Isotopes and Teeth: Human Movement in two Medieval Danish Cemetery Populations

by

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Abstract

The mobility patterns of two medieval Danish populations were investigated using oxygen isotopic analysis. Oxygen isotopic data were collected from the dental enamel of 26 individuals, 13 from the urban cemetery, Ole Worms Gade, and 13 from rural Sejet, both located in Central Denmark. Phosphate was chemically isolated as an oxygen analyte and analyzed using Thermal Combustion Elemental – Mass Spectrometry (TC/EA-MS) in order to minimize the effects of diagenesis on the oxygen isotopic composition of enamel. Diagenesis of the dental tissues was also investigated using spectroscopic and microscopic techniques. Secondary Ion Mass Spectrometry (SIMS) was explored as an alternate method of obtaining isotope data for these materials. Isotope data revealed three possible migrants. Results are interpreted in the context of the shifting socioeconomic climate in medieval Europe.

This work is dedicated to the burial populations of Sejet and Ole Worms Gade and those working to shed light on their individual and collective histories.

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Chapter 1: Introduction

Analysis of various physical, biological, and chemical characteristics of archaeological human remains has contributed in no small way to the study of past human behaviour. A multitude of long-established and new methods have enabled paleodemography, paleopathology, and paleodietary studies, among others, to become promising and active fields within physical anthropology. Katzenberg and Saunders (2000) introduce their edited volume on the subject stating that, "the cornerstone of biological anthropology is the interaction of culture and human biology" (2000, p. ix). The questions researchers ask of human skeletal remains are in large part the same questions of cultural activity and human behaviour that have been asked of the archaeological and historic records for centuries. Human remains provide the opportunity to examine past cultures and human behaviour through indicators of diet, health, movement, and interaction at the scale of the individual. This is an opportunity rarely provided by other archaeological or historical remains.

There is a lengthy historical relationship between analytical chemistry and archaeological materials that has proven invaluable to bioarchaeological analysis. Pollard, Batt, Stern, and Young (2007) write about chemists working in the late 1700s who investigated the chemical composition of archaeological materials such as coins and residues. The relationship between archaeological investigation and chemical analysis has strengthened significantly in the centuries since. Advancements in analytical capabilities and precision are ongoing, facilitating investigation on an increasingly smaller scale.

The measurement of natural variations in light stable isotope ratios has long been recognized as an important tool with a range of applications from palaeoclimatological reconstructions to archaeological dietary studies. The analytical methods were developed for use on geochemical questions; however, methodological advances in recent decades have enabled reliable analysis of carbon, nitrogen, oxygen and strontium from biological tissues in archaeological contexts, each used to contribute to our understanding of past life ways (e.g. White et al., 1998; Wright and Schwarcz, 1999; Richards et al., 2002; Price et al., 2004). While a number of elements that have more than one stable isotope can provide information about human diet and health, oxygen isotopes are of interest here as they can be used to provide information about human movement. Meteoric (rain) water ends up in rivers, steams and lakes and is consumed by humans. The oxygen isotopic ratio of meteoric water varies geographically as a result of a number of factors related to the hydrological system, such as geographic distance from water sources, temperature, and elevation (Dansgaard, 1964). Human tissue reflects this variability and serves as a geographic indicator as a result of the incorporation of oxygen from meteoric water, consumed by the individual, into forming tissues (Longinelli, 1984). In mammalian tissues, oxygen isotopes can be analyzed from any of the oxygen-bearing components of "bio-apatite", found in teeth and bones, and more rarely preserved in keratinous tissues (Lee-Thorp, 2008). Vital effects (e.g., metabolic processes) complicate the use of oxygen isotope studies on human remains, and biological tissues more generally (Schoeninger & Moore, 1992; Urey, Lowenstam, Epstein, & McKinney, 1951). A number of factors involved in biomineralization, such as tissue turnover, body temperature, dietary complexity, and species-specific proportions of water intake (drinking vs. food-sourced),

must be navigated and controlled. These initially unrecognized complications have led to a field of research distinct from that in the geological sciences where the methods were pioneered (Schoeninger & Moore, 1992).

