank 1, L14=PCR blank 2, L15=λ *Hin*d III

The cloning attempt produced ten colonies for MPY86 623, three colonies for MPY77 754, seven colonies for MPY86 2905, six colonies for MPY86 4282 but none for MPY86 4851. None of MPY86 2905's colonies were successful, but all of MPY77 754's colonies were, along with two of 623's and four of 4282's, and all nine clones were purified, sequenced and sent off to the University of Oxford for analysis.

Table 26. Summary of MPY86 & MPY77 Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	716	×				
DISH	2748	×				
DISH	2905	✓	✓	7	0	×
DISH	366	×				
DISH	4282	✓	✓	6	4	✓
DISH	4851	✓	✓	0	×	
No	623	✓	✓	10	2	✓
No	754	✓	✓	3	3	✓
No	799	×				
No	2376	✓	×			
No	3447	×				

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.7 19/91 Blackfriars Friary, Gloucester Samples

Eight of the Blackfriars, Gloucester tooth samples provided sufficient dentine upon extraction – DISH samples 19/91 122, 257, 302 and 420 and non-DISH samples 19/91 92, 104, 212, and 233. These samples were amplified by PCR but the first attempt showed a possible contamination of one of the extraction blanks for all samples except 19/91 212. The PCR was repeated for all except 19/91 212. No contamination was found and DNA was present in samples 19/91 92, 104, 233, 257, 302, and 420 (see Figure 39) as well as 19/91 212. These were purified and cloned.

Cloning produced 14 colonies for 19/91 92, six colonies for 19/91 104, six for 19/91 212, five for 19/91 233, two for 19/91 257, six for 19/91 302 and six for 19/91 420. Of these, all of the clones, except one of 19/91 212's and one of 19/91 233's, were successful and were purified, sequenced and sent off to the University of Oxford for analysis.

Table 27. Summary of 19/91 Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	56	✗				
DISH	257	✓	✓	2	2	✓
DISH	302	✓	✓	6	6	✓
DISH	420	✓	✓	6	6	✓
Possible	122	✓	✗			
No	92	✓	✓	14	14	✓
No	104	✓	✓	6	6	✓
No	*143	✗				
No	212	✓	✓	6	5	✓
No	233	✓	✓	5	4	✓

*No tooth to extract DNA from

The results of the sequencing are presented in a single alignment in Appendix A.

8.3.1.8 YFH Fishergate House, York Samples

Two of the four Fishergate House, York tooth samples produced sufficient dentine during the extraction attempts – one DISH sample YFH 308 and one non-DISH sample YFH 310. The first PCR attempt produced some possible contamination and the repeat PCR yielded DNA in sample YFH 308 (see Figure 39). This sample was purified and cloned.

Table 28. Summary of YFH Extractions

	Sample	Extraction	DNA	No. of Clones	No. of Successful Clones	Sequenced
DISH	28	✗				
DISH	308	✓	✓	0	✗	
No	159	✗				
No	310	✓	✗			

The cloning attempt did not produce any colonies for sample YFH 308, and hence there were no sequencing results.

8.3.2 Sequencing Results

The sequencing results are presented as a single alignment in Appendix A.

Chapter 9: Discussion of Results, Aetiology of DISH, and Future Directions

This chapter will first present a brief discussion of the expected results from the stable isotope analysis based on the data presented in earlier chapters (Chapters 2, 3, and 4), before discussing the actual stable isotope data. It will then explore the links between DISH and diet, and DISH and protein, and finally summarise the inferences made from the data whilst exploring other potential explanations for the observations made.

Within the aDNA section, a brief discussion of the limitations of carrying out the analysis will be followed by a discussion to establish the authenticity of the sequenced samples. The sequencing data will then be interpreted and subsequently discussed with respect to whether DISH has a genetic component.

9.1 Expectations of Dietary Analysis

Medical studies associate hyperinsulinaemia (excess insulin) with DISH and cite obesity as being a contributor to hyperinsulinaemia, and therefore a risk factor for the development of DISH. The type of diet that would lead to obesity or weight gain includes one that contains more calories than is needed by the body to survive, or one that has an imbalance of dietary components (carbohydrate, fat, protein). The three monastic orders in this research were similar in terms of dietary components but key differences existed. On the basis of the documentary evidence (see sections 3.3.2 and 3.4.2), the Dominicans and pre-fifteenth century Cistercians consumed much less protein than their Augustinian and post-fifteenth century Cistercian counterparts, the former group apparently consuming no red meat at all and thereby subsisting on mostly brown bread, pottage, vegetables and fruit. This type of diet would not have led to obesity although it could have impacted on their health in other ways, leading to dietary deficiencies in certain vitamins and minerals normally gained through animal protein. The other

monastic orders, the Augustinians and the post-fifteenth century Cistercians were less frugal. They consumed substantially more meat and fish, possibly as much as 23% of their dietary intake, eating both permitted and prohibited meat and mostly marine fish. When estimating the amount of protein that this food represents, however, it appears to be within the recommended limits for good health. Therefore, it is not expected that the Augustinians or post-fifteenth century Cistercians experienced any ill-effects due to protein intake.

Whether any of these monastic groups were eating too much of the foods they subsisted on, and whether this contributed to the development of DISH, is a different matter, and one that is difficult to discern. It is known that religious orders gave away a portion of their meals to the poor and the needy (Harvey 1993:64, White 1993), but how much was available to begin with is unknown. Harvey (1993:35) estimates the calorie intake of the Benedictines as being 4807kcal during Lent, when fasting was continual, and 6207kcal outside of Lent. She estimates that if they gave away 40% of their food to the poor (as is estimated in the literature) and consumed the remainder themselves, the monks would have a calorie intake of 3724kcal outside Lent, some 1200kcal more than is required for an average man with a low level of activity (see Department of Health 1991). As discussed in Chapter 3, the Augustinians, Cistercians and Dominicans were not as wealthy, nor did they consume as great a quantity of food as the Benedictines, but, even if their intake was only half of that discussed above, there would still be a sufficient excess in calories to result in weight gain, and possibly obesity. However, using the documentary evidence as a guide, it would seem that the pre-fifteenth century Cistercians and the Dominicans were, owing to their frugal lifestyles, unlikely to be consuming calories in excess of their daily requirements. It is more likely that if any orders were to be plagued by obesity that it would be the Augustinian and post-fifteenth century Cistercians. Thus it would be expected that these two groups would be distinctive from the rest on the basis of quantity of food eaten.

Unfortunately, dietary isotopic analysis cannot distinguish between different quantities of food eaten, only different types of food eaten. Therefore, the latter debate about excess calories being consumed by certain orders that may have

lead to obesity cannot be resolved within the scope of this research. The differences in protein consumption between the different orders are, however, possible to discern providing such differences exist and it is expected that some orders, such as the Cistercians and Dominicans might stand out. It is also expected, providing a link between DISH and diet does exist, that the DISH samples would exhibit similar dietary profiles to each other, and different profiles to those without DISH.

9.2 Comparison with Other Contemporary Late Medieval Sites

Table 29. Comparison of Stable Isotope Data with Other Contemporary Sites

Sites	Mean $\delta^{13}C$ Value	Mean $\delta^{15}N$ Value
St Giles/Warrington/Towton[1]	-19.4 +/- 0.6‰ (1σ)	12.2 +/- 0.9‰ (1σ)
Timberhill, Norwich[2]	-19.3 +/- 0.3‰ (1σ)	11.1 +/- 0.3‰ (1σ)
Wharram Percy, Yorkshire[3]	-19.2 +/- 1.0‰ (1σ)	n/a
St Andrew's Priory, Fishergate, York[4]	-19.1 +/- 0.6‰ (1σ)	12.8 +/- 1.3‰ (1σ)
Kokksijde, Belgium[5]	-19.1 +/- 0.5‰ (1σ)	11.1 +/- 0.9‰ (1σ)
All samples (this study)	**-20.0** +/- 0.6‰ (1σ)	**13.1** +/- 1.0‰ (1σ)
DISH samples (this study)	**-19.9** +/- 0.6‰ (1σ)	**13.4** +/- 0.8‰ (1σ)
Non-DISH samples (this study)	**-20.1** +/- 0.6‰ (1σ)	**12.7** +/- 1.1‰ (1σ)
Monastic samples (this study)	**-20.4** +/- 0.6‰ (1σ)	**13.2** +/- 0.9‰ (1σ)
Non-monastic samples (this study)	**-20.0** +/- 0.6‰ (1σ)	**12.6** +/- 1.1‰ (1σ)
Post-medieval, All Saints, York ($C16^{th}$-19^{th})[6]	-18.8 +/- 0.4‰(1σ)	12.6 +/- 1.2‰(1σ)
Roman high status, Poundbury ($C4^{th}$)[7]	-18.2 +/- 0.3‰ (1σ)	10.1 +/- 1.1‰ (1σ)
Roman low status, Poundbury ($C\ 4^{th}$)[7]	-19.5 +/- 0.5‰ (1σ)	9.3 +/- 1.3‰ (1σ)
Anglo-Saxon, Berinsfield ($C\ 5^{th}$-7^{th})[8]	-20.1 +/- 0.2‰ (1σ)	9.8 +/- 0.7‰ (1σ)

References: [1]Müldner and Richards 2005, [2]Bayliss *et al* 2004, [3]Mays 1997, [4]Müldner and Richards 2007b, [5]Polet and Katzenberg 2003, [6]Müldner and Richards 2007a, [7]Richards *et al.* 1998, [8]Privat *et al.* 2002

The carbon and nitrogen isotope data from this study compare well with other medieval sites. The mean for the samples in this study falls within two standard deviations of other published isotope data for British medieval sites (see Table 1), with the exception of the Timberhill site in Norwich, but this latter site spans more than just the late medieval period (E Popescu, pers.comm., July 2008) so it is an exception. The small standard deviations of 0.3‰ for both carbon and

nitrogen at Timberhill might be another reason that data from this study lie outside the Timberhill site, although the mean values for carbon and nitrogen at Timberhill do fit within the data from other late medieval sites. In general, though, the DISH/non-DISH data are similar to other late medieval sites and show that the samples used in this study are not exceptional and are representative of the late medieval population in Britain.

9.3 Discussion of Isotope Results

9.3.1 Individual Sites

Within individual sites there are no significant differences between those with DISH and those without in either carbon or nitrogen. This may be a valid statistical outcome but it is also possible that the sample sizes are too small to detect any difference between the groups.

Unfortunately, the Chichester samples from the medieval leprosy hospital did not produce any usable isotope data so it is not possible to assess whether data from this site differed from the other sites. It was inferred from the documentary and archaeological evidence that the carbon and nitrogen results for the samples would be clustered as these individuals were institutionalised and therefore likely eating the same diet. However, these ideas could not be explored as no data were produced for this site.

9.3.1.1 Blackfriars Ipswich

There is a possibility that the Ipswich Blackfriars skeletons are monks rather than lay persons. The burials at this site included males, females and some juveniles, and showed little evidence of spatial patterning; it is likely that the friars and the lay benefactors were interred together in the same cemetery. According to the documentary data (see Chapter 3), the Dominicans had a secular "third order"

who were both lay persons and monks, lived at home, were allowed to be married, and could combine secular occupations with religious life. These secular monks may have been buried in the same cemeteries as women and children, and therefore the burials from Ipswich thought to be possible lay persons may in fact be monks. Whether these monks were still consuming meals with the regular members of the order is unclear though, so it is not known whether the dietary analysis would be affected.

With these points in mind, the statistical tests were recalculated to incorporate Ipswich Blackfriars (IAS 4801) as a monastic site. The results, in terms of significance, for nearly all tests, including the more informative comparisons – monastic versus non-monastic, non-monastic with and without DISH, monastic with and without DISH– were unchanged so it makes little difference whether the Ipswich data is classed as monastic or non-monastic. The one test that does change is the comparison of the Dominican orders with Augustinian. For this test, as the Ipswich samples are added to the Dominican side of the equation, the test becomes significant for carbon ($p=0.001$), whereas before it was approaching significance. The results are interesting for the monastic and non-monastic comparisons as moving the Ipswich samples to the monastic category means that the monastic sample size increases from 25 to 36 but that the non-monastic sample size decreases from 21 to 10 resulting in a greater imbalance in terms of sample size. Even with the change in sample size, however, there is still no difference detected between the two groups in either carbon or nitrogen which mirrors the original findings.

Since no important differences were found amongst the original data when re-classifying the Ipswich data as monastic, the possibility of Ipswich being a monastic site is not discussed any further and the samples from this site remain in the non-monastic category.

9.3.1.2 DISH and Non-DISH Individuals

Assessing whether there are any differences in diet between those with DISH and those without is not straightforward. A statistical test of the differences between those skeletons with and without DISH shows a significant difference in nitrogen, signifying differences in protein consumption, but testing all the male skeletons with and without DISH does not (see Table 2 for a summary). It seems odd that these results contradict each other as both groups are made up of similar samples, the only difference being the absence of the female samples from the male group. It could be that this small group of six female samples skews the data when examining all the skeletons together but, looking at the monastic data, which are all males, there are statistically significant differences in nitrogen between those with DISH and those without. All of these comparisons are made up of relatively large (minimum n=11) and quite equal sample sizes.

Comparisons of DISH and non-DISH samples at individual sites, amongst females, and amongst non-monastic skeletons do not yield any significant differences in either carbon or nitrogen but it should be acknowledged that these groups of samples are smaller and, in some cases, consist of very unbalanced DISH and non-DISH samples. However, in all plots, there is a trend for the DISH samples to plot higher $\delta^{15}N$ values than the non-DISH samples, which suggests that differences between the DISH and non-DISH groups do exist, even if the sample sizes are too small to detect this statistically.

Table 30. Summary of Key Data for Samples with and without DISH

Test	Data	Significant Difference in C	Significant Difference in N
Mann-Whitney	Skeletons with and without DISH	✗	✓
	Monastic skeletons with and without DISH	✗	✓
	Male skeletons with and without DISH	✗	✗

9.3.1.3 Monastic and Non-Monastic Sites

There are no statistically significant differences in either carbon or nitrogen found between monastic and non-monastic groups. Since Blackfriars Ipswich is in the non-monastic category, the numbers are quite balanced between the monastic and non-monastic groups with 25 monastic and 21 non-monastic samples. Looking at the scatterplot, the non-monastic samples appear to have similar ranges in carbon and nitrogen values to the monastic samples, so perhaps no real dietary differences exist.

An absence of differences between the monastic and non-monastic groups is not that surprising given that fasting regulations were meant to be upheld by all members of society, not just the religious. However, one would expect that the monastic orders would be much more dedicated in their abidance by the monastic Rule than secular society, and that this would result in some differences between monastic and non-monastic groups. The Rules of St. Benedict and St. Augustine, which monastic communities were meant to adhere to, prohibited meat from being eaten as it was thought to invoke carnal desire and distract the mind from prayer (Tobin 1995:136). Fish could be substituted, however, and also game on certain occasions (Gasquet 1922:52; Lawrence 1990:31,79). The documentary evidence on how closely these rules were adhered to is unclear, however, with accounts of monastic communities sending out dogs to hunt deer and thus 'transform' them into game, tales of extravagant feasts, or stories of monks making claims that they were ill and staying in the infirmary where meat was allowed in order to fortify themselves after a period of illness (Burton 2000:167, Gasquet 1922:58-59, Waldron 1985). Many of these accounts come from the wealthier monastic houses, such as the Benedictines, many of the other orders being more austere and adhering to the rules more strictly.

As discussed in Chapter 4 (section 4.2.1.1), one would also expect the lower classes to be substituting meat with dried fruit, eggs or cheese, rather than fish (Woolgar 1999:90), which would result in lower $\delta^{15}N$ values for those sites that

include individuals from lower socio-economic backgrounds (all sites except Blackfriars Friary, Ipswich) but this has not manifested as a difference between monastic and non-monastic groups either. Blackfriars Friary (Ipswich) does, indeed, appear to have higher $\delta^{15}N$ values, and higher mean values, than the other non-monastic sites, but there are more isotope data for this site than all the other non-monastic sites combined so this may be a biased inference.

When comparing the different monastic orders to each other, there are some significant differences in carbon between some of the orders. Cistercians, when compared to both Augustinian sites, show a significant difference in $\delta^{13}C$ but not $\delta^{15}N$, and the Dominican site when compared with both Augustinian sites is approaching significant for $\delta^{13}C$ ($p=0.064$). A significant result is likely prevented by the small sample size of three for site 19/91 (Dominican). Visually, there are clear differences between all three groups and the isotopic data seem to mirror the results of the dietary analysis from the documentary and archaeological evidence explored in Chapter 3. On the basis of the aforementioned data, it was expected that the Dominicans and the pre-15th century Cistercians would differ from the Augustinians. Although the Cistercian samples cannot be identified as either pre- or post-15th century, on the basis of these data their status is still unclear as they group with neither the Augustinians nor the Dominicans.

As demonstrated in Chapter 3, all the monastic orders had different attitudes to fasting and different degrees of adherence to the monastic Rule. It was expected that the Cistercians, being one of the more austere regimes in this study and having strict prohibitions against eating meat prior to the 15th century, would have shown differences in nitrogen when compared to the other orders. The differences in carbon are surprising and perhaps reflect differences in consumption of terrestrial and marine foods. In light of the documentary data, which suggests Augustinians ate more meat than the other orders, as the Augustinian samples are shifted towards more negative carbon values than the Cistercian and Dominican samples it could be that the protein sources eaten by the Austin Canons were more terrestrial – i.e. that they were consuming terrestrial meat protein and the Cistercians and Dominicans were consuming fish

protein. The absence of statistically significant differences in nitrogen could possibly be explained by the consumption of marine fish by the Dominicans and Cistercians (whose $\delta^{13}C$ values are less negative) and omnivore protein by the Augustinians which have high nitrogen values but lower carbon values.

The differences in carbon could also be reflecting differences in geography. The Dominican site of Blackfriars, Gloucester is classed in this study as a coastal site whilst the Augustinian sites of Merton Priory and St. James Abbey Northampton, are both classed as inland sites. The Cistercian site of St Mary Graces, albeit a London site and hence classified here as 'urban', fits in with the coastal values for the other coastal sites so can be considered a coastal site (see section 9.3.1.5 below). Therefore, the comparison of the Cistercian and Augustinian sites (St Mary Graces v Merton Priory and St James Abbey) is actually a comparison of coastal and inland sites (8 coastal v 16 inland) as is the comparison of the Dominican and Augustinian sites (Gloucester Blackfriars v Merton Priory and St James Abbey; 5 coastal v 16 inland).

9.3.1.4 Males and Females

Although the female samples are small in number (n=6), on the scatterplot of males and females they appear to have a similar spread in nitrogen values to that of the males and a spread in carbon values that seems to be more tightly clustered. The statistical tests show no significant difference in either carbon or nitrogen but there is an imbalance in the number of samples – 40 males and six females – so this may influence the detection of any differences between the groups.

The other various permutations of male and female comparisons – Males with DISH and females with DISH, males without DISH and females without DISH, females with DISH and without DISH, males with DISH and without DISH – do not show any differences in carbon or nitrogen.

9.3.1.5 Geographic comparisons

The comparison of inland and coastal sites (without London/York sites) shows a clear difference between the groups both visually and statistically. A significant difference is found in carbon, but not nitrogen, and, when looking at the scatterplot, two distinct groups along the x-axis can be seen but no differences in nitrogen are visually apparent. These data seem to suggest that there are clear dietary differences between the inland and coastal groups and a likely explanation for this is in the consumption of marine and freshwater fish. The coastal sites are shifted to less negative carbon values than the inland sites, suggesting that they were consuming more marine fish than the inland sites.

Adding in the urban sites of London's Royal Mint Sites and York's Fishergate House does not demonstrate any clear differences between urban and non-urban diets. As many of the Royal Mint site samples fit with the coastal data, the comparison of urban and inland becomes a comparison of coastal and inland and results in a significant difference in carbon. The comparison of urban and coastal sites results in no significant differences, nor does the comparison of urban with both coastal and inland sites combined. These data suggest that the urban category, despite having access to more resources than the other sites, is not a separate dietary category from coastal or inland, and the urban sites merely fit in with their respective geographic locations.

9.3.2 Does a relationship between DISH and diet exist?

The relationship between DISH and diet is a rather complex one to answer. On the basis of the isotopic evidence, there do appear to be correlations between DISH and nitrogen levels – nitrogen being statistically significantly higher in the DISH group, thus indicating consumption of animal protein. However, whilst this correlation exists for the total group of all skeletons, and for monastic skeletons in the original data set, it does not exist amongst male skeletons. This finding suggests that DISH is not the reason for the differences in nitrogen in the other groups since, if a link between DISH and diet indeed exists, it would be

expected to be seen in all groups of skeletons with and without DISH, including the males for which DISH has a high prevalence rate. Male skeletons account for the majority of the dataset, totalling 40 samples, so it is surprising, and quite important, that this group of individuals do not exhibit any statistically significant differences in diet between those with DISH and those without.

It was suggested earlier in the chapter (section 9.3.1.2) that these differences in the statistical outcomes might be due to small groups of samples (females or non-monastic males) influencing the results. As can be seen from the addition of the May and June 2007 data to the original data set, five extra samples can change the results of the statistical tests from significant to non-significant. Unfortunately, the number of female skeletons is too small to assess any sex-related dietary differences and there appear to be no isotopic differences between monastic and non-monastic diet.

In consideration of the 'osteological paradox' as described by Wood *et al.* (1992), it is possible that the male individuals who do not exhibit any of the bone changes associated with DISH died before they could develop any signs of the condition and therefore look the same, isotopically, as those males who have DISH. On the other hand, if we consider DISH to be a syndrome with the bone changes as one manifestation of the condition, then it is possible that those without the characteristic bone changes of DISH may still have had the condition but were resistant to any skeletal changes and thus could still look isotopically similar to those who did develop the bone changes associated with DISH.

However, despite the lack of statistically significant evidence for a difference between DISH and non-DISH samples, there is clear visual evidence of such a difference. In all plots, the general trend is for DISH samples to plot higher in terms of $\delta^{15}N$ values than non-DISH samples. This separation between DISH and non-DISH samples suggests that individuals with DISH *do* have a different diet than individuals without DISH, and that this diet includes foods rich in animal protein, and foods of a higher trophic level, than those without DISH.

Before discussing the links between DISH and protein, there are several other influences on nitrogen levels, other than diet, that could account for the differences observed between the groups. Many of these relate to a monastic lifestyle. These are poor quality collagen, disease, dehydration, and fasting.

9.3.2.1 Poor quality collagen

It is known that poor collagen yields of less than 5% of the sample weight can elevate carbon and nitrogen values. White and Schwarcz (1994) tested the relationship between yields of less than 5% and the resulting carbon and nitrogen values and found a positive correlation for both, indicating that yield can affect the stable isotope results. Any samples used in this research study which had poor yields *and* poor C/N ratios were discarded. Fewer than ten samples with poor yields were included in the study but even these had greater than 3.5% yields. Some authors maintain that when using ultrafiltration, any yield greater than 0.1% is considered valid as the ultrafiltration greatly reduces the resulting amount of collagen (Fischer *et al.* 2007). If collagen yields were affecting the results it would be expected that both carbon and nitrogen would be affected and in this case it is only nitrogen that is affected. It is not likely that poor collagen yield is a factor in this case.

9.3.2.2 Disease

Katzenberg and Lovell (1999) demonstrated that pathological bone can affect the outcome of carbon/nitrogen stable isotope analysis. They sampled from bones with various pathological lesions ranging from healed fractures to osteomyelitis and concluded that nitrogen values were higher in those samples that came from the affected parts of the bone in comparison to the unaffected (normal) bone. In this study of DISH, although primarily a study of disease, none of the bone sampled was affected by DISH and there were no observable active or healed lesions on the ribs. The only possible exception might be the samples from the Royal Mint Black Death Cemetery, as these individuals likely died of plague.

However, it is unlikely that an infection with such a fast disease course could affect the isotope results as it would not have had a chance to affect the skeleton. Again, none of the samples chosen came from ribs with observable active or healed lesions so it is unlikely that disease is influencing the difference in nitrogen values observed in this research. However, it is possible, reflecting on the idea of some individuals with DISH not exhibiting the skeletal changes associated with the condition, that a normal looking bone could, in actuality, be diseased, and thus produce elevated $\delta^{15}N$ levels.

9.3.2.3 Dehydration

Animal studies have shown that periods of drought or dehydration can affect nitrogen levels in the body (Ambrose and deNiro 1987). When water-stressed, ^{14}N is preferentially excreted (through urea) thereby elevating ^{15}N and thus elevating the ^{15}N isotope values obtained. It has been hypothesised that water-stress can also elevate nitrogen levels in humans (White and Armelagos 1997). According to the documentary evidence, monastic communities had very proscribed daily routines with specific periods set aside for eating and drinking (Burton 2000:160-161). As they were not allowed to drink freely, and drank mostly beer and very little water, it is likely that the monks were dehydrated. The lay population also had the potential to be dehydrated, particularly the upper classes. Although the lower classes and peasants drank both water and beer, the upper classes certainly did not drink water, choosing to drink alcoholic beverages instead (Harvey 1993:66, Labarge 2003:102). It is possible that dehydration may have influenced the statistically significant differences seen in the nitrogen isotope values but, given that there is no difference in $\delta^{15}N$ seen between monastic and non-monastic groups, it seems an unlikely explanation.

9.3.2.4 Fasting

Long periods of fasting have also been known to increase $\delta^{15}N$ levels (see Macko *et al.* 1999). In a process similar to water stress, a reduction in nutrients and/or

food intake means that nitrogen has to be obtained from proteins broken down in the body rather than the food ingested, thereby causing an enrichment of ^{15}N over ^{14}N (Hobson *et al.* 1993). Assuming that the strict monastic regimes were followed, monastic communities fasted several times throughout the year, especially during Easter, with some orders consuming only bread and water (Hinnebusch 1965:359). In addition, they had a restricted food intake for most of the winter as they were allowed only a single meal during the winter months (Knowles 1948:18). These periods of fasting could possibly lead to increased nitrogen levels amongst some individuals within the monastic orders. However, no differences between monastic and non-monastic groups have been found, nor have any differences in nitrogen been found between the different monastic orders themselves so fasting is an unlikely explanation for the high nitrogen levels seen in this data.

9.3.3 DISH and Protein

No other studies have specifically looked at DISH using stable isotope analysis but some have looked at correlations between DISH and isotope values as part of larger projects. Müldner and Richards (2007b) found that four male individuals from St. Andrew's Priory, Fishergate in York (Stroud and Kemp 1993) had carbon and nitrogen values that were above the mean for males at the site but this was not statistically significant.

The DISH samples in this study have a mean $\delta^{13}C$ value of -19.9‰ and mean $\delta^{15}N$ value of 13.4‰ with a range of -20.7‰ to -18.2‰ in carbon and 10.7‰ to 14.4‰ in $\delta^{15}N$. These data are very similar in range to those found for late medieval sites by Müldner and Richards (2005). For the sites of St Giles, a hospital; Warrington, an Austin Friary; and Towton, a mass grave; they reported a range of -20.6‰ to -18.1‰ in $\delta^{13}C$ and 10.5‰ to 14.9‰ for $\delta^{15}N$, with an average of -19.4‰ for $\delta^{13}C$ and 12.2‰ for $\delta^{15}N$.

The DISH samples have a higher $\delta^{15}N$ mean than the aforementioned sites and also a higher $\delta^{15}N$ mean than the mean for all samples in this study of monastic

and lay populations – 13.4‰ versus 13.1‰. Müldner and Richards (2005) interpreted the isotope data at their sites as having $\delta^{13}C$ values that were too terrestrial for the high $\delta^{15}N$ values they were seeing and suggested that the individuals were eating omnivore protein (most likely pork) or freshwater fish rather than marine resources. As the data for the DISH samples are similar, it is logical that the same interpretation could apply to this data. The documentary and archaeological evidence does not suggest that pork was a prominent meat amongst monastic communities, but it was commonly consumed by the lay population. With 14 monastic DISH samples and nine non-monastic DISH samples, it is possible that freshwater fish was influencing the high $\delta^{15}N$ values more than pork consumption but carbon and nitrogen stable isotope analysis is not specific enough to determine which protein source is responsible for the data produced. Therefore, we cannot say with any certainty that the fasting regulations were being upheld with large amounts of fish being substituted for meat. However, it is also possible that the high $\delta^{15}N$ values represent a mixture of marine and freshwater sources which could result in high $\delta^{15}N$ values, a marine signature, but terrestrial $\delta^{13}C$ signatures. If this is the case, then it would indicate fasting regulations were being adhered to.

9.3.4 Possible Outcome with an Expanded Data Set

It is unfortunate that the original sample size became reduced over the course of the research. With a larger data set it would be possible to determine whether the patterns observed within the individual sites, male and female data, for DISH samples to plot higher in $\delta^{15}N$ than non-DISH samples, are statistically significant. A larger data set might also help address the question of why nitrogen levels are significant amongst all skeletons with and without DISH but not males with and without DISH. The differences between the monastic orders might also become clearer, resulting in a statistically significant outcome for all comparisons, and the female data might also yield some interesting results.

Although the Durham data were determined to be both inconsistent and invalid due to differences between previous runs of samples and measurements for the

standards that differed from expected values, the differences in results between the two datasets show clearly that interpretation depends very heavily on the machines used to generate the data. Researchers must be careful to ensure that the equipment used, and the data obtained, are valid and consistent through the use of standards and duplicate runs of samples.

9.3.5 Isotope Analysis Summary

To summarise the key findings:

- A comparison of all DISH and non-DISH samples yields a significant difference in $\delta^{15}N$
- No significant difference between DISH and non-DISH samples at individual sites or amongst males or females but patterns of high $\delta^{15}N$ values for DISH samples
- No significant differences between monastic and non-monastic samples
- Significant difference in $\delta^{15}N$ between monastic DISH and monastic non-DISH
- Significant differences in $\delta^{13}C$ between different monastic orders
- Significant differences in $\delta^{13}C$ between coastal and inland sites

There is no doubt that the DISH samples have a higher average $\delta^{15}N$ value than the non-DISH samples but it is not clear what this represents. Typically, high $\delta^{15}N$ values suggest marine food consumption but, as the mean $\delta^{13}C$ value has not shifted along the x-axis and remains typical of a terrestrial signature, this interpretation is not straightforward. As suggested in section 9.3.3, the $\delta^{15}N$ values could be a reflection of omnivore protein consumption, freshwater fish consumption, or a combination of both freshwater and marine fish consumption. If freshwater of marine fish are being consumed then it seems likely that the monastic rules were being upheld; if it is pork, this is less likely. There is support for both sides of the argument. The isotopic comparisons of the different monastic orders appears to support this idea of dietary differences between monastic groups and shows clear differences between the Augustinians, Cistercians, and Dominicans, the latter group suggesting a mixing line between terrestrial and marine protein, and thus supporting the idea of religious fasting and consumption of fish. However, as the documentary evidence also suggests

that orders such as the Augustinians were consuming large amounts of animal protein and defying the monastic regulations on diet, and their isotopic signature appears quite terrestrial but with some high $\delta^{15}N$ values, then it seems more likely that these could be attributed to omnivore protein.

It should be noted that, even though the DISH samples have a higher average $\delta^{15}N$ value, the $\delta^{15}N$ values seen amongst the DISH samples are not consistently higher; there are still many samples with DISH who have lower $\delta^{15}N$ values than those without DISH. Nevertheless, the statistically significant difference in $\delta^{15}N$ values between the DISH and non-DISH samples, and the pattern of higher $\delta^{15}N$ values in all the other remaining DISH/non-DISH comparisons, indicates that there is a link between DISH and protein. It is difficult to align this link between protein and DISH with the current theories in the bioarchaeological literature that suggest that this link is a result of consumption of a lot of animal protein and a lot of calories. As there is some evidence that some of the monastic orders were, as the documentary data suggest, following the monastic Rule in terms of fasting and substituting meat with fish, it seems unlikely that they would have been disobeying the Rule in non-fasting periods by eating high calorie, high animal protein foods. It also seems equally unlikely that a diet consisting of fish and grains with a moderate amount of meat would lead to the bone changes of DISH when this condition is most often associated with obesity and hyperinsulinaemia.