The medieval period in Denmark is marked by an agrarian crisis as well as the devastation of the Black Death Plague (Yoder, 2006). These events may have had severe impacts on many aspects of people's lives, and may have impacted different populations in unique ways. In this study, oxygen isotopes are measured in dental material. Dental enamel, a tissue formed in childhood, is analyzed from a sample of adult individuals, reflecting the geographic locale of the individuals during their childhood. Great demographic, environmental, and social changes were weathered in the Middle Ages, and adaptations can be seen in everyday agricultural and trade activities among others (Poulsen, 1997; Raoult et al., 2000; Yoder, 2006). Oxygen isotopic analysis will investigate whether human mobility can be detected in individuals from the study sample. In this way, the relationship between small-scale social behaviour, investigated at the level of the individual, and broader environmental and social changes can be considered, adding to the understanding of the shifting socioeconomic climate of the period.

Chapter 2: Literature Review

Medieval Denmark: Sources of Information

Archaeology and physical anthropology are well suited to increase our understanding of life in medieval Denmark at the individual level. Physical anthropology often stands alone in its ability to investigate life from the perspective of the individual as well as the population. There are numerous studies of diet, health, and disease of material from several medieval mortuary sites in Denmark (e.g. Boldsen, 1998, 2005; Yoder, 2010). Such studies help to connect general socio-economic trends occurring, to the people living at the time, and to determine whether socio-economic stressors differentially impacted populations or sub-populations. In order to contextualize any bioarchaeological findings, it is important to begin with an understanding of what is currently understood about life in medieval Denmark and the sites from which material is being examined.

Historical documentation is a valuable source of information, though limited in its utility as a source of information about daily life. For instance, written accounts of activities from peasant farms are not known from before the 16th century (Corsi, 2008; Poulsen, 1997). Questions of representativeness and reliability arise when dealing with historic documentation. As Yoder points out, "no matter how well researched, historical documentation can only illuminate what the people living at the time saw fit to record" (2006, p. 3). Medieval laws, records, and images should be used as a guide for creating hypotheses and models about medieval activities, while archaeological research can

reveal unrecorded aspects of medieval life and confirm the representativeness of realities expressed in written documents.

With over 50 Danish towns with known medieval history, there is an abundance of medieval material to be investigated. There have been several excavations in both rural and urban settings at a variety of scales, mostly prompted by development projects (Roesdahl, 2004). Also abundant in Denmark are burial sites, many dating to the medieval period. The first 150 years of the medieval period in Scandinavia saw the construction of several thousand Catholic churches with accompanying cemeteries (Boldsen, 1996). Many burial grounds and churches were abandoned in the 14th century following the Black Death, or nearer the end of the Middle Ages, while others are still in use today (Kieffer-Olsen, 1993). An immense number of graves have been excavated, and the human remains belonging to them have become available for analysis, such as in the present study.

Archaeological Setting: Medieval Denmark

The medieval period in Denmark is significantly shorter than that of the rest of Europe. Dating from the end of the Viking period in 1050 to the Danish Reformation in 1536, Denmark's medieval period is known to have been a tumultuous time, especially in the 14th century. The Great Famine (1315), the Black Death plague (1350), and the Late Medieval Agrarian Crisis (1350) are a few of the notable events which must be considered as interplaying factors in social, economic, and demographic realities at the time (Yoder, 2006). There has been much research into each of these factors and a

general understanding of the impacts on life at the population level has been reached with the help of historic documents, and archaeological excavations.

The population of Denmark increased through much of the medieval period. Between A.D. 1000 and 1300 the population is estimated to have doubled from 750,000 people to 1.5 million (Jordan, 1996; Yoder, 2006). Many factors likely contributed to this growth in population. Around the turn of the 11th century Danish communities became more sedentary. This was likely related to the establishment of parish systems with churches being built, and a strengthening feudal system seen across Europe. In Denmark specifically, a transition to increased sedentism involved a transition from an economy driven by raids as seen in the Viking period, to peaceful home-based economies (Poulsen, 1997). The adoption of a crop rotation system after the 12th century also prevented farmers and communities from needing to move to fresh land once present fields had been exhausted (Yoder, 2006).