It could be that the high $\delta^{15}N$ values amongst DISH samples bear no relationship to diet. As mentioned previously, bioarchaeologists may be mistaking an absence of bone changes for an absence of disease and thus, in sampling from what appears to be healthy bone, may actually be sampling diseased bone which could produce higher $\delta^{15}N$ values. High $\delta^{15}N$ values have also been shown to be associated with bone *loss* rather than bone deposition. In a study by White and Armelagos (1997), it was suggested that high $\delta^{15}N$ levels could be used as a marker of osteopenia (pre-stage to osteoporosis) as they appear to show a correlation with bone loss. Perhaps the high $\delta^{15}N$ values seen in some DISH/non-DISH comparisons is reflecting a physiological process rather than a dietary one and high $\delta^{15}N$ values are produced whenever any bone changes take place, whether this be bone loss or bone deposition? Why this would manifest as

statistically significant amongst all DISH/non-DISH individuals but not amongst males with and without DISH is unknown.

The fact that the pattern of high $\delta^{15}N$ values for DISH samples occurs in all groups – males, females, monastic and non-monastic – suggests that this is not a monastic phenomenon. It is highly possible that the suggested correlation between DISH and monastic cemeteries is simply a reflection of the demography of the monastic cemeteries. Since monastic communities are made up of older males, one would expect to find more DISH present amongst the population as DISH is a condition that affects older individuals more than it does younger and males more than females.

9.3.5.1 Other theories that may account for the occurrence of DISH

One theory that has emerged from this research is one that links sleep deprivation with DISH. According to a 20-year research study on sleeping patterns and health, there is a strong correlation between lack of sleep and obesity with those getting two to four, five, and six hours sleep being 235%, 60%, and 27%, more likely to be obese, respectively (Gangswisch *et al.* 2005). From examining the timetables (*horarium*) of the different orders it is apparent that during the summer they all had less than six hours sleep a night. The Dominicans had the fewest number of hours rest, with less than four hours sleep, as they went to bed at 8:15pm and rose at midnight for *matins*. The Cistercians had approximately five and a half hours a night and the Augustinians probably a similar amount – their records cannot be found but the Benedictines had approximately five hours sleep and the Austin Canons were probably similar to them and the Cistercians. With these figures in mind, all the orders were at least 60% more likely to be obese, with the Dominicans possibly being as much as 235% more likely to be obese. These figures suggest that the orders had an increased chance of developing obesity, albeit perhaps only for half the year, even without any additional influences of diet or exercise. There is also a link between hyperinsulinaemia, the condition that is thought to link to DISH, and lack of sleep. Those with four hours of sleep or less a night, such as is the case with the

Dominicans, have a 30% reduction in their insulin response to glucose which could mean that they have a greater potential to develop DISH.

As discussed in Chapter 3, the daily activities of the monastic orders are also likely to have contributed to their weight. The Austin Canons, appear to have been the most active with their daily tasks alleged to have included digging the garden, sowing seeds, weeding, ploughing, reaping and mowing, amongst others (Burton 2000:165). However, they balanced these outdoor activities with more sedate indoor ones such as making wooden spoons and candlesticks, preparing parchment, and writing and correcting books (*ibid.*). The Cistercians left most of their agricultural labour to the *conversi* to carry out and the Dominicans did little exercise, having replaced manual labour with studying and writing books (Burton 2000:164, Hinnebusch 1965:339). Based on these accounts it is probably fair to say that the monks, friars, and canons did not live an active life; with so much time dedicated to prayers and the reading of the liturgy it is unlikely that they had much time for any other activities. With this in mind, they were likely undertaking little exercise and would have been prone to gaining weight, another potential source of hyperinsulinaemia, and hence another potential link to DISH.

However, despite these potential dispositions to gaining weight we cannot say that the monks were *obese* as there is no real evidence of this. A study using DISH as a marker of obesity found a greater association of obesity-related osteoarthritis with monastic skeletons than secular ones, but did not find any significant differences in BMI (body mass index, calculated as weight in kilograms divided by height in meters squared) between monastic and secular assemblages (see Patrick 2005). An average BMI of 24.47 for monastic individuals and 24.64 for secular individuals, placed them in the 'acceptable' weight range and not 'obese' categories defined by the World Health Organisation (see Patrick 2005:323). However, despite 11 of 12 calculations for BMI and weight falling in the normal range for adults and not the obese one, Patrick concludes that monks have higher rates of obesity-related pathology than secular counterparts, and also skeletons that were "built to cope" with the increased body weight. It was thus proposed by Patrick (2005:376) that obesity

in monks was a real phenomenon in the late medieval period but, given that no difference in BMI was found between those with DISH and those without, it is difficult to believe that this can be true. In fact, trying to determine if DISH is related to obesity by using DISH as a measure of obesity is flawed because all samples with DISH will then be deemed to have been obese.

9.4 Discussion of DNA data

The original goal of the aDNA analysis was to perform a kinship analysis of the skeletal remains using short tandem repeats or microsatellites but it was later realised that analysis of the maternal lineages through mtDNA would likely be more successful. Upon arrival at the University of Manchester, and after discussion with experts in the field, it became apparent that a thorough mtDNA analysis of the remains in question would not be possible within the short space of time available (three months). As such, a new strategy was devised whereby the samples would be analysed with one primer at a time and then assessed as to whether any potentially related individuals were present. The amount of time and energy that would be needed to do a full mtDNA analysis would require several years of work, something that was not anticipated by this researcher in the initial stages of the project. As a result, the allotted time for the aDNA research was doubled to six months. Even in the short time available, a considerable amount was achieved and the aDNA analysis was very successful, yielding 31 sequences from 74 potential samples.

9.4.1 Contamination and Post-Mortem Damage

Ancient DNA is subject to post-mortem damage and as such aDNA sequences are often full of random mutations that occur as hydrolytic deamination and depurination take place. Some of these nucleotide positions that mutate more readily and more frequently than others, known as "hotspots", have been studied in terms of their mutation rates to determine if the degree of post-mortem damage, and hence random mutation, might be impacting upon the designation

of haplogroups (see Gilbert *et al.* 2003). The sites that have been identified as having high mutation rates and that are present amongst the samples analysed in this study are – 16172, 16183, 16189, 16192, 16204, 16223, and 16270. Of these hotspots, only three are the sole mutations used to assign a haplogroup – 16189, 16192, and 16270 – 16189 and 16270 for U5b, 16192 and 16270 for U5a, and 16270 for U5. It is certainly possible that the samples designated as U5b, U5a, and U5 – samples 19/91 104, 19/91 233, and EL00 3141, respectively – are simply products of post-mortem degradation but considering that five of five clones for 19/91 104 and six of six clones for EL00 3141 have the same mutations it is perhaps unlikely. Sample 19/91 233 has a consensus sequence in only two of four clones and so is less convincing.

It is generally considered by biomolecular specialists that teeth are less susceptible to contamination than bone. Although no bone was used in the aDNA analysis here, the isotope samples were all derived from bone and therefore we can make some inferences based on the success of the collagen extraction and C/N ratios. Out of a total of 83 collagen isotope samples, 46 were successful (55%) and out of a total of 51 extracted DNA samples, 31 were successful (61%) which suggests there is no difference between them.

There was little contamination identified amongst the aDNA samples which could be attributed to the use of teeth rather than bone as a sample material. Although this researcher was familiar with the techniques and analysis of genetics and microbiology, she had never worked with ancient DNA material. It was quite impressive, therefore, that no contamination with the researcher's own DNA took place in the final sequenced material. As mentioned in Chapter 7, the anti-contamination methods were quite stringent with separate laboratories, specialist clothing and gloves, and special decontamination methods for the material itself, but it would still be possible to find evidence of contamination in spite of all these measures. Three samples had one or more clones with one of this researcher's mutations (C-T transition at 16223) but, as these were not accompanied by this researchers' other mutations, the mutations were thought to be endogenous rather than contamination.

The sequencing alignments were also checked for evidence of contamination by other researchers working in the ancient DNA labs at the University of Manchester. Two samples, EL00 3095 and 19/91 302, had a T-C transition at position 16172, known to be a mutation shared by researcher AB at the University of Manchester. EL00 3095 contained the T-C mutation in only three of six clones and, although other random mutations were present, as there was no other consensus sequence it is possible that this represents contamination by researcher AB. Sample 19/91 302 had several other consensus mutations, in addition to 16172, that are not part of AB's haplogroup so this sequence was thought to be endogenous rather than contamination.

Unfortunately, it is not possible to rule out the possibility that the sequences obtained in this study represent those of the initial excavator or curator of the skeletal material. To explore this idea, multiple samples would have to be obtained and the aDNA analysis repeated for each sample (extraction, purification, cloning, sequencing etc) in order to authenticate it; this could not be carried out within the time frame of this research. Nevertheless, no other samples showed signs of contamination – either by presence of mutations shared by other researchers in the lab, or by presence of multiple consensus sequences – which supports the idea that what has been sequenced is ancient DNA.

9.4.2 Degradation

Considering that both aDNA and stable isotope fields have to contend with issues of degradation and deterioration, there might be some links to be made between those samples that are too degraded for aDNA work and those that are too degraded for stable isotope work. However, upon comparing the two groups of samples there does not appear to be any correlation between poor C/N ratios and unsuccessful sequences. Of the 37 isotope samples that failed to produce collagen or had C/N ratios falling outside the acceptable range, 18 were still successful in producing sequencing data. Of the 46 isotope samples that had C/N ratios within the acceptable range, 13 also produced successful sequences. Vice versa, of those 20 samples from which DNA could be extracted but which failed

to produce sequences, only four failed to produce any collagen and a further four produced collagen but did not produce C/N ratios within the accepted range. Therefore, there do not appear to be any clear links between DNA degradation and collagen deterioration. However, it must be pointed out that the aDNA samples were obtained from teeth, and the isotope samples obtained from bone. They are different materials with differing susceptibility to deterioration and contamination. Nevertheless, it would be expected that since all the material, whether bone or tooth, was in the same location and subject to the same burial conditions that there would be some comparison possible.

9.4.3 Interpretation of the Sequencing Data

The 141 readable clone sequences represent 31 DNA samples from Merton Priory in Surrey, Gloucester Blackfriars, Chichester's Hospital of St James and St Mary Magdalene, Hereford Cathedral, Ipswich Blackfriars, St James' Abbey in Northampton, St Mary Graces in London, and the Royal Mint Black Death cemetery in London. Only one site, York's Fishergate House, failed to produce any sequencing data. None of these samples share identical sequences indicating that there is no evidence of maternal relatedness on the basis of this data.

Although not identical, the sequences did present some common mutations with 16270, 16192, and 16189 appearing several times. Some of the clones give consensus sequences that can be identified as specific haplogroups (Macaulay and Richards 2008, accessed February 2008; Richards and Macaulay 2000:142). Gloucester Blackfriars sample 104 has mutations at positions 16189 and 16270 which are characteristic of haplogroup U5b, whilst sample 233 has mutations at positions 16192 and 16270, which are characteristic of haplogroup U5a. These are both western Eurasian haplogroups. Gloucester Blackfriars sample 302 has 6 of 6 clones with mutations at positions 16172, 16222, and 16261. These mutations are three of the six mutations that make up the haplogroup J1b1. The other mutations occur at positions 16069, 16126, and 16145 and as primer mtC only covers the region 16147-16294, these would be undetected by the sequencing analysis. It is possible, therefore, that 19/91 302 is haplogroup J1b1,

another western Eurasian haplogroup. Two other samples give consensus sequences – CH86 45 and EL00 3141. CH86 45 has eight of eight clones with mutations at 16192, 16256, and 16270 all of which are characteristic of U5a1, a western Eurasian haplogroup. EL00 3141 has six of six clones with a mutation at point 16270 which is characteristic of U5, also a western Eurasian haplogroup.

Table 31. Identifiable Haplogroups

Sample	Consensus Mutations	No. of Clones	Haplogroup
19/91 104	183 a->c, 189 t->c, 270 c->t	5 of 5	U5b
19/91 233	192 c->t, 270 c->t	2 of 4	U5a
19/91 302	172 t->c, 222 c->t, 261 c->t	6 of 6	J1b1
CH86 45	192 c->t, 256 c->t, 270 c->t	8 of 8	U5a1
EL00 3141	270 c->t	6 of 6	U5

Considering that these samples are from the late medieval period in England, it is not surprising that the individuals presented western Eurasian haplogroups. The remaining sequences either have random mutations at inconsistent points or have no mutations and thus look identical to the Cambridge Reference Sequence (CRS). The CRS is haplogroup H, which represents approximately half of all European lineages and, therefore, it was deemed unlikely that samples with sequences that look the same as CRS would be related individuals, although this is not impossible (Richards *et al.* 1998). Had any of the other samples yielded identical sets of mutations, the samples would have been tested using additional primers that would have expanded the section of HVR1 analysed, and thus allow any additional shared mutations to be identified and inferences made as to whether any of the individuals were related. As this was not the case, these further steps were not necessary.

9.4.4 Genetic Links with DISH

The osteoarchaeological and biomolecular literature suggests that DISH might occur within related individuals. As such, it was expected that some of the individuals with DISH in this study, particularly those in the parish cemeteries, might be related and share haplogroups. Unfortunately, only eight individuals

out of the 31 that were successfully extracted and sequenced were from non-monastic sites so the chance of finding related individuals was reduced. However, although the monastic sites were not expected to contain many related individuals it would not have been impossible to find such relationships. It was common for monastic recruitment to take place in the nearby town so local families often had ties to the nearby monastic houses and, as men could become monks at any age, they may have had wives or children living in the town as well as parents or brothers and sisters (Lawrence 1990:37). It is possible that these family members may have requested burial at the monastery/friary and so related individuals could have been buried within the same monastic site.

Several studies suggest genetic factors for the aetiology of DISH (Havelka *et al.* 2002, Havelka *et al.* 2001, Pappone *et al.* 1996, Sarzi-Puttini and Atzeni 2004, Pappone *et al.* 2005, Crubezy 1996, Gorman *et al.* 2005) though none have yielded any conclusive results. There are also a few published examples of DISH or similar disorders occurring in families (Gorman *et al.* 2005, Matsunaga *et al.* 1999) which supports the theory that a genetic component may exist. Some of the studies suggest that genetic links exist through the HLA (human leukocyte antigen) as it is common in spondyloarthropathies but DISH has not been commonly associated with this antigen so there must be an alternate explanation.

It was one of the goals of this study to examine the samples with DISH to determine if any might be related. If the samples with DISH had turned out to be related to one another it would suggest that DISH was genetic, and more specifically, that it was x-linked. A sex-linked recessive gene on the x-chromosome would result in males expressing the condition more than females as they have only one x-chromosome. Therefore, given the predominance of DISH in males, it makes sense that DISH could be an x-linked recessive condition. However, on the basis of this data there is no evidence for this. Out of the 31 samples that were successfully sequenced, only 10 were from samples with DISH so unfortunately this idea has not been thoroughly explored. Further samples and DNA analysis is needed in order to gain some conclusive results.

9.4.5 Summary of DNA analysis

To summarise the key findings:

- The sequences obtained appear genuine and show little sign of contamination
- 31 of 74 samples were successfully sequenced, 10 of which had DISH
- Five different haplogroups were identified; all Western Eurasian
- There was no evidence of maternal relatedness amongst the samples

The aDNA analysis performed here demonstrates that extraction and sequencing of late medieval skeletal material is possible, even within a short space of time. Despite these skeletal collections being well-researched and having been curated and stored in a variety of ways, viable mtDNA still remained. Nuclear DNA appeared to be damaged and unusable for sexing and other genetic analyses but the mitochrondrial genome was a viable option and researchers should bear this in mind when designing their research questions. The advancement in anti-contamination and amplification methods means that DNA analysis can be a very useful analytical tool for osteoarchaeologists.

The genetic analysis undertaken in this research project provides no evidence for DISH being maternally related. This, however, does not mean that a genetic link does not exist for DISH. There are other ways in which a disease or condition can be genetically conferred, for example, it could be an autosomal rather than sex-linked condition, but these could not be explored within the scope of this project. Even with the data that was presented here, there is no conclusive answer to the question of a maternal link for DISH as the sample size was quite small; it is still an idea to be explored by further research.

9.5 Aetiology of DISH

The idea that DISH is related to monastic diet was one first proposed on the basis of evidence from skeletons excavated from Merton Priory that found a higher prevalence amongst these individuals than in living populations and proposed a disregard for the austere dietary regime of the monastic Rule was to blame

(Waldron 1985). This interpretation was based upon clinical evidence at the time which suggested that DISH may be related to diabetes, and documentary evidence suggesting that monks were eating great amounts of food, mostly meat, and avoiding the dietary regulations imposed upon them. This was followed up with a further article by Rogers and Waldron in 2001, providing more evidence of DISH in late medieval monastic populations and, again, suggesting that monastic diet was a key factor in its aetiology.

Mays (2006:184) has since pointed out that the initial study by Waldron (1985), is statistically flawed as the high prevalence rate for DISH at Merton Priory is based upon only three samples. He demonstrates that DISH is just as prevalent in the non-monastic site of Wharram Percy, a rural site consisting of medieval peasants who show signs of nutritional stress. As demonstrated earlier in this study (see Chapter 2), DISH occurs throughout many different time periods and in many different populations, not all of them monastic or likely to be consuming great quantities of food. It seems unlikely that diet is the common link in all these populations and yet the theory of a gluttonous monastic diet and high prevalence rates of DISH amongst monastic populations pervades. Although the idea of a high calorie, high meat protein monastic diet is only a theory to explain DISH, this type of diet is normally associated with upper class/high status groups and yet it has now often extrapolated as proof that DISH is a condition/disorder indicative of high status. This is a rather dangerous assumption to make given that the monastic diet theory and its relationship to DISH has, before now, never been tested. Bioarchaeologists need to act with caution before accepting a theory such as this.

It is strange to think that the ideas proposed by Waldron in 1985 existed for 15 years before being questioned (Mays 2000b) or before any attempts were made to determine if it was indeed based in fact. The idea of monastic sites having higher prevalence rates of DISH may be a bias within the bioarchaeological data. The late medieval period in Britain has the most skeletons (16,327 versus 7122 in the early medieval and 5716 in the Roman period), and the second most number of sites (63 versus 72 early medieval and 52 Roman), of all the periods in British archaeology (see Roberts and Cox 2003:28) so it seems logical that more cases

of DISH will be found in this period of history. Bioarchaeology, as a discipline, needs to return to a more scientific approach to data and attempt to incorporate all forms of evidence – biological, clinical, documentary, and archaeological – as this study has attempted to do, before making inferences about diseases in the past.

9.6 Future Research

Ultimately, this subject would benefit from further research. New developments in ageing methods (Falys *et al.* 2006) may be able to identify those over 60 years of age and thus help us to gain a better understanding of the progressive nature of DISH. A larger sample size and data set for both the stable isotope and aDNA analysis may help to give more definitive answers to the questions asked of these data. The sample size should be determined on the basis of the statistical tests that will be used to analyse the data. Ideally, the data should include a balance of monastic and non-monastic samples, and a balance of DISH and non-DISH samples, from a variety of geographic locations within the late medieval period. Preferably, the sites, from which these samples are taken, should have detailed documentary and archaeological evidence for diet so that the stable isotope analysis can be more easily interpreted, and inferences made as to whether individuals with DISH are eating the same quantities and types of food. Unfortunately, stable isotope analysis cannot answer the questions about calorie intake nor quantity of food consumed; these can only be answered with documentary, or sometimes archaeological, evidence.

The aDNA analysis can be enhanced by obtaining greater numbers of samples from individuals with DISH from the same cemeteries, preferably non-monastic cemeteries. More time is also required to carry out the detailed analysis needed to obtain more meaningful results. Obtaining DISH samples from the same cemetery greatly enhances the chance of finding a family group or groups and determining whether DISH was present within that family/those families.

Other methods of analysis might be more useful for exploring the aetiology of DISH. Stable isotope analysis is limited in terms of the information it can give and the level of detail. Unfortunately, it is not subtle enough to determine which specific protein sources have been consumed and in what proportions and, without this level of detail, it remains difficult to answer the question of whether DISH is related to protein intake. New methods of stable isotope analysis such as sulphur may be able to help with such interpretations. When used in conjunction with carbon and nitrogen stable isotopes analysis, $\delta^{34}S$ values can help to distinguish whether marine or freshwater protein was being consumed (Richards *et al.* 2003), which may help to add another dimension to the dietary analysis. There are also other analytical techniques, being newly applied to palaeopathology, that can detect pathological biomarkers using Matrix Assisted Laser Desorption Ionization tandem Time of Flight (MALDI TOF/TOF) mass spectrometry and HPLC which may provide some interesting results (Mark *et al.* 2006, Minnikin *et al.* 2006).

Although the dietary and genetic theories still merit further investigation, some other ideas came out of this study which may provide interesting avenues of research. The ideas of sleep deprivation and posture being linked to weight gain and hyperinsulinaemia are concepts that have never been explored in conjunction with monastic data or DISH. The sleep deprivation theory is particularly interesting as it results in hyperinsulinaemia, which is thought to be linked with DISH, but hyperinsulinaemic people are not necessarily obese. Given the findings of this study, that monastic orders may not have been obese, and that all groups of society, not just monks, show evidence of DISH, it is possible that this sleep deprivation theory could have some merit.

Chapter 10: Conclusion

10.1 Chapter Summaries

This first section of the conclusion will summarise the findings of Chapters 2 through 9.

Chapter 2, the "DISH, Diabetes, Obesity" chapter, demonstrated that although occurrences of DISH have been frequently reported amongst late medieval European religious communities, DISH is not confined to any one time period or archaeological context. However, owing to the reported high prevalence rates of DISH in late medieval religious communities, and the suggestions that the reason for this is that these communities were plagued by obesity, type II diabetes, and consumption of large amounts of meat, it was logical to explore these relationships. Within the clinical literature, there were no statistically significant correlations between DISH and diabetes but there were possible links between DISH and hyperinsulinaemia, and some strong correlations with increased weight. Although the potential for obesity exists amongst monastic communities, based on the historical documents, it was concluded that much of this potential depended upon the components of their diet.

Chapter 3, the "Monastic Orders, Lifestyle and Diet" chapter, demonstrated that there were some important differences between monastic orders in terms of both diet and lifestyle that could have affected their propensity to develop DISH. There is evidence that not all monastic communities had the potential for obesity or consumed lots of excess calories, and that there were clear differences in terms of protein consumption, especially between the Augustinian and Dominican orders.

Chapter 4, the "Medieval Diet and Lifestyle" chapter, explored the data available for secular diet amongst the different socio-economic groups in the late medieval period. The nutritional quality of the diet clearly declined as a person moved up

the social scale and, should the links between DISH and protein consumption be true, the diet of the upper classes was deemed to be most likely to contribute to the development of DISH. Some limitations were highlighted; that the differences in diet between different sectors of late medieval society were mostly differences in proportions of food eaten rather than consumption of different foods, which is difficult to differentiate with the use of stable isotope analysis.

Chapter 5, the "Stable Isotopes and Diet" chapter, provided an overview of the field of stable isotope analysis as it pertains to reconstructing past diet. It discussed how this type of analysis could be used to explore one of the potential causes of DISH, that is, diet, and the limitations of such analysis. It concluded that the stable isotope data for monastic populations might be difficult to interpret as they are difficult to place on the social (and hence dietary) scale – the question is: does their diet resemble that of the lower classes or the upper?

Chapter 6, the "Ancient DNA (aDNA) Analysis" chapter, demonstrated how aDNA analysis can be used to explore the question of whether DISH has a genetic component. It provided an overview of the use of aDNA analysis within palaeopathology, the limitations of such analysis, and also demonstrated how little has been done in bioarchaeology to look at potential genetically inherited diseases in the past.

Chapter 7, the "Materials and Methods" chapter, outlined the methodology for this study. It also highlighted the difficulties in obtaining suitable skeletal data for this study and problems that were encountered with using the scientific protocols.

Chapter 8, the "Results" chapter, presented the results of the research. A discussion of the problems encountered with the stable isotope data was also included.

Chapter 9, the "Discussion of Results" chapter, interpreted the data presented in the "Results" chapter. The major conclusions drawn from the isotope data were:

that DISH samples appeared to plot higher in terms of $\delta^{15}N$ values than non-DISH samples that this trend is seen in all data, not just the monastic data that this is significant when comparing all DISH samples to all non-DISH samples obtained in this study and, that differences between the Augustinians, Cistercians, and Dominicans were seen isotopically.

Alternative explanations to diet were considered for the high $\delta^{15}N$ values – that the levels seen could be affected by the pathological lesions. Alternative explanations were also considered for the possible link between DISH and protein – that it could be due to sleep deprivation and hyperinsulinaemia. It was concluded that more data was needed in order to determine whether the pattern of high $\delta^{15}N$ values was statistically significant for all data.

The major conclusions from the aDNA data were that the sequences appeared genuine and endogenous and that, on the basis of the data obtained, DISH is not maternally linked. It was also pointed out that it is still possible for DISH to have a genetic component and that, ultimately, more data are needed.

10.2 Objectives of this Study

The purpose of this research was to test two of the major hypotheses surrounding the aetiology of DISH:

1. That DISH may be related to diet
2. That DISH may be genetically linked;

Essentially, the objectives in terms of dietary analysis were three-fold:

1. To compare the diets of monastic and non-monastic, DISH and non-DISH populations
2. To identify the components of these diets and proportions, if possible
3. To establish, if any, links between animal protein and DISH.

The objective of the aDNA analysis was to determine whether individuals with DISH were maternally related.

10.2.1 Were the Objectives Met?

The objectives were met in the sense that the two major hypotheses - whether DISH was related to diet, and whether DISH was genetically linked - were tested. However, both hypotheses could have been better explored with a larger sample size. The initial goal of this study was to compare monastic and non-monastic sites from within the same geographic area, in order to reduce the number of variables being tested – i.e. to obtain samples that would have had access to similar food resources. Some of the initially proposed sites were key sites for the medieval period and ones that had high prevalence rates for DISH but, unfortunately, several of these sites were unavailable due to other researchers taking priority, or skeletal collections being moved/re-housed. The fact that many of the sites were unpublished also posed problems, both with access and with trying to gain background information for the sites, especially in terms of diet. The lack of specificity with regards to foods eaten within different sites meant that the proportions of foods consumed by different groups could not be explored.

One of the major issues with trying to establish whether DISH was related to diet was the sample size for the stable isotope analysis. Initially, there were 93 rib samples for analysis but, owing in part to the problems with the original laboratory analysis in the Department of Earth Sciences at Durham University, this sample size was halved to 46 samples. The original samples had been run and re-run so many times that many did not have any collagen left for further analysis, for some samples there was only a small amount of bone remaining that could be used to derive more collagen (and this did not produce enough to be efficiently analysed), and some had no bone remaining to do any analysis. Of course, some of the samples were simply not good quality and failed to fall within the acceptable range for C/N ratios. The original sample size would have posed no problems with establishing whether comparisons between different groups of samples were statistically significant or not as they would have been a large enough data set to have been robust. The problem with having a smaller sample size is that it becomes difficult to determine whether a comparison that

does not produce a statistically significant result is real or due to a small sample size. This has made interpretation of the stable isotope data difficult.

The issue with trying to establish whether DISH was genetically linked is down to time rather than sample size, although a larger sample size of genetically viable samples would have been beneficial. To carry out a detailed genetic analysis of the individuals sampled for this study would be a PhD thesis in itself. The eventual methodology of using mtDNA to test for maternal relatedness was the quickest and simplest way of determining whether there was any potential for a genetic link for DISH. Unfortunately, many of the samples that produced viable DNA and good sequences were from monastic individuals without DISH, so exploring the question of whether individuals with DISH were related was difficult. There was, however, nothing that could have been done to improve the number of successful sequences, within the sample size obtained and within the time frame given; this was simply the nature of aDNA work.

10.2.2 Major Interpretations of the Data

10.2.2.1 Is DISH Related to Diet?

Based on the findings of this study, the question of whether DISH is related to diet is not straightforward. The general pattern of DISH samples producing higher $\delta^{15}N$ for all comparisons strongly suggests that there is a dietary difference between individuals with DISH and individuals without DISH. However, although the comparison of all 46 samples with and without DISH, and all monastic samples with and without DISH, give a statistically significant difference for $\delta^{15}N$, the other comparisons are not significant. It is also possible that this pattern of high $\delta^{15}N$ values for individuals with DISH is in some way related to the disease process.

10.2.2.2 Is DISH Genetically Linked?

The answer to this question is "possibly". Based on the data obtained in this study, there is no evidence for individuals with DISH being maternally related. However, the number of DISH samples for which sequences were obtained was small, so this is not a definitive answer. More data is needed in order to explore this question fully.

10.3 Conclusion

Based on the present study, there does appear to be evidence to support the theory that DISH is related to diet. However, this difference between the dietary patterns of those with DISH and those without is not restricted to the monastic samples. All samples - male, female, monastic and non-monastic, high and low status – display the same isotopic differences between those with DISH and those without. On the basis of this evidence, it would seem that the idea of DISH being a monastic phenomenon, and one indicative of high status, to be unjustifiable.

Bioarchaeologists also need to re-visit the concept of how disease affects stable isotope measurements. Although Katzenberg and Lovell (1999) investigated this briefly with pathological bone specimens, it needs to be looked at in more depth and the $\delta^{15}N$ values compared between diseased and non-diseased skeletons, including sampling from sites without any macroscopic signs of lesions or disease, whilst controlling for diet. Isotope analysis, in general, needs more time to develop as a discipline and the techniques of dietary analysis need to be refined before we can apply this to complex questions of disease and diet. At present, carbon and nitrogen stable isotopes are not sophisticated enough to provide the level of detail required by bioarchaeologists for these investigations.

Overall, this study provides a starting point for the investigation into the aetiology of DISH. Given the many different links with the condition (metabolic, dietary, genetic etc) and the many different sectors of society affected, it seems that DISH may in fact have multiple aetiologies. All of these avenues of research

should be investigated before bioarchaeologists can begin to understand, and make inferences about, this very interesting condition that we call DISH.