Favourable climate and increased arability of land have also been identified as important factors in this early stage of medieval history throughout Europe. The first two centuries of the medieval period, which were the warmest years since the 2nd century A.D., are referred to as the Medieval Warm period. It has been suggested that during this time average summer temperatures were 0.7°C to 1°C higher than the average temperatures in 20th century. The growing season was extended, marginal lands became arable and thus habitable, and production was increased overall (Fagan, 2000; Yoder, 2006). It is estimated that individual farms increased from 2-5 hectares to 15 hectares in cultivated areas from the Viking to the medieval period (Hybel & Poulsen, 2007). It is also suggested, however, that the average temperatures in the northern hemisphere were

only 0.2°C warmer during the Medieval Warm Period relative to the Little Ice Age that followed in the 14th century (Hybel & Poulsen, 2007). The overall impact of the Medieval Warm Period is uncertain and may have only been felt acutely in geographically marginal areas. Regardless of the role of climate, agricultural production was revitalized during this period with the construction of mills, the clearing of previously unused land, and an increased focus on livestock production (Poulsen, 1997). The major crops grown in medieval Denmark were cereal grains. In northern Europe, from the 8th to 11th centuries leading up to the medieval period, cereal grains increased from one third of the caloric intake of the peasant population to three quarters (Hunt & Murray, 1999). Barley and winter rye dominated and some regions exported grain continuously throughout the medieval period (Yoder, 2006). Mixed agriculture involving some animal husbandry not only helped to fertilize crops, but was appropriate for Denmark's soils which are a mixture of the pastoral lands characteristic of the rest of Scandinavia, and the arable lands of mainland Europe (Poulsen, 1997). Increased agricultural efficiency related to the use of mills and draught animals, as well as favourable climate, not only increased productivity and permitted population growth, but also freed up more time that was devoted to other tasks. This resulted in specialization in crafts by individuals no longer required in the fields (Hunt & Murray, 1999: 19).

Farmland in the early medieval period was largely owned by religious institutions and nobility; however, both freeholders and semi-free tenants worked the land according to a variety of agreements (Poulsen, 1997). Hunt and Murray describe this as a "productive tension of cooperation/compulsion between the lords and peasants of Europe" (1999, p. 18). Slavery continued on farms operated by both peasants and lords

until the 12th century and many farm operations involved large tracts of land. Peasant farming activities gradually increased and spread, facilitating a reduction in the dominance of large-scale farming operations managed by the wealthy. These became the dominant operations by the 14th century and were largely independent and family-operated (Poulsen, 1997). It was also around the 14th/15th centuries that Lords became more involved in agricultural production.

Population growth in Northern Europe was accompanied by steady economic growth; however, the latter slowed before the former. This resulted in economies unable to sustain the continued population growth, and eventually, as demands on resources increased and could no longer be sustained, populations became increasingly stressed (Jordan, 1996). Economic growth was largely tied to agricultural yield and success. Even in good years, grain yields were less than 4 grains harvested per grain sown meaning that one poor season could increase the price of grain. Communities could endure one or two low yielding seasons; however, in cases of multi-year periods of poor weather, be it drought, early frosts, or infestations, communities were sure to suffer, and famine was likely (Hybel & Poulsen, 2007).