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Appendix A: aDNA Sequence Alignment

The results of the sequencing were combined and presented here as a single alignment. EL00 3127 and EL00 3266 were not available in electronic format so their raw data is not presented here.

```
                  16150       16160       16170       16180       16190
                  ...|....|....|....|....|....|....|....|....|....|....|.
CRS               CCACC TGT AGT ACAT AAAAACCC  AAT CCA CA T C  AAAAC
Rosa              ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-1        ..... ... . . .... ........ ... ... .. . .  .....
19/91 92-2        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-3        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-4        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-5        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-6        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-7        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-8        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-9        ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-10       ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-12       ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-13       ..... ... ... .... ........ ... ... .. . .  .....
19/91 92-14       ..... ... ... .... .... ... ... ... .. . .  .....
19/91 104-1       ..... ... ... .... ........ ... ... .. . .  ...C.
19/91 104-2       ..... ... ... .... ........ ... ... .. . .  ...C.
19/91 104-3       ..... ... ... .... ........ ... ... .. . .  ...C.
19/91 104-5       ..... ... ... .... ........ ... ... .. . .  ...C.
19/91 104-6       ..... ... ... .... ........ ... ... .. . .  ...C.
19/91_212-1       ..... ... ... .... ........ ... ... .. . .  .....
19/91_212-2       ..... ... ... .... ........ ... ... .. . .  .....
19/91_212-3       ..... ... ... .... ........ ... ... .. . .  .....
19/91_212-5       ..... ... ... .... ........ ... ... .. . .  .....
19/91_212-6       ..... ... ... .... ........ ... ... .. . .  .....
19/91_233-1       ..... ... ... .... ........ ... ... .. . .  .....
19/91 233-2       ..... ... ... .... ........ ... ... .. . .  .....
19/91 233-3       ..... ... ... .... ........ ... ... .. . .  .....
19/91 233-5       ..... ... ... .... ........ ... ... .. . .  .....
19/91 257-1       ..... ... ... .. . ........ ... ... .. . .  .....
19/91 257-2       ..... ... ... .... ........ ... ... .. . .  .....
19/91_302-1       ..... ... ... .... ........ ..C ... .. . T  .....
19/91_302-2       ..... ... ... .... ........ ..C ... .. . .  .....
19/91_302-3       ..... ... ... .... ........ ..C ... .. . .  .....
19/91_302-4       ..... ... ... .... ........ ..C ... .. . .  .....
19/91_302-5       ..... ... ... .... ........ ..C ... .. . .  .....
19/91_302-6       ..... ... ... .... ........ ..C ... .. . .  .....
19/91_420-1       ..... ... ... .... ........ ... ... .. . .  .....
19/91_420-2       ..... ... ... .... ........ ... ... .. . .  .....
19/91_420-3       ..... ... ... .... ........ ... ... .. . .  .....
19/91_420-4       ..... ... ... .... ........ ... ... .. . .  .....
19/91_420-5       ..... ... ... .... ........ ... ... .. . .  .....
19/91_420-6       ..... ... ... .... ........ ... ... .. . .  .....
CH86 45-3         ..... ... ... .... ........ ... ... .. . .  .....
CH86 45-4         ..... ... ... .... ........ ... ... .. . .  .....
CH86 45-5         ....A ... ... .... ........ ... T.TA.. . T  .....
CH86 45-6         ..... ... ... .... ........ ... ... ..C .  .....
CH86 45-7         ..... ... ... .... ........ ... ... .. . .  .....
CH86 45-8         ..... ... ... .... ........ ... ... .. . .  .....
CH86 45-9         ..... ... ... .... ........ ... ... .. . .  .....
CH86 45-10        ..... ... ... .... ........ ... ... .. . .  .....
EL00-3005-1       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3005-2       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3073-2       ..... ... ... .... .... ... ... ... .. . .  .....
EL00-3073-3       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3079-1       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3079-2       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3079-3       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3079-4       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3079-5       ..... ... ... .... ........ ... ... .. . .  .....
EL00-3079-6       ..... ... ... .... ........ ... ... .. . .  .....
EL00_3095-2_      ..... ... ... .... ........ ... ... .. T.  .....
EL00_3095-3       ..... ... ... .... ........ ... ... .. . .  .....
EL00_3095-4       ..... ... ... .... ........ ... ... .. . .  .....
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EL00_3095-5
EL00_3095-6
EL00_3095-7
EL00_3095-8
EL00-3100-1
EL00-3100-2
EL00-3100-4
EL00-3100-9
EL00_3116-2
EL00_3116-3
EL00_3116-4
EL00_3116-5
EL00_3116-6
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EL00_3141-2_
EL00_3141-3
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EL00_3141-6_
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EL00_3285-2
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EL00_3285-5
EL00_3285-6_
EL00_3285-7
EL00_3285-8
EL00_3285-9
EL00_3285-10
He93A-606-2
He93A-606-3
He93A_1997-1
He93A_1997-2
He93A_1997-3
He93A_1997-4
He93A_1997-5
IAS 4801_1451-2
IAS 4801 1762-1
IAS 4801 1762-2
IAS 4801 1762-3
IAS 4801 1762-4
IAS 4801 1762-5
IAS 4801 1762-6
IAS 4801 1762-7
IAS 4801 1762-8
IAS 4801 1762-9
IAS 4801 2005-2
IAS 4801 2005-3
IAS 4801 2005-4
IAS 4801 2005-5
IAS 4801 2005-6
IAS 4801 2005-7
IAS 4801 2005-8
IAS 4801 2005-9
IAS 4801_2642-1_
IAS 4801_2642-2_
IAS 4801_2642-3_
IAS 4801_2642-4_
IAS 4801_2642-5
IAS 4801_2642-6
MIN 86 BD_5960-5
MIN 86 LG_12356-1_
MIN 86 LG_12356-2
MIN 86 LG_12356-4_
MIN 86 LG_12356-5_
MIN 86 LG_12687-4_
MIN 86 LG_12687-5
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MIN 86 LG_12687-10    .....-...-...-....-........--...-..A..-.-.--.....
MPY 86_623-3          .....-...-...-....-........--...-..A..-.-.--.....
MPY 86_623-5          .....-...-...-....-........--...-...-..-.-.--.....
MPY 86_754-1          .....-...-...-....-........--...-...-..-.-.--.....
MPY 86 754-2          .....-...-...-....-........--...-...-..-.-.--.....
MPY 86 754-3          .....-...-...AT...A........--...-...G..-.-.--.....
MPY 86_4282-1_        .....-...T...-....T........--...T...-..-.T.--.....
MPY 86_4282-4_        .....-...-...-....-........--...-...-..-.-.--.....
MPY 86_4282-5_        .....-...T...-....-........--...T...-..-.T.--.....
MPY 86_4282-6_        .....-...-...-....-........--...-...-..-.-.--.....
MPY 86_16344-1        .....-...-...-....-........--...-...-..-.-.--...G.
MPY 86 16344-9        .....-...-...-....-........--...-...-..-.-.--...G.

                      16200        16210        16220        16230        16240
                      ...|....|....|....|....|....|....|....|....|....|.
CRS                   CCCCTCCCC---AT-GCTTAC-AAGCAAG--TAC-AGCAA---------T-
Rosa                  .........---..-......-.......--...-.....---------.-
19/91 92-1            .........---..-......-.......--...-.....---------.-
19/91 92-2            .........---..-......-.......--...-.....---------.-
19/91 92-3            .........---..-......-.......--...-.....---------.-
19/91 92-4            .........---..-......-.......--...-.....---------.-
19/91 92-5            .........---..-A.....-.......--...-.....---------.-
19/91 92-6            .........---..-......-.......--...-.....---------.-
19/91 92-7            .........---..-......-.......--...-.....---------.-
19/91 92-8            .........---..-......-.......--...-.....---------.-
19/91 92-9            .........---..-A.....-A......--...-.....---------.-
19/91 92-10           .........---..-......-.......--...-.....---------.-
19/91 92-12           .........---..-......-.......--...-.....---------.-
19/91 92-13           .........---..-......-.......--...-.....---------.-
19/91 92-14           .........---..-......-.......--...-.A...---------.-
19/91 104-1           ....C....---..-......-.......--...-.....---------.-
19/91 104-2           ....C....---..-......-.......--...-.....---------.-
19/91 104-3           ....C...C---..-......-......A--...-.....---------.-
19/91 104-5           ....C...C---..-......-.......--...-.....---------.-
19/91 104-6           ....C....---..-......-A......--...-.....---------.-
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19/91_212-3           .........---..-......-.......--...-.....---------.-
19/91_212-5           .........---..-......-.......--...-.....---------.-
19/91_212-6           .........---..-......-.......--...-.....---------.-
19/91_233-1           .......T.---..-......-.......--...-....G---------.-
19/91 233-2           .........---..-......-.......--...-.....---------.-
19/91 233-3           .........---..-......-.......--...-.....---------.-
19/91 233-5           .......T.---..-......-.......--...-.....---------.-
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19/91 257-2           .........---..-......-.......--...-.....---------.-
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19/91_302-2           .........---G.-......-.......--...-.....---------.-
19/91_302-3           .........---..-......-.......--...-.....---------.-
19/91_302-4           .........---..-......-.......--...-.....---------.-
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19/91_302-6           .........---..-......-.......--...-.....---------.-
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19/91_420-3           .........---..-......-.......--...-.....---------.-
19/91_420-4           .........---..-......-.......--...-.....---------.-
19/91_420-5           .........---..-......-.......--...-.....---------.-
19/91_420-6           T........---..-......-.......--...-.....---------.-
CH86_45-3             .......T.---..-......-.......--...-.....---------.-
CH86 45-4             .......TT---..-......-.......--...-.....---------.-
CH86 45-5             .......T.---..-.T....-.......--...-.....---------.-
CH86 45-6             .......T.---..-......-...GC.AG-...-.....---------.-
CH86 45-7             .......T.---..-......-.......--...-.....---------.-
CH86 45-8             .......T.---..-......-.......--...-.....---------.-
CH86 45-9             .......T.---..-......-.......--...-.....---------.-
CH86 45-10            .......T.---..-......-.......--...-.....---------.-
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EL00-3005-1          .........~~..~~.......T...~~...~~.....~~~~~~~~~~.~
EL00-3005-2          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3073-2          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3073-3          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3079-1          .T..C....~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3079-2          .T..C....~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3079-3          .T..C....~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3079-4          .T.....T.~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3079-5          .T..C....~~..~~.......A.....~~...~~.....~~~~~~~~~~.~
EL00-3079-6          .T..C....~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3095-2_         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3095-3          .........~~..~~.............~~...~~.....~~~~~~~~T~.~
EL00_3095-4_         .........~~..A..............~~...~~.....~~~~~~~~~~.~
EL00_3095-5          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3095-6          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3095-7          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3095-8          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3100-1          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3100-2          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3100-4          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00-3100-9          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3116-2          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3116-3          ......TT.~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3116-4          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3116-5          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3116-6          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3141-1_         ....~....~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3141-2_         .........~~..~~.......A.....~~...~~.....~~~~~~~~C~.~
EL00_3141-3          .........~~..~~.............~~...A...~~~~~~~~~~.~
EL00_3141-4          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3141-5_         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3141-6_         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-1          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-2          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-4          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-5          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-6_         .........~~..~~.....A...A...~~...~~.....~~~~~~~~~~.~
EL00_3285-7          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-8          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-9          .........~~..~~.............~~...~~.....~~~~~~~~~~.~
EL00_3285-10         .........~~..~~.....A.......~~...~~.....~~~~~~~~~~.~
He93A-606-2          .........~~..~~.....A...A...~~...~~.....TACAGCAA~~.~
He93A-606-3          .........~~..~~.....A...A...~~...~~.....~~~~~~~~~~.~
He93A_1997-1         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
He93A_1997-2         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
He93A_1997-3         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
He93A_1997-4         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
He93A_1997-5         .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801_1451-2      A..TA...AC~~.C~~.C.C.ACCTGT..~~...~~.TA..~~~~~~~~~~.~