A shift took place after the Medieval Warm Period in Europe, known as the Little Ice Age. This period was characterized by cool winters, hot summers, drought and flooding. Beginning in the early 14th century in Iceland and Greenland and later in the century elsewhere, including Denmark, this period wreaked havoc on populations accustomed to the productive and relatively consistent climate of the Medieval Warm Period (Fagan, 2000). Weather became a growing concern and crops failed, bringing about starvation and famine. The year 1316 was the worst recorded year for grains in the

medieval period, and the famine of 1315-1317, caused by extreme rainfall, was the most severe (Hybel & Poulsen, 2007; Yoder, 2006). This event is extensively reported in many areas of northern Europe; however, Danish reports are less complete and inconsistent with regards to this famine. The period from 1330 to 1390 appears to be have been particularly cold in Scandinavia (Vahtola, 2003). Grain production in Denmark's west is known to have stagnated in the 14th century and abandoned farms were reported as early as 1315, in particular in peripheral areas that were less arable (Poulsen, 1997; Vahtola, 2003). Agriculturalists became more involved in commercial economic pursuits such as the export of cattle, and on the islands, coastal areas persisted, taking advantage of fishing and trading opportunities when inland, settlements were being abandoned (Poulsen, 1997; Vahtola, 2003). However, cattle were affected, because feed increasingly became difficult to produce, and disease was spreading (Fagan, 2000; Yoder, 2006). Some years, and even periods of a dozen years, saw drier summers and warmer temperatures as a respite from persistent flooding. However, inconsistent weather continued in large part until the 1800s, making agricultural success tenuous and prompting the characterization of this period as the Late Medieval Agrarian Crisis (Yoder, 2006).

Poor weather and resulting food shortages were not the only cause of the recurring famines in the medieval period. Commercial sanctions and wars also contributed to restricting access to sufficient food and Denmark was not immune to these realities. If poor harvests and food shortages did not extend over a significant geographical area, trading activities were often prompted to lessen the impact on affected

communities (Hybel & Poulsen, 2007). As such, a combination of agricultural and commercial shortages, or sustained and expansive shortages, was the most devastating.

Despite plagues in previous centuries, nothing prepared European populations for the vast destruction of the plague of 1348-1350, now referred to as the Black Death. The Black Death is believed to have killed one third of Europe's population during this first outbreak alone (Aberth, 2005; Benedictow, 2006). The plague reignited once a decade for the next century, though with less force, and estimates put average death rates at 50% of Europe's population overall.

The Black Death followed closely behind decades of famine. As such, population growth had already been stunted and populations declined heavily during the plague years. Land prices, and thus rent decreased as production and population decreased leaving more land available (Vahtola, 2003; Yoder, 2006). The plague is commonly regarded as causing a deceleration and decline of medieval society and culture. However, a second line of thought considers it an inevitable result of overpopulation in Europe. More recently, historians have sought to emphasize the industriousness and resilience of the surviving populations, who, despite great adversity and dwindling populations managed to increase the efficiency of existing systems and reinvigorate existing cultural practices (Aberth, 2005). The challenges faced and the significant population decline recorded during the plague years are reflected in the many churches that were abandoned at this time. Though the total number continues to grow, abandoned churches number in the thousands, and probably in the tens of thousands, in Denmark alone. The mid 15th century saw the first signs of recovery, and population levels began to recover in the 16th century (Yoder, 2006).

It is generally understood that the population growth in the early medieval period was accompanied by increasing urbanization. Scandinavia lagged behind Europe with respect to urbanization, though Denmark developed more rapidly than other Scandinavian countries, with 50-60 towns identified before the end of the 13th century. At this time, towns in Sweden counted 15, and Finland had only one or two (Benedictow, 1996). Urbanized communities made up less than 5% of Danish parishes in 1200, with this number doubling before the end of the Middle Ages (Boldsen, 1996). Urbanization was motivated by both changes in agriculture and economy as well as political changes (Corsi, 2008). The human movement of individuals within and between mortuary populations is of interest to this study. With both urban and rural samples, human movement associated with urbanization may be identifiable.

Migration theory in archaeology

Human migration has implications for cultural interaction, economic progress, the spread of disease, and much more, intimately connecting it to many areas of anthropological research. Despite this, archaeology has skirted the subject on and off for decades (Burmeister, 2000). The popularity of migration as an explanation for culture change has risen and fallen in archaeological studies over the recent past. A movement away from what some termed 'hyperdiffusionism', or culture change explained by population diffusion, occurred when empirical evidence for human movement could not be presented (Clark, 1994). Until recently, migration could not be demonstrated as a past activity, and to date is incompletely understood in most archaeological cultures.