IAS 4801_1762-1      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-2      ...T.....~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-3      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-4      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-5      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-6      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-7      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-8      .........~~..~~.....A.......~~...~~.....~~~~~~~~~~.~
IAS 4801 1762-9      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-2      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-3      .....T..T~~..~~.T...T.......~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-4      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-5      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-6      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-7      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-8      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
IAS 4801 2005-9      .........~~..~~.............~~...~~.....~~~~~~~~~~.~
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IAS 4801_2642-1_       .........   .. ....... .......  ... .....          .
IAS 4801_2642-2_       .........   .. ....... .......  ... .....          .
IAS 4801_2642-3_       .....T..TCC .. ....... .......  ... .....          .
IAS 4801_2642-4_       ....C....   .. ....... .......  ... .....          .
IAS 4801_2642-5_       .........   .. ....... .......  ... .....          .
IAS 4801_2642-6        .........   .. ....... .......  ... .....          .
MIN 86 BD_5960-5       .........   .. .....TT.......  ... .....          T.
MIN 86 LG_12356-1_     .........   .. ...... C.AGC.AGT... ...C.AT         .
MIN 86 LG_12356-2_     .........   ..T...... ....C.AGT...C...C.AT         .C
MIN 86 LG_12356-4_     .........   .. ....... .......  ... .....          T.
MIN 86 LG_12356-5_     .........   .. ....... .......  ... .....          T.
MIN 86 LG_12687-4_     ATAAAAA..CAGTG  GG..GG.TA.C.ACAA..CTA.CCACAAGGG
MIN 86 LG_12687-5_     .........   .. ....... .......  ... .....          .
MIN 86 LG_12687-10     .........   .. ....... .......  ... .....          .
MPY 86_623-3           .........   .. ....... .......  ... .....          .
MPY 86_623-5           .........   .. ....... .......  ... .....          .
MPY 86_754-1           .........   .. ....... .......  ... .....          .
MPY 86 754-2           .........   .. .A..... .......  ... .....          .
MPY 86 754-3           .........   .. .A..... .......  ... .....          .
MPY 86_4282-1_         .........   .. ....... .......  ... .....          T.
MPY 86_4282-4_         .........   .. ....... .......  ... .....          .
MPY 86_4282-5_         .........   .. ....... .......  ... .....          T.
MPY 86_4282-6_         .........   .. ....... .......  ... .....          .
MPY 86_16344-1         .........   .. ....... .......  ... .....          .
MPY 86 16344-9         .........   .. ....... .......  ... .....          .


                        16250     16260     16270     16280     16290
                       ...|....|....|....|....|....|....|....|....|....|.
CRS                    CAACCCT CAA CT AT C ACACAT CAACTG CAACTCCAAAG CCAC
Rosa                   .....T. ... .. .. . ...... ...... ........... ....
19/91 92-1             ....... ... .. .. . ...... ...... ..........A ....
19/91 92-2             ....... ... .. .. . ...... ...... ..........A ....
19/91 92-3             ....... ... .. .. . ...... ...... ........... ....
19/91 92-4             ....... ... .. .. . ...... ...... ........... ....
19/91 92-5             ....... ... .. .. . ...... ...... ........... ....
19/91 92-6             ....... ... .. .. . ...... ...... ........... ....
19/91 92-7             ....... ... .. .. . ...... ...... ........... ....
19/91 92-8             ....... ... .. .. . ...... ...... ........... ....
19/91 92-9             ....... ... .. .. . ...... ...... ........... ....
19/91 92-10            ....... ... .. .. . ...... ...... ..........A ....
19/91 92-12            ....... ... .. .. . ...... ...... ........... ....
19/91 92-13            ....... ... .. .. . ...... ...... ........... ....
19/91 92-14            ....... ... .. .. . ...... ...... ........... ....
19/91 104-1            ....... ... .. .. . ...... .....A ...........G....
19/91 104-2            ....... ... .. .. . ...... ...... ........... ....
19/91 104-3            ....... ... .. .. . ....T. ...... ...........A....
19/91 104-5            ....... ... .. .. . ...... ...... ...........G....
19/91 104-6            ....... ... .. .. . ...... .....AG..........AG....
19/91_212-1            .......T... .. .. . ......T...... ........... ....
19/91_212-2            .....T. ... .. .. . ...... ...... .C......... ....
19/91_212-3            .....T. ... .. .. . ...... ...... ........... ....
19/91_212-5            .....T. ... .. .. . ......T...... ........... ....
19/91_212-6            .....T. ... .. .. . ......T...... ........... ....
19/91_233-1            ....... ... .. .. . ...... ...... ........... ....
19/91 233-2            T...T.C ... .. .. . .T.... ...T.. ...T.TT.... T...
19/91 233-3            ....... ... .. .. . ...... ...... ........... ....
19/91 233-5            ....... ... .. .. . ...... ...... ........... ....
19/91 257-1            ....... T.. .. .. . ...... ...... ........... ....
19/91 257-2            ....... ... .. .. . ...... ...... ........... ....
19/91_302-1            ....T.. ... .. ..T. ......T...T.. ........... ....
19/91_302-2            ....T.. ... .. .. . ...... ...... ........... ....
19/91_302-3            ....T.. ..G .. .. . ...... ...... ..........A ....
19/91_302-4            ....T.. ... .. .. . ...... ...... ........... ....
19/91_302-5            .G..T.. ... .. .. . ...... ...... ........... ....
19/91_302-6            ....T.. ... .. .. . ...... ...... .....T..... ....
19/91_420-1            ....... ... .. .. . ...... ...... ........... ....
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19/91_420-2          ..........................................................
19/91_420-3          ..........................................................
19/91_420-4          ..........................................................
19/91_420-5          ..........................................................
19/91_420-6          .......T..................................................
CH86 45-3            ..........................................................
CH86 45-4            ..........................................................
CH86 45-5            ..........................................................
CH86 45-6            .........G................................................
CH86 45-7            ..........................................................
CH86 45-8            ..........................................................
CH86 45-9            ..........................................................
CH86 45-10           ..........................................................
EL00-3005-1          ..........................................................
EL00-3005-2          ..........................................................
EL00-3073-2          ..........................G...............................
EL00-3073-3          ......CT..................G...............................
EL00-3079-1          ..........................................................
EL00-3079-2          ..........................................................
EL00-3079-3          ..........................................................
EL00-3079-4          ..........................................................
EL00-3079-5          ..........................................................
EL00-3079-6          ..........................................................
EL00_3095-2_         ..........................................................
EL00_3095-3          .........T..T.....T.....................G.....................
EL00_3095-4_         ..........................................................
EL00_3095-5          ..........G...............................................
EL00_3095-6          ..........................................................
EL00_3095-7          ..........................G...............................
EL00_3095-8          ..........................................................
EL00-3100-1          ..........................................................
EL00-3100-2          ..........................................................
EL00-3100-4          ..........................................................
EL00-3100-9          ..........................................A...............
EL00_3116-2          ..........................................................
EL00_3116-3          ..........................................................
EL00_3116-4          ..........................................................
EL00_3116-5          ..........................................................
EL00_3116-6          ..........................................................
EL00_3141-1_         ..........................A...............................
EL00_3141-2_         .........T.....T..........................................
EL00_3141-3          ..........................................................
EL00_3141-4          ..........................................................
EL00_3141-5_         ..................T.......................................
EL00_3141-6_         .G........................................................
EL00_3285-1          ..........................................................
EL00_3285-2          ..........................................................
EL00_3285-4          T.........................................................
EL00_3285-5          ..........................................................
EL00_3285-6_         ..........................................................
EL00_3285-7          ..........................................................
EL00_3285-8          ..........................................................
EL00_3285-9          ..........................................................
EL00_3285-10         ..........................................................
He93A-606-2          ..........................................................
He93A-606-3          ..........................................................
He93A_1997-1         ..........................................................
He93A_1997-2         ..........................................................
He93A_1997-3         ..........................................................
He93A_1997-4         ..........................................................
He93A_1997-5         ..........................................................
IAS 4801_1451-2      .....ACT.G.GAT.G..A...A.CCT.C.CA..C.....CCTGTAGT
IAS 4801 1762-1      ...T......................................................
IAS 4801 1762-2      ...T......................................................
IAS 4801 1762-3      ...T......................................................
IAS 4801 1762-4      ...T......................................................
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IAS 4801 1762-5      ...T... ... .. .. . ...... ...... ...........G....
IAS 4801 1762-6      ...T... ... .. .. . ...... ...... ..........A ....
IAS 4801 1762-7      ...T... ... .. .. . ...... ...... ........... ....
IAS 4801 1762-8      ...T... ... .. .. . ...... ...... ........... ....
IAS 4801 1762-9      ...T... ... .. .. . ...... ...... ..........A ....
IAS 4801 2005-2      ....... ... .. .. . ...... ...... ...........C.A .
IAS 4801 2005-3      ....... ... .. .. . ...... ...... ........... ....
IAS 4801 2005-4      ....... ... .. .. . ...... ...... ........... ....
IAS 4801 2005-5      ....... ... .. .. . ...... ...... ........... ....
IAS 4801 2005-6      ....T.. ... .. .. . ...... ...... ..........G....
IAS 4801 2005-7      ....... ... .. .. . ...... ...... ........... ....
IAS 4801 2005-8      ....... ... .. .. . ...... ...... ........... ....
IAS 4801 2005-9      ....... ... .. .. . ...... ...... ........... ....
IAS 4801_2642-1_     .....T. ... .. .. . ...... ...... ........... ....
IAS 4801_2642-2_     .....T. ... .. .. . ...... ...... ........... ....
IAS 4801_2642-3_     ....... ... .. .. . ...T..T...... ........... .T.T
IAS 4801_2642-4_     ....... ... .. .. . ...... ...... ........... ....
IAS 4801_2642-5_     .....T. ... .. .. . ...... ...... ........... ....
IAS 4801_2642-6      .....T. ... .. .. . ...... ...... ........... ....
MIN 86 BD_5960-5     ....TTC ... .. ..T ......T...... ........... ....
MIN 86 LG_12356-1_   ....... ... .. .C . ......T...... ........... ....
MIN 86 LG_12356-2_   ....... ... .. .C .C......T...... ........... ....
MIN 86 LG_12356-4_   ....... ... .. .C . ......T...... ........... ....
MIN 86 LG_12356-5_   ....... ... .. .C . ......T...... ........... ....
MIN 86 LG_12687-4_         .GAATTC .AG C         .CA.TGGC.G.CG .TACT..TGG.T
MIN 86 LG_12687-5_   .....T. ...T. .. . ...... ...T.. ........... ....
MIN 86 LG_12687-10   ....... ... .. .. . ...... ...... ........... ....
MPY 86_623-3         ....... ... .. .. . ...... ...... ........... ....
MPY 86_623-5         ....... ... .. .. . ...... ...... ........... ....
MPY 86_754-1         ....... ... .. .. . ...... ...... ........... ....
MPY 86 754-2         ....... ... .. .. . ...... ...... ........... ....
MPY 86 754-3         ....... ... .. .. . ...... ...... ........... ....
MPY 86_4282-1_       .....T. ... .. ..T. ......T...... ........... ....
MPY 86_4282-4_       ....... ... .. .. . ......T...... ........... ....
MPY 86_4282-5_       ....... ... .. ..T. ......T...... ........... ....
MPY 86_4282-6_       ....... ... .. .. . ......T...... ........... ....
MPY 86_16344-1       ......C ... .. .. . ...... ...... ........... ....
MPY 86 16344-9       ......C ... .. .. . ...... ...... ........... ....

                       16300     16310     16320     16330     16340
                     ...|....|....|....|....|....|....|....|....|....|.