Past human migration can appear unpredictable and chaotic, and has often been treated this way in traditional archaeological research (Anthony, 1990). Much research has focused on the supposed result of movement, avoiding the more difficult to identify causes, and the movement itself. Anthony (1990) argues that traditional methodologies are not based in a solid understanding of migration as a human behaviour, and that "archeological methods for recognizing migration were never related to or drawn from any explicit set of postulates setting forth how migrations worked" (1990, p. 896). This lack of theoretical framework has hindered the explicit testing of migration models. Anthony (1990) suggests that human movement must be treated as an orderly and intentional activity. Emphasis should concentrate on push and pull factors, economic and otherwise, which are further mitigated by factors such as access to information and transportation, thus linking human movement to other human activities from which it stems or to which it gives rise (Anthony, 1990).

Not all modern definitions or concepts of migration may be applicable in scale to past migrations, and Clark (1994) warns against using modern understandings of human movement as analogies for past migrations. It has become commonplace to forego providing a definition when discussing migration in an archaeological context, and understandings of the concept must be gleaned from what is implied (e.g. Leach, Lewis, Chenery, Müldner, & Eckardt, 2009; Price et al., 2004). One definition likely to be applicable to the realities of small populations, whether modern or historic, is one that Anthony puts forward: "a behavior that is typically performed by defined subgroups (often kin-recruited) with specific goals, targeted on known destinations and likely to use familiar routes" (1990, pp. 895–6).

In addition to a general definition, Anthony suggests the use of several theories of migration produced in the social sciences, such as leapfrogging, migration streams, and return migration (1990). Understanding the possible archaeological impacts of these various patterns are crucial to interpreting possible migration in the archaeological record; however, the fact that the archaeologically visible result of a variety of activities, such as trade, innovation, and migration will overlap must not be overlooked, so as not to oversimplify interpretations.

This study will investigate human movement at the individual level, and to the extent possible, the population level. A focus on the individual is not incongruent with the definition presented by Clark, as it is individuals who make up the subgroups to which he refers. At the time of writing, Anthony did not have access to data that could clearly identify an individual migrant. Most migration studies focus on cultural material and seek to identify outside influence on the record. This may be a change in artifact style or type, or a new method of production. The causes of such changes are difficult to identify, and trade and innovation compete with migration and diffusion as leading explanations in individual archaeological contexts.

The mortuary record provides several opportunities to study human movement. The potential for scientific study of human remains will be discussed in detail later; however, this was not always available to archaeologists. Mortuary artifacts are unique because they are likely to not only reflect the culture of the community and actions of the surviving members, known as extrinsic factors, but also to reflect factors specific to the deceased individual, known as intrinsic factors (Parker Pearson, 2000). Although others control extrinsic factors, an individual's identity as an outsider may be widely recognized,

and thus can be reflected as deviations from typical rituals in the mortuary preparation and artifacts (Alekshin et al., 1983).

There is a substantial assumption made in many migration studies that co-burial equates with co-residence and vice versa (Spence, 1974). This is not always true, as cases of individuals being returned to their birth-home for burial have been observed both ethnographically and historically (Parker Pearson, 2000). As such, a solid understanding of human movement into and out of a cemetery population is one important factor to consider in demographic studies based on cemetery samples. Genetics and inherited non-metric skeletal traits have been used to try to tease out information about post-marital residential status and other forms of migration (Spence, 1974, Tomczak & Powell, 2003). Though these studies may miss migrants who returned to their birthplace, and present other methodological challenges, they have proven useful, in particular in combination with artifactual evidence.

As illustrated, migration may reduce the representativeness that the burial population maintains of the regional population demographics that are being reconstructed. Alternately, burial population demographics may help to reveal human movement in past populations when sex and age distributions are outside of those expected. The community of Lund, located in Scania underwent significant expansion and urbanization in the early medieval period with accelerated building of churches and houses. This required labour from outside the town, and adult male immigrants filled this need. This piece of human movement history was gleaned in part from demographic distributions in the cemetery population of Lund which contained a disproportionate number of males, and an overall higher age at death (Benedictow, 1996).

Generally, migration is motivated by stresses in the home region and attractive circumstances at the destination; however, these features may be difficult to identify (Anthony, 1990). A basic definition of migration, or human movement, will be used here: the movement of people from their home community to another destination for an extended period of time. It is unnecessary to further define the term at this time, because the type of migration being considered will not preclude the identification of others in the study being conducted. This is because it is the possible difference in geographies of and within individuals that is being analyzed. As such, the data should reflect any type of migration, and leaves the interpretation of migration type to other forms of archaeological investigation.

Cases where migrants can be identified, but the movement itself remains invisible should be seen as evidence of sufficient push from the place of origin, and/or pull from the destination unhindered and/or aided by other factors. This may promote the testing of further hypotheses regarding these push and pull factors. Additionally, factors promoting or deterring human movement may differentially impact various subgroups of a population. Demographic information about individuals found in the archaeological record to have migrated may help tailor research aimed at identifying promoting factors.

This study seeks to identify the migration of individuals using oxygen stable isotopes in childhood dental tissues. These data will be compared at the population level to identify outsiders. Once these individuals are identified, those with possible migrant status could be compared to the demographic profile, health status, and movement pattern of other individuals in order to consider any possible patterns. While a full analysis of both sites being considered in this study is beyond the scope of this project, every effort

will be made to use available data in combination, keeping in mind that "only the study of human skeletal remains in combination with the archaeological data can provide the data necessary to answer questions regarding actual population movement" (Blom, Hallgrimsson, Keng, Lozada, & Buikstra, 1998, p. 240).

Migration in medieval Denmark

Human movement was an integral part of the Viking period in Denmark and elsewhere. In addition to agricultural activity, local trade and craft production, economic activity consisted largely of raiding and trading and much activity occurred in landing sites (Corsi, 2008). Reports of long distance movement are not as prominent in the medieval period; however, evidence for trade exists, and human movement is being investigated. As noted earlier, the medieval period in Denmark was punctuated by a series of catastrophic events, the most consequential of which were the Great Famine, the Late Medieval Agrarian Crisis, and the Black Death (Yoder 2006). These and other events had demographic consequences, including human movement into and out of a variety of areas which provided more or less opportunity at any given time throughout the Middle Ages (Yoder 2006).

Food production is closely tied to human habitation and success in a given locale, with the arability of land frequently dictating where and in what density populations may settle. While grain cultivation dominated subsistence practices at the beginning of the medieval period, some focus began gradually shifting to animal husbandry in the middle of the 14th century. Yoder's (2010) dietary study of medieval Denmark suggests that the middle medieval period was witness to significant change in urban diet, suggested to be

the result of disruption in food product exchange during this tumultuous time. This subsistence shift along with increasing urbanization and related spread of infectious diseases likely contributed to the population crisis in the 14th century which marked the end of centuries of socio-economic growth in Denmark.

As noted earlier, prior to the adoption of isotope studies in archaeology, human movement was difficult to study empirically in past populations. This was certainly the case in studies of medieval populations for which registers and other documentation were not made or have not survived. Despite challenges, attempts have been made to discuss the subject of human movement in medieval Denmark in theoretical terms. A study of human movement was made for model populations by Boldsen (1989) using marriage laws and palaeodemographic information as the bases for computer simulations. These simulations outlined the required migration rates for model populations of 75, 150, and 300 people. The requirements for migration were those that would supplement the population in order for the population to be maintained without violating marriage regulations as set out by the Catholic Church. A birth rate greater than the rate of death is considered to be the basic requirement for population stability. Unsurprisingly, the models demonstrated that smaller populations require a greater level of immigration than larger communities in order to maintain population size while avoiding familial intermarriage (Boldsen, 1989). It should be noted that the models discussed are considerations of required movement. As such, they do not reflect the realities of individual choice or population dynamics that may be impacted by geographic and other constraints. For instance, it would be logical to assume that human movement from rural to urban is to be expected in a period when centers were becoming increasingly

urbanized, and where urbanized centers presented pull factors for those living in rural areas and vice versa. This will not be present in simulations such as the one outlined; however, it is these migrants that might be expected in the sample analyzed in the present study. This simulation is a valuable model with which to compare migration data from populations of comparable size.