CRS                   CCCT  CACC CA CT A GGA  TACC      AACAAA         C
Rosa                  ....  .... .. .. . ...  ....      ......         .
19/91 92-1            ....  .... .. .. . ...  ....      ......         .
19/91 92-2            ....  .... .. .. . ...  ....      ......         .
19/91 92-3            ....  .... .. .. . ...  ....      ......         .
19/91 92-4            ....  .... .. .. . ...  ....      ......         .
19/91 92-5            ....  .... .. .. . ...  ....      ......         .
19/91 92-6            ....  .... .. .. . ...  ....      ......         .
19/91 92-7            ....  .... .. .. . ...  ....      ......         .
19/91 92-8            ....  .... .. .. . ...  ....      ......         .
19/91 92-9            ....  .... .. .. . ...  ....      ......         .
19/91 92-10           ....  .... .. .. . ...  ....      ......         .
19/91 92-12           ....  .... .. .. . ...  ....      ......         .
19/91 92-13           ....  .... .. .. . ...  ....      ......         .
19/91 92-14           ....  .... .. .. . ...  ....      ......         .
19/91 104-1           ....  .... .. T. . ...  ....      ......         .
19/91 104-2           ....  .... .. T. . ...  ....      ......         .
19/91 104-3           ....  .... .. T. . ...  ....      ......         .
19/91 104-5           ....  .... .. T. . ...  ....      ......         .
19/91 104-6           ....  .... .. T. . ...  ....      ......         .
19/91_212-1           ....  .... .. .. . ...  ....      ......         .
19/91_212-2           ....  .... .. .. . ...  ....      ......         .
19/91_212-3           ....  ..T. .. .. . ...  ....      ......         .
19/91_212-5           ....  .... .. .. . ...  ....      ......         .
19/91_212-6           ....  .... .. ..T. ...  ....      ......         .
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19/91 233-1       ....  ....  ..T.  ....  ....  ......
19/91 233-2       ....  ...T T.  ..  ....  ....  ......
19/91 233-3       ....  ....  ..  ..  ....  ....  ......
19/91 233-5       ....  ....  ..T.  ....  ....  ......
19/91 257-1       ....  ....  ..T.  ....  ....  ......
19/91 257-2       ....  ....  ..  ..  ....  ....  ......
19/91_302-1       .T..  ....  ..  ..  ....  ....  ...C..A
19/91_302-2       .T..  ....  ..  ..  ....  ....  ......
19/91_302-3       .T..  ....  ..  ..  ....  ....  ......
19/91_302-4       .T..  ....  ..  ..  ....  ....  ......
19/91_302-5       .T..  ....  ..  ..  ....  ....  ......
19/91_302-6       .T..CT....  ..  ..  ....  ....  ......
19/91_420-1       ....  ....  ..  ..  ....  ....  ......
19/91_420-2       ....  ....  ..  ..  ....  ....  ......
19/91_420-3       ....  ....  ..  ..  ....  ....  ......
19/91_420-4       ....  ....  ..  ..  ....  ....  ......
19/91_420-5       ....  ....  ..  ..  ....  ....  ......
19/91_420-6       .TT.  ....  ..T.  ....  ....  ......
CH86 45-3         ....  ....  ..T.  ....  ...C..  ......
CH86 45-4         ....  ....  ..T.  ....  ....  ......
CH86 45-5         ....  ....  ..T.  ....  ....  ......
CH86 45-6         ....  ..G.C .GT.  .T..  ..TACCGTC..T..C
CH86 45-7         ....  ..A.  .CAT.  ....  ...C....  ....
CH86 45-8         ....  T...  .TAT.  ....  ...C....  ....
CH86 45-9         ....  ....  ..T.  ....  ....  ......
CH86 45-10        ....  ....  ..T.  ....  ....  ......
EL00-3005-1       ....  ....  ..  ..  ....  ....  ......
EL00-3005-2       T...  ....  ..  ..  ....  ....  ......
EL00-3073-2       ....  ....  ..  ..  ....  ....  ......
EL00-3073-3       ....  ....  ..  ..  ....  ....  ......
EL00-3079-1       ....  ....  ..  ..  ....  ....  ......
EL00-3079-2       ....  ....  ..  ..  ....  ....  ......
EL00-3079-3       ....  ....  ..  ..  ....  ....  ......
EL00-3079-4       ....  ....  ..  ..  ....  ....  ......
EL00-3079-5       ....  ....  .G  ..  ..A.  ....  ......
EL00-3079-6       ....  ....  ..  ..  ....  ....  ......
EL00_3095-2_      ....  ....  ..  ..  ....  ....  ......
EL00_3095-3       ....  ....C .  ..T.  ...AT....  A.C....  CCNACT
EL00_3095-4_      ....  ....  ..  ..  ....  ....  ......
EL00_3095-5       ....  ....  ..  ..  ....  ....  ......
EL00_3095-6       ....  ....  ..  ..  ....  ....  ......
EL00_3095-7       ....  ....  ..  ..  ....  ....  ......
EL00_3095-8       ....  ....  ..  ..  ....  ....  ......
EL00-3100-1       ....  ....  ..  ..  ....  ....  ......
EL00-3100-2       ....  ....  ..  ..  ....  ....  ......
EL00-3100-4       ....  ....  ..  ..  ....  ....  ......
EL00-3100-9       ....  ....  ..  ..  ....  ....  ......
EL00_3116-2       ....  ....  ..  ..  ....  ....  ......
EL00_3116-3       ....  ....  ..  ..  ....  ....  ......
EL00_3116-4       ....  ....  ..  ..  ....  ....  ......
EL00_3116-5       ....  ....  ..  ..  ....  ....  ......
EL00_3116-6       ....  ....  ..  ..  ....  ....  ......
EL00_3141-1_      ....  ....  ..T.T.  ....  ....  ......
EL00_3141-2_      ....C.....  ..T.T.  ...AT....AACCA...CT.CCCACCAAAG
EL00_3141-3       ....  ....  ..T.  ....  ....  ......
EL00_3141-4       ....  ....  ..T.  ....  ....  ......
EL00_3141-5_      ....  ....  ..T.  ....  ....  ......
EL00_3141-6_      ....  ....  ..T.  ....  ....  .....
EL00_3285-1       ....  ....  ..  ..  ....  ....  ......
EL00_3285-2       ....  ....  ..  ..  ....  ....  ......
EL00_3285-4       ....  ....  ..  ..  ....  ....  ......
EL00_3285-5       ....  ....  ..  ..  ....  ....  ......
EL00_3285-6_      ....  ....  ..  ..  ....  ....  ......
EL00_3285-7       ....  ....  ..  ..  ....  ....  ......
EL00_3285-8       ....  ....  ..  ..  ....  ....  ......
EL00_3285-9       ....  ....  ..  ..  ....  ....  ......
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EL00_3285-10
He93A-606-2
He93A-606-3
He93A_1997-1
He93A_1997-2          T.T.
He93A_1997-3
He93A_1997-4
He93A_1997-5
IAS 4801_1451-2       A.A.AAA.A.  .CA..
IAS 4801 1762-1
IAS 4801 1762-2
IAS 4801 1762-3
IAS 4801 1762-4
IAS 4801 1762-5
IAS 4801 1762-6
IAS 4801 1762-7
IAS 4801 1762-8
IAS 4801 1762-9           C..  T.                C.
IAS 4801 2005-2       ...C
IAS 4801 2005-3       ...C
IAS 4801 2005-4       ...C
IAS 4801 2005-5       ...C
IAS 4801 2005-6       ...C
IAS 4801 2005-7       ...C
IAS 4801 2005-8       ...C
IAS 4801 2005-9       ...C    ...T
IAS 4801_2642-1_
IAS 4801_2642-2_
IAS 4801_2642-3_      T.
IAS 4801_2642-4_
IAS 4801_2642-5_
IAS 4801_2642-6_
MIN 86 BD_5960-5            .CA.CT.     T.       A.C.
MIN 86 LG_12356-1_    ..TCTC.          ..GAT.     NA.CNN..  C NCNACN
MIN 86 LG_12356-2_    .T.. C....C..    ..GAT.     CN...NN.ACC CCNAC.
MIN 86 LG_12356-4_    .T..       ..C .T.  ...A      A.C.
MIN 86 LG_12356-5_    .T..    ....C..C .T.  ... A.
MIN 86 LG_12687-4_    C.GA GCTCGGTACC AAGCTT GGCGTAATCATGGTC.TAGCT
MIN 86 LG_12687-5_
MIN 86 LG_12687-10
MPY 86_623-3
MPY 86_623-5                    ..G..
MPY 86_754-1
MPY 86_754-2          T...
MPY 86_754-3          T...
MPY 86_4282-1_                        T.       A.C.
MPY 86_4282-4_                  ..T.
MPY 86_4282-5_                        T.       A.C.
MPY 86_4282-6_                  ..T.
MPY 86_16344-1_
MPY 86_16344-9_

                      16350       16360       16370       16380       16390
                      ...|....|....|....|....|....|....|....|....|....|.
CRS                   CTACC AC
Rosa                  .....C..CCTTAACAGTNCATGGTACATAAAGCCATTTACCGTACATAG
19/91 92-1            .....C. CAAG   GG    CGA TTTNTG    GNGA TATCCAT
19/91 92-2            .....C. CAAG   GG    CGA TTTCTG    GGGN TNTCCAT
19/91 92-3            .....C. CAAG   GG    CGA TTCTG    CAGGA TATCCATC
19/91 92-4            .....C. CAAG   GG    CGGATTTCTG   CAGGA TATCCAT
19/91 92-5            .....C. CAAG   GG    CGA NTTCTG   CAGGN TNTCCAT
19/91 92-6            .....C   AAG   GG    CGA TTTCTG   NAGGN TNTCCNT
19/91 92-7            .....C. CAAG   GG    CGA TTTCTG   CAGGN TATCCAT
19/91 92-8            .....C. CAAG   GG    CGATTTCTG    CAGGA TATCCATC
19/91 92-9            .....C. CAAG   GG    CGA TTTCTG   G GGA TATCCAT
19/91 92-10           .....C. CAAG   GG    CGNATTTCTG    NGGN TNTCCAT
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19/91 92-12          .....C. CAAG  GG   CGATTTCTG    AGGA TNTCCATC
19/91 92-13          .....C. CAAG  GG   CGA TTTCTG   GGGA TATCCNT
19/91 92-14          .....C. CAAG  GG   CGAATTCTG   CAGGA TATCCATC
19/91 104-1          .....C. CAAG  GG   CGA TTTCTG   ANGA TATCCAT
19/91 104-2          .....C. CAAG  GG   CGA TTTCTG   NAGGA TATCCAT
19/91 104-3          .....C. CAAG  GG   CGA TTTCTG   CAGGN TNTCCAT
19/91 104-5          .....C. CAAG  GG   CGA TTTCTG   CAGGN TNTCCAT
19/91 104-6          .....C. CAAG  GG   CGA TTTCTG   GGGN TATCCAT
19/91_212-1          .....C..CTTA       GGNATTACTCGAACNAAA CNCTACTC
19/91_212-2          .....C. CCNGA GGGC  GAATTTCT GCNAGA TATTCCAT
19/91_212-3          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
19/91_212-5          .....C. CNNAGGGGGC  GNAATTTCT GCNAGNA TNATTCTN
19/91_212-6          .....C. CNGA  GGGC  GNAATTTCT GCNAGAT NATTCTNA
19/91_233-1          .....C. CAAG  GG   CGA TTTCTG   GGGN TNTCCAT
19/91 233-2          .....C. CAAG  GG   CGA  TTCTG   CAGGN TNTCCA T
19/91 233-3          .....C. CAAG  GG   CGA TTTNTG   GGGN TNTCCNT
19/91 233-5          .....C. CAAG  GG   CGA TTTCTG   CAGGA TATCCAT
19/91 257-1          .....C. CAAG  GG   CGATTTCTG   NAGGA TATCCATC
19/91 257-2          .....C. CAAG  GG   CGATT CTG   CAGGA TATCCATC
19/91_302-1          .....C. C AG  NGGCG  NAA TTTCT GCNAGNA TNTTCTNT
19/91_302-2          .....C. CA G  GGC   GAATTTCT GCNAGA TATTCCNA
19/91_302-3          .....C. CAAG  GG   CGAATTC TG  CAGA  TATCCATC
19/91_302-4          .....C. CA G  GGC   GAATTTCT GCNAGA TATCC  A
19/91_302-5          .....C. CAAG  GGC   GAATTTCT GCNAGA TATTCCAT
19/91_302-6          .....C. CAAG  GGC  GNAATTTCT GCGAGNA TNTTCTNT
19/91_420-1          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
19/91_420-2          .....C. CAAG  GG   CGAATTN TG  CAGA  TATCCATC
19/91_420-3          .....C. CAAG  GGC   GAATTTCT GCNAGA TAT  CCA
19/91_420-4          .....C. CAAG  GGC   GAATTTCT GCNAGA TATTCCAT
19/91_420-5          .....C. CAAG  GGC   GAATTTCT GCNAGA TATCCATC
19/91_420-6          .....C. CAAG  GG   CGAATTC TG  CAGA  TATCCATC
CH86 45-3            .....C. CAAG  GGCA     ATTNGTG  NGGNG     GTCC
CH86 45-4            .....C. CAAG  GG   CGAAGTTNTG  NAGGN TNTCCNAT
CH86 45-5            .....C. CAAG  GGC   GAATTCCAG  CACACGTGGCGGCCGG
CH86 45-6          TGG..TT CAAT  GGTACAAAAANNAGGG  GGGGA CTNNCGTNC
CH86 45-7            .....C. CA G  GGCN     ACTTNNG  GGGG  TTTCCNTCN
CH86 45-8            .....C. CAAG  GGCG     ANTTNTG  CNGGG NGTCCATCG
CH86 45-9            .....C. CAAG  GG   CGA TTTCTG   CNGGG TGTCCNT
CH86 45-10           .....C. CAAG  GG   CGA TTTCTG   CNGGG TGTCCAT
EL00-3005-1          .....C. CAAG  GG   CGA ATTCTG   CAGGN TNTCCNNT
EL00-3005-2          .....C. CAAG  GG   CGATT CTG   CAGGA TATCCATC
EL00-3073-2          .....C. CAAG  GGC  GNAGTTCTG   CAGNG TNTCCATC
EL00-3073-3          .....C. CAAG  GGC   GAATTCCAG  CACAC TGGCGGCCG
EL00-3079-1          .....C. CAAG  GG   CGATTCTG   CANGA TATCCATC
EL00-3079-2          .....C. CAAN  GGG  CGA GTTCTG  CAAGN TNTCCAT
EL00-3079-3          .....C. CAAG  GGG  CGA TTTCTG  CAGAN NNTCCTT
EL00-3079-4          .....C. CAAG  GG   CGA TTTCTG  CANGA TNTCCAT
EL00-3079-5          .....C. CAAG  GG   CGA ATTCTG  CANGN TNTCCAT
EL00-3079-6          .....C. CAAG  GG   GCGATTCTG   CANGA TATCCATC
EL00_3095-2_         .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3095-3          .CNA.NT NAGG  GGGCC GNAATTTCCTGCNAGNAT TATTCNCNAT
EL00_3095-4_         .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3095-5          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3095-6          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3095-7          ..C..AG CAAG  GGG  CGAATTAATG  NAGA  TATCCATCC
EL00_3095-8          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00-3100-1          .....C. CAAG  GGG  CGATTTCTG   CAGGA TATCCATC
EL00-3100-2          .....C. CAAG  GG   CGATT CTG   CAGGN TATCCNTC
EL00-3100-4          .....C. CAAG  GG   CGAATTCTG   CAGGN TATCCATC
EL00-3100-9          .....C. CAAG  GG   CGAATTCTG   CANGA TATCCATC
EL00_3116-2          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3116-3          .....C. CAAG  GGC   GANTTANG   GGGA  TATCCATC
EL00_3116-4          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3116-5          .....C. CAAG  GGC   GAATTCTG   CAGA  TATCCATC
EL00_3116-6          .....C. CAAG  GG   CGAATTC TG  CAGA  TATCCATC
EL00_3141-1          .....C. CAA   GGGC  GNAATTTCT GCGAGNA TNATTCTN
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EL00_3141-2_        GGCGGNT NAGG  GGGC  GNAATTTCCTG CNAGNA TNATTCTN
EL00_3141-3         .....C. CA G  GGC    GAATTTCT  GCNAGA  TATCC  N
EL00_3141-4         .....C. CAAG  GGC    GAATTTCT  GCNAGA  TAT  CCA
EL00_3141-5_        .....C. CAAG  GGC    GAATTTCT  GCNAGA  TATTCCNA
EL00_3141-6_        .....C. CA G  GGC    GAATTTCT  GCNAGA  TATCC  A
EL00_3285-1         .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
EL00_3285-2         .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
EL00_3285-4         .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
EL00_3285-5         .....C. CAAG  GGC    GAATTNTG    CAGA  TATCCATC
EL00_3285-6_        .....C. CAAG  GG   CGAATTC TG    CAGA  TATCCATC
EL00_3285-7         .....C. CAAG  GGN    GAATTCTG    CNGA  TATCCATC
EL00_3285-8         .....C. CAAG  GG   CGAATTC TG    CAGA  TATCCATC
EL00_3285-9         .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
EL00_3285-10        .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
He93A-606-2         .....C. CAAG  GG   CGA ATTCTG    CAGGN TNTCCAT
He93A-606-3         .....C. CAAG  GG   CGA NTTCTG    CANGA TATCCAT
He93A_1997-1        .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
He93A_1997-2        .....C. GCAA  GGG   CGAATTC TG    CAGA  TATCCATC
He93A_1997-3        .....C.GCAAG  GGC    GAATTNTG    CAGA  TATCCATC
He93A_1997-4        .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
He93A_1997-5        .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