Stable Isotopes

An isotope of an element has the same number of protons, but different number of neutrons in the nucleus, and therefore a different mass (Schoeller, 1999; Schoeninger & Moore, 1992). Natural variation in the ratio of the heavier and lighter isotopes of an element has long been recognized. The measurement of these variations is an important tool with many applications. While many analytical methods were developed within the field of geochemistry, archaeologists have adopted and adapted these for archaeometric use, and the incorporation of stable isotopic studies into the interpretation of excavated human remains is now commonplace.

Stable isotopes do not degrade with time as radioactive, unstable, isotopes do, and thus, the ratio of heavy to light isotopes in a reservoir remains constant in the absence of reactions which may cause fractionation. Fractionation is defined as the partitioning of the isotopes between phases (gases, liquids and solids). Fractionation can be caused by either kinetic or equilibrium processes (Schoeller, 1999). Schoeller describes the resulting isotope abundance ratios present at various stages in an element's journey as an "isotopic fine structure" (1999, p. 667). Although in some forms of research this complexity may be seen to be cumbersome, and has in the past caused problems with interpretation (eg.

Hall, 1967), it also enables the interpretation of not only the source or final destination of an element, but reaction mechanisms and environments as well (Schoeller, 1999).

As general rule, the fractionation of the isotopes of the lighter elements (e.g., H, Li, B, C, O, N, S) is more pronounced than in the isotopes of heavier elements (e.g., Fe, U) due to the relative difference in masses between the light and heavy isotopes (Urey, 1947). The first measurement of hydrogen and oxygen isotope ratios in the hydrological cycle occurred in the 1930s through density measurements of water and air (Sharp, 2007). Isotopic measurement has progressed to include an impressive range of elements, opening up a wide variety of research questions (e.g. Fenner, 2008; Nakamura, Schoeller, Winkler, & Schmidt, 1982; Sharp, Atudorei, Panarello, Fernández, & Douthitt, 2003; White et al., 1998).

Stable isotopes in archaeology: Human remains

Human remains recovered from archaeological sites provide a unique opportunity to reconstruct the biological life history of an individual. The study of stable isotopes in archaeological remains has become a well-recognized means of reliably gaining information about an individual's diet and movement. Hard tissues such as bone and teeth stand the greatest chance of being preserved in the archaeological record, and maintaining their original isotopic values. Isotopic investigation was first applied to archaeological problems in the 1970s. Carbon was the first isotope employed, immediately followed by Nitrogen, through use in dietary studies (DeNiro & Epstein, 1978, 1981; Van Der Merwe & Vogel, 1978; Vogel & Van Der Merwe, 1977). Other isotopes followed, including oxygen, making use of a wider range of preserved biological

tissues. Overall, the analysis of human bones and teeth has provided access to a vast set of data that, ideally, speaks directly to the activities of individuals during their lifetime.

Bones and teeth are largely composed of bioapatite, the biological form of apatite, which also exists in geological and artificial forms. Some forms of bioapatite are similar to hydroxyapatite, $Ca_5 (PO_4)_3 (OH)$, which contains a hydroxyl ion. This apatite form, unique to mammalian tissues, is composed of 38% phosphorus, and 18% calcium with traces of sodium and magnesium (Burton, 2008; Hillson, 1996). Mammalian hard tissues are distributed across a spectrum of hydroxyapatite-like apatites with varying degrees of substitutions and crystal sizes (Lee-Thorp, 2002). In mammalian tissues, oxygen isotopes can be analyzed from bone collagen or one of the oxygen-bearing components of bioapatite for the purpose of studying human movement and origin. It has been rightly pointed out that the characteristics that differentiate tissues, including the non-mineral/non-apatitic components, result in different functional properties in life as well as in post-mortem contexts (Lee-Thorp, 2002). Choice of sample tissue must take into account life function and development as well as composition and structure that may influence the susceptibility of a tissue to various diagenetic agents.