IAS 4801_1451-2     .....C. CAAG  GGN    GAATTCTG    CAGA  TATCCATC
IAS 4801_1762-1     .....C. CAAG  GG   CGAATTCTG    CANGN TATCCATC
IAS 4801_1762-2     .....C. CAAG  GG   CGN TTTCTG    CANGN NNTCCAT
IAS 4801_1762-3     .....C. CAAG  GG   CGA NTTCTG    CAGGN NNTCCAT
IAS 4801_1762-4     .....C. CAAG  GG   CGA ATTCTG    CANGN NNTCCAT
IAS 4801_1762-5     .....C. CAAG  GG   CGA ATTCNG    NAGGN TNTCCAT
IAS 4801_1762-6     .....C. CAAG  GG   CGA ATTCTG    CA GN TGTCCNT
IAS 4801_1762-7     .....C. CAAG  GG   CGA TTTCTG    NGGGG TCTCCATC
IAS 4801_1762-8     .....C. CAAG  GGC    GAATTNNG     NGGT TATCCATN
IAS 4801_1762-9     .....C. CAAG  GGCG     ATTTNTG    NGGGG NCTCCATNA
IAS 4801_2005-2     .....C.TCAAG  GGC    GTTTTTNNG    GGGNA NNTCCNTC
IAS 4801_2005-3     .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
IAS 4801_2005-4     .....C. CAAG  GG   CGN ATTCTG    CAAGA TNTCCAT
IAS 4801_2005-5     .....C. CAAG  GG   CGA ATTCTG    CAGGA TATCCAT
IAS 4801_2005-6     .....C. CAAG  GG   CGA GTTCTG    CAANN  ATCCAT
IAS 4801_2005-7     .....C. CAAG  GGG  CGA TTTCTG    CAANN TATCCTT
IAS 4801_2005-8     .....C..AAGG  GGC    GANTT CTG    CANGA TATCCATC
IAS 4801_2005-9     .....C. CAAG  GG     CGATTCTG    CANGA TATCCATC
IAS 4801_2642-1_    .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
IAS 4801_2642-2_    .....C. CAAG  GGC   GAA TTTCT  GCNAGAT N TTCTNT
IAS 4801_2642-3_    .....C. CAGG    GC    GAATTTCT  GCNAGAT TATTCTNA
IAS 4801_2642-4_    .....C. CAAG  GGC    GAATTTCT  GCNAGA  TATTCCAT
IAS 4801_2642-5     .....C. CA G  GGC    GAATTTCT  GCNAGA  TATTCC A
IAS 4801_2642-6     .....C. CAAG  GGC    GAATTCTG    CAGA  TATCCATC
MIN 86 BD_5960-5    .....C. CCAG  GGGCG  GNAATTTCT  GCNAGNA TNTTCTNT
MIN 86 LG_12356-1_  .CNA.NT NAGN  GGGC   GNAATTTCNTGGCNAGNA TNNTCCCN
MIN 86 LG_12356-2   .CNANNT NAGN  GGGC   GNAATTTCNTGGCNAGNA TNATTCCNAT
MIN 86 LG_12356-4_  .....C. CCAA  GGGC   GNAATTTCT  GCNAGNA TTATTCTNA
MIN 86 LG_12356-5_  .....CT ACAG  GGC    GAAATTTCT  GCNAGAT TATTCTNT
MIN 86 LG_12687-4_    GTTTC.TGANGNG                GCTNTTANA   TNNNA NNTCCGC
MIN 86 LG_12687-5   .....C. CAAG  GGC    GAATTCCAG   CACAC TGGCGGCCG
MIN 86 LG_12687-10  .....C. CAAG  GGC    GAATTCCAG   CACAC TGGCGGCCG
MPY 86_623-3        .....C. CAAG  GGC    GAATTCCAG   CACAC TGGCGGCCG
MPY 86_623-5        .....C.GCAAG  GGC    GAATTCTG    CAGA  TATCCATC
MPY 86_754-1        .....C. CAAG  GG   CGA TTTCTG    CANGN TNTCCAT
MPY 86_754-2        .....C. CAAG  GG   CGANTTCTG    CAGGA TATCCATC
MPY 86_754-3        .....C. CAAG  GGC    GNNGTTCTG    NNGGT TGTCNNNT
MPY 86_4282-1_      .....C. CCAA  GGGC   GAAATTTCCT GCNAGNA TTATTCTNA
MPY 86_4282-4_      .....C. C AA  GGGC   GNAATTTCT  GCNAGNA TNATTCTNA
MPY 86_4282-5_      .....C. CAAG  GGC    GAAATTTCT  GCGAGNA TTTTCTNT
MPY 86_4282-6_      .....C. CA A  GGGC   GNAATTTCT  GCNAGAT NATTCTNT
MPY 86_16344-1      .....C. CAAG  GG   CGA ATTCTG    CAGGN TNTCCAT
MPY 86_16344-9      .....C. CAAG  GG   CGAAATCTG    CAGGA TNTCCATC
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Appendix B: University of Alaska Raw Stable Isotope Data

DISH?	SITE	SKELETON	sample	weight	N signal	C signal	Conc N	Conc C	C/Nmass	C/N atomic	dN	dC
DISH	19/91	56	01A	0.33	2.39	2.12	13.55	46.81	3.46	4.03	11.90	-21.09
			01B	0.31	2.14	2.05	12.90	48.23	3.74	4.36	11.28	-21.22
NO	19/91	92	02A	0.34	2.59	2.03	14.25	43.48	3.05	3.56	9.27	-20.23
			02B	0.34	2.34	1.97	12.88	42.23	3.28	3.83	8.82	-20.16
NO	19/91	104	03-A	0.35	3.55	3.84	17.81	51.58	2.90	3.38	11.36	-19.55
			03-B	0.33	2.51	2.79	13.34	39.66	2.97	3.47	11.42	-19.77
NO	19/91	143	04-A	0.34	2.28	3.05	11.75	42.13	3.59	4.18	12.00	-21.52
			04-B	0.33	2.55	3.33	13.55	47.39	3.50	4.08	12.00	-21.41
NO	19/91	212	05-A	0.33	1.76	2.47	9.22	36.22	3.93	4.58	12.61	-22.65
			05-B	0.3	1.12	1.65	6.45	26.69	4.14	4.83	12.54	-23.15
NO	19/91	233	06A	0.28	2.22	1.91	14.85	49.77	3.35	3.91	13.06	-19.72
			06B	0.3	1.88	1.78	11.74	43.17	3.68	4.29	12.43	-19.81
DISH	19/91	257	07A	0.34	2.98	2.07	16.38	44.40	2.71	3.16	13.08	-18.81
			07B	0.34	2.83	2.08	15.55	44.72	2.88	3.36	12.61	-18.83
DISH	19/91	302	08A	0.33	1.62	1.78	9.18	39.29	4.28	4.99	10.62	-20.81
			08B	0.33	1.77	1.79	10.00	39.47	3.95	4.60	10.55	-20.90
DISH	19/91	420	09A	0.33	2.49	2.01	14.09	44.38	3.15	3.67	13.67	-18.10
			09B	0.33	2.35	1.83	13.31	40.49	3.04	3.55	13.69	-18.38
DISH	CH86	18	10A	0.34	2.79	2.51	15.33	53.84	3.51	4.10	11.85	-20.17
			10B	0.34	2.52	2.16	13.88	46.38	3.34	3.90	11.86	-20.02
NO	CH86	23	11A	0.32	2.23	1.99	13.00	45.47	3.50	4.08	11.05	-20.08
			11B	0.33	2.17	1.98	12.31	43.76	3.55	4.15	11.25	-20.17
NO	CH86	45	12A	0.32	2.38	2.02	13.93	46.13	3.31	3.86	10.46	-20.71
			12B	0.32	2.52	2.12	14.72	48.34	3.28	3.83	10.22	-20.70
DISH	CH86	123	13A	0.36	2.59	2.20	13.44	44.57	3.32	3.87	11.69	-20.45
			13B	0.34	2.76	2.36	15.18	50.56	3.33	3.89	11.77	-20.65
NO	CH86	175	14A	0.31	1.86	1.75	11.19	41.07	3.67	4.28	9.85	-21.21
			14B	0.28	1.92	1.66	12.82	43.28	3.38	3.94	10.48	-21.14
DISH	CH86	357	15A	0.31	1.81	1.71	10.91	40.18	3.68	4.30	11.52	-20.75
			15B	0.36	1.76	1.60	9.12	32.35	3.55	4.14	11.95	-20.74
NO	EL00	3005	16-A	0.28	1.59	2.38	9.83	41.15	4.19	4.88	10.78	-23.07
			16-B	0.28	1.52	2.29	9.44	39.60	4.19	4.89	10.88	-23.03

DISH?	SITE	SKELETON	sample	weight	N signal	C signal	Conc N	Conc C	C/Nmass	C/N atomic	dN	dC
NO	EL00	3014	17A	0.31	2.13	1.95	12.85	45.78	3.56	4.16	12.21	-21.18
			17B	0.34	2.20	1.94	12.08	41.72	3.45	4.03	12.58	-21.25
NO	EL00	3019	18A	0.33	2.75	2.17	15.59	47.94	3.08	3.59	12.92	-20.87
			18B	0.35	2.52	2.10	13.48	43.70	3.24	3.78	11.71	-20.34
DISH	EL00	3027	19A	0.33	2.70	2.22	15.32	49.02	3.20	3.73	12.23	-20.77
			19B	0.3	2.48	2.06	15.43	50.03	3.24	3.78	12.36	-20.94
DISH	EL00	3039	20A	0.33	2.72	2.22	15.43	49.09	3.18	3.71	13.13	-20.23
			20B	0.34	2.45	2.01	13.46	43.11	3.20	3.74	13.03	-20.30
NO	EL00	3068	21A	0.33	2.49	2.20	14.11	48.58	3.44	4.02	12.66	-21.05
			21-B	0.35	2.80	3.24	14.03	43.51	3.10	3.62	12.53	-19.97
NO	EL00	3073	22A	0.36	2.00	2.27	10.38	45.91	4.42	5.16	13.45	-22.80
			22B	0.32	1.43	1.69	8.36	38.44	4.60	5.37	13.29	-23.39
NO	EL00	3079	23A	0.32	2.39	1.93	13.97	44.05	3.15	3.68	12.11	-20.38
			23B	0.34	3.18	2.40	17.47	51.57	2.95	3.44	12.83	-20.30
DISH	EL00	3090	24A	0.34	2.62	2.03	14.42	43.61	3.02	3.53	13.50	-20.21
			24B	0.35	2.69	2.11	14.37	43.90	3.06	3.56	13.39	-20.23
NO	EL00	3094	25A	0.3	2.47	1.96	15.40	47.65	3.09	3.61	12.26	-20.64
			25B	0.32	2.63	2.10	15.39	47.86	3.11	3.63	12.15	51.63
NO	EL00	3095	26A	0.33	2.01	1.87	11.38	41.28	3.63	4.23	12.59	-22.01
			26B	0.34	1.81	1.65	9.95	35.30	3.55	4.14	12.90	-21.89
DISH	EL00	3096	27-A	0.36	2.82	3.43	13.76	44.69	3.25	3.79	12.60	-20.15
			27-B	0.31	2.50	2.84	14.14	43.10	3.05	3.56	13.44	-20.21
DISH	EL00	3099	28A	0.34	2.43	2.02	13.34	43.39	3.25	3.79	12.83	-20.73
			28B	0.31	2.39	2.01	14.44	47.40	3.28	3.83	12.58	-20.83
NO	EL00	3100	29A	0.31	2.36	1.89	14.25	44.46	3.12	3.64	12.77	-20.56
			29B	0.32	2.89	2.28	16.86	51.92	3.08	3.59	12.85	-20.42
DISH	EL00	3116	30A	0.32	2.72	2.11	15.89	48.00	3.02	3.52	13.13	-20.53
			30B	0.33	3.05	2.40	17.29	53.13	3.07	3.58	12.96	-20.46
DISH	EL00	3121	31A	0.33	2.48	1.92	14.07	42.54	3.02	3.53	13.60	-20.42
			31B	0.36	3.03	2.36	15.71	47.87	3.05	3.55	13.41	-20.36
DISH	EL00	3127	32A	0.32	2.21	1.86	12.90	42.43	3.29	3.84	13.52	-20.91
			32B	0.3	2.06	1.75	12.86	42.56	3.31	3.86	13.39	-20.96

DISH?	SITE	SKELETON	sample	weight	N signal	C signal	Conc N	Conc C	C/Nmass	C/N atomic	dN	dC
NO	EL00	3141	33A	0.32	2.50	2.04	14.60	46.45	3.18	3.71	13.36	-20.35
			33B	0.34	2.61	2.13	14.36	45.60	3.17	3.70	13.49	-20.51
NO	EL00	3145	34A	0.32	2.10	1.69	12.29	38.42	3.13	3.65	12.89	-20.44
			34B	0.31	2.05	1.64	12.33	38.48	3.12	3.64	12.68	-20.44
DISH	EL00	3266	35-A	0.32	2.44	2.87	13.38	42.09	3.15	3.67	12.37	-20.21
			35-B	0.34	2.29	2.75	11.80	37.93	3.21	3.75	12.07	-20.23
DISH	EL00	3271	36A	0.34	2.48	2.01	13.63	43.07	3.16	3.69	12.60	-20.53
			36B	0.34	2.65	2.18	14.58	46.76	3.21	3.74	12.66	-20.63
DISH	EL00	3285	37A	0.32	2.08	1.65	12.18	37.55	3.08	3.60	13.88	-20.71
			37B	0.31	2.16	1.81	13.01	42.51	3.27	3.81	13.36	-20.70
DISH	EL00	3292	38-A	0.35	2.70	3.12	13.53	41.89	3.10	3.61	10.67	-19.99
			38-B	0.32	2.20	2.66	12.08	39.11	3.24	3.78	10.08	-20.07
NO	He93A	606	39A	0.29	2.14	1.73	13.77	43.60	3.17	3.69	11.14	-20.61
			39B	0.29	2.21	1.87	14.22	46.95	3.30	3.85	11.19	-21.22
DISH	He93A	1664	40A	0.31	2.62	1.99	15.82	46.86	2.96	3.46	12.23	-19.93
			40B	0.33	3.24	2.47	18.38	54.57	2.97	3.46	12.24	-19.85
DISH	He93A	1997	41A	0.33	2.27	1.86	12.87	41.08	3.19	3.72	11.02	-20.89
			41B	0.31	2.25	1.85	13.55	43.50	3.21	3.74	10.97	-20.93
NO	He93A	2073	42A	0.31	2.52	2.00	15.21	47.10	3.10	3.61	10.70	-20.97
			42B	0.31	2.23	1.81	13.44	42.58	3.17	3.70	10.66	-21.07
NO	IAS 4801	1391	43A	0.33	2.23	1.78	12.62	39.27	3.11	3.63	13.29	-20.16
			43B	0.31	2.02	1.65	12.19	38.74	3.18	3.71	13.10	-20.13
NO	IAS 4801	1415	44A	0.28	2.07	1.80	13.80	46.88	3.40	3.96	14.82	-20.59
			44B	0.31	2.41	2.09	14.54	49.15	3.38	3.94	14.89	-20.52
DISH	IAS 4801	1417	45A	0.33	2.61	2.05	14.81	45.22	3.05	3.56	13.04	-20.02
			45-B	0.32	2.38	2.72	13.05	39.89	3.06	3.57	12.73	-19.96
DISH	IAS 4801	1457	46-A	0.31	2.21	2.41	13.36	41.35	3.10	3.61	13.50	-20.36
			46-B	0.33	2.30	2.46	13.06	39.76	3.04	3.55	13.54	-19.98
DISH	IAS 4801	1757	47-A	0.35	2.71	3.11	13.61	41.72	3.07	3.58	13.72	-20.20
			47-B	0.36	2.73	3.16	13.28	41.20	3.10	3.62	13.47	-20.14
NO	IAS 4801	1762	48-A	0.32	2.19	2.43	12.83	40.47	3.15	3.68	13.78	-20.20
			48-B	0.35	2.30	2.56	12.33	38.89	3.15	3.68	13.74	-20.30

DISH?	SITE	SKELETON	sample	weight	N signal	C signal	Conc N	Conc C	C/Nmass	C/N atomic	dN	dC
DISH	IAS 4801	1799	49-A	0.31	2.09	2.40	12.66	41.14	3.25	3.79	13.32	-20.48
			49-B	0.36	1.43	1.64	13.78	43.69	3.17	3.70	13.01	-20.33
NO	IAS 4801	1872	50-A	0.33	2.88	3.11	15.30	44.30	2.90	3.38	13.81	-19.64
			50-B	0.35	3.09	3.34	15.50	44.82	2.89	3.37	13.78	-19.59
NO	IAS 4801	1919	51-A	0.35	2.81	3.18	15.06	48.38	3.21	3.75	13.25	-20.50
			51-B	0.31	2.67	2.93	16.15	50.37	3.12	3.64	13.37	-20.16
NO	IAS 4801	1933	52-A	0.33	2.24	2.25	12.72	36.34	2.86	3.33	13.38	-19.57
			52-B	0.3	1.33	1.47	15.40	47.45	3.08	3.60	13.18	-20.05
DISH	IAS 4801	2005	53-A	0.31	1.18	1.27	13.41	40.15	2.99	3.49	14.40	-19.44
			53-B	0.34	1.45	1.57	15.24	45.62	2.99	3.49	14.39	-19.47
NO	IAS 4801	2508	54-A	0.32	1.38	1.54	15.57	47.56	3.05	3.56	13.73	-19.31
			54-B	0.34	1.71	1.89	18.21	55.35	3.04	3.55	13.91	-19.22
DISH	IAS 4801	2591	55-A	0.35	1.76	1.91	18.35	54.35	2.96	3.46	13.75	-19.86
			55-B	0.33	1.11	1.23	12.38	37.48	3.03	3.53	13.68	-19.97
NO	IAS 4801	2640	56-A	0.3	1.46	1.61	18.07	54.18	3.00	3.50	13.81	-19.36
			56-B	0.34	2.27	1.75	12.51	37.62	3.01	3.51	14.04	-19.53
NO	IAS 4801	2642	57-A	0.34	2.60	2.08	14.31	44.70	3.12	3.64	14.13	-20.09
			57-B	0.32	2.32	1.96	13.53	44.58	3.29	3.84	14.04	-20.41
DISH	IAS 4801	2654	58-A	0.31	2.52	2.75	14.24	41.63	2.92	3.41	13.90	-20.10
			58-B	0.31	1.17	1.33	14.25	43.34	3.04	3.55	13.57	-20.07
NO	MIN86 BD	5960	59-A	0.33	2.59	2.84	13.78	40.46	2.94	3.42	14.17	-18.96
			59-B	0.31	1.12	1.27	13.60	41.45	3.05	3.55	13.68	-19.01
DISH	MIN86 BD	6412	60-A	0.34	1.84	1.84	10.11	39.47	3.90	4.56	13.21	-22.08
			60-B	0.36	1.88	1.85	9.75	37.50	3.84	4.48	13.32	-21.94
NO	MIN86 BD	8341	61-A	0.36	2.90	2.22	15.06	44.96	2.99	3.48	11.92	-20.93
			61-B	0.31	2.66	2.05	16.03	48.23	3.01	3.51	11.96	-20.94
DISH	MIN86 BD	11944	62-A	0.32	2.49	1.91	14.56	43.51	2.99	3.49	12.98	-19.92
			62-B	0.35	2.69	2.10	14.37	43.80	3.05	3.56	12.81	-19.89
NO	MIN86 LG	10177	63-A	0.35	2.03	1.90	10.86	39.55	3.64	4.25	13.71	-22.11
			63-B	0.33	2.02	1.91	11.43	42.14	3.69	4.30	13.83	-22.14
NO	MIN86 LG	10348	64-A	0.33	1.59	1.40	8.99	30.89	3.44	4.01	12.22	-20.95
			64-B	0.32	2.09	1.80	12.24	41.13	3.36	3.92	12.68	-21.08

DISH?	SITE	SKELETON	sample	weight	N signal	C signal	Conc N	Conc C	C/Nmass	C/N atomic	dN	dC
NO	MIN86 LG	10420	65-A	0.36	1.71	1.77	8.88	35.94	4.05	4.72	13.21	-22.27
			65-B	0.29	1.61	2.41	9.61	40.27	4.19	4.89	13.05	-22.20
NO	MIN86 LG	12356	66-A	0.29	2.16	2.67	13.09	43.19	3.30	3.85	13.45	-20.71
			66-B	0.28	2.33	2.61	14.62	43.84	3.00	3.50	13.46	-20.08
NO	MIN86 LG	12520	67-A	0.35	3.05	3.29	15.29	44.14	2.89	3.37	12.20	-19.76
			67-B	0.33	2.88	3.13	15.32	44.49	2.90	3.39	12.17	-19.78
NO	MIN86 LG	12687	68-A	0.36	2.54	1.92	13.19	38.98	2.95	3.45	12.60	-20.28
			68-B	0.34	2.51	1.88	13.78	40.41	2.93	3.42	12.49	-20.26
DISH	MIN86 LG	13518	69-A	0.34	2.48	1.82	13.62	38.97	2.86	3.34	14.23	-19.75
			69-B	0.35	2.73	2.04	14.56	42.59	2.93	3.41	14.08	-19.81
DISH	MIN86 LG	13663	70-A	0.34	2.44	1.91	13.40	41.05	3.06	3.57	14.24	-19.45
			70-B	0.33	2.66	2.04	15.07	45.19	3.00	3.50	14.26	-19.37
DISH	MIN86 LG	16098	71-A	0.35	1.96	1.70	10.45	35.42	3.39	3.95	14.30	-20.81
			71-B	0.3	2.50	2.10	15.59	51.00	3.27	3.82	14.10	-20.88
DISH	MIN86 LG	16311	72-A	0.33	2.61	1.96	14.78	43.34	2.93	3.42	13.70	-19.25
			72-B	0.35	2.55	1.91	13.62	39.84	2.92	3.41	13.77	-19.30
DISH	MIN86 LG	16332	73-A	0.3	1.98	1.53	12.34	37.31	3.02	3.53	13.58	-19.90
			73-B	0.31	2.42	1.85	14.61	43.59	2.98	3.48	13.60	-19.97
DISH	MIN86 LG	16344	74-A	0.3	2.35	2.58	13.73	40.46	2.95	3.44	13.26	-19.73
			74-B	0.31	2.21	1.72	13.35	40.51	3.03	3.54	13.20	-19.95
DISH	MPY86	716	75-A	0.33	2.71	3.09	14.43	43.97	3.05	3.56	14.36	-20.55
			75-B	0.32	2.30	2.66	12.60	39.11	3.10	3.62	14.33	-20.69
NO	MPY86	754	76-A	0.29	1.49	1.31	9.61	32.91	3.42	3.99	13.09	-21.39
			76-B	0.25	1.55	1.34	11.56	39.17	3.39	3.95	12.87	-21.49
NO	MPY86	799	77-A	0.32	2.15	1.71	12.54	39.08	3.12	3.64	13.81	-20.00
			77-B	0.35	2.71	2.14	14.49	44.61	3.08	3.59	13.92	-19.96
DISH	MPY86	2905	78-A	0.32	2.31	1.78	13.50	40.61	3.01	3.51	13.98	-19.61
			78-B	0.32	2.49	2.06	14.56	46.95	3.23	3.76	13.97	-20.14
NO	MPY86	3447	79-A	0.32	2.57	1.89	15.00	43.12	2.87	3.35	13.18	-19.56
			79-B	0.31	2.49	1.84	15.02	43.29	2.88	3.36	13.12	-19.66
DISH	MPY86	4851	80-A	0.35	2.71	2.90	13.60	38.96	2.86	3.34	11.68	-19.47
			80-B	0.31	2.33	1.74	14.07	40.98	2.91	3.40	11.62	-19.60

DISH?	SITE	SKELETON	sample	weight	N signal	C signal	Conc N	Conc C	C/Nmass	C/N atomic	dN	dC
DISH	YFH	28	81-A	0.34	2.05	1.65	11.30	35.30	3.13	3.65	12.70	-20.91
			81-B	0.35	2.28	1.84	12.18	38.26	3.14	3.67	12.76	-20.95
NO	YFH	308	83-A	0.32	2.26	1.80	13.19	40.95	3.10	3.62	13.46	-20.40
			83-B	0.33	2.89	3.30	15.20	48.34	3.18	3.71	12.99	-20.21
NO	YFH	310	84-A	0.3	1.93	2.34	11.16	37.78	3.38	3.95	13.36	-21.07
			84-B	0.35	1.19	1.46	12.83	42.30	3.30	3.85	13.33	-21.08