Although teeth are somewhat complex due to the different tissues involved, once separated, they can be effectively used for chemical analysis. Enamel is almost entirely inorganic (mineral). It is composed of densely packed, well-formed hydroxyapatite crystals, making it significantly more resistant to chemical alteration than the less densely packed hydroxyapatite in bone (Burton, 2008). For this reason, enamel is increasingly chosen for chemical studies, and the merit of this choice will be evaluated. Dentin and cementum are similarly composed with less well developed crystalinity and higher

organic content (Hillson, 1996; Roche, Ségalen, Balan, & Delattre, 2010). Dental tissues are unique in their utility for bioarchaeological study, because both enamel and dentin do not undergo tissue turnover in the way that skeletal tissues do and thus their chemistry is preserved from childhood when the tissues formed. Cementum also does not turn over, but continues to form throughout life as a reaction to dental trauma and natural stress, acting to secure the dental root in the surrounding alveolar bone, thus reflecting a more generalized lifetime chemistry (Hillson, 1996). Because of these characteristics, dental tissues offer the possibility of comparing isotopes from different stages of an individual's life (Balasse, Bocherens, Mariotti, & Ambrose, 2001; Price, Bentley, Luning, Gronenborn, & Wahl, 2001; Price, Johnson, Ezzo, Ericson, & Burton, 1994; Richards et al., 2002).

The earliest isotopic studies of biological tissues aimed to understand paleoenvironments. Oxygen isotope fractionation factors were developed for many mineral-water pairs and were used as geothermometers (Epstein, Buchsbaum, Lowqenstam, & Urey, 1953; Kolodny, Luz, & Navon, 1983; Longinelli & Nuti, 1973a, 1973b; Longinelli, 1965, 1966, 1984; Tudge, 1960; Urey, 1947). Bioapatites have proven useful for an ever-increasing suite of isotopic investigations (Kohn & Cerling, 2002). Isotopic compositions in bioapatites reflect the environment in which an organism lived as well as the physiological filters within the organism that result in isotopic fractionation. The study of bioapatites has been used to investigate various factors that make up these two complex contributions (e.g. Koch, 1998; Lee-Thorp, 2008).

Archaeological migration and oxygen isotopes

There are three isotopes of oxygen: ^{16}O, ^{17}O, and ^{18}O. Oxygen-17 is the least abundant (0.04%) (Schoeninger, 1995). It can largely be disregarded in studies of oxygen isotopes of terrestrial materials because its relationship to ^{16}O co-varies with that of ^{18}O, and ^{17}O anomalies are not observed in large mammals (Gehler, Tütken, & Pack, 2011). Masses 16 and 18 are much more abundant, at 99.8% and 0.2% respectively, and are commonly used in isotopic research. Variation in $^{18}O/^{16}O$ ratios in water is related to the hydrologic cycle. The basic principle is that the lighter isotope, ^{16}O, will more readily partition into the vapour phase, while the heavier isotope, ^{18}O, will more readily go into the liquid phase. Precipitation requires a reduction in temperature, and as temperature drops, the heavier isotope is rained out and the $^{18}O/^{16}O$ ratio changes in the vapour phase. The resulting ratio of heavy to light isotopes is greater in initial rainwaters than those released as a weather system moves farther from its source (Dansgaard, 1964). As such, the geographical location of meteoric water (e.g., ground water, streams, and lakes), local temperature, the number of local precipitation events, and altitude, largely determine the oxygen isotopic composition of water (Craig, 1961; Longinelli, 1984; Luz & Kolodny, 1985). While isotope ratios vary geographically, they are not unique to specific locations, as similar conditions may produce similar ratios across a wide region, or in two geographically distinct areas.

Migration has been approached in many ways through the archaeological record, and advancements in archaeometric methods now permit the investigation of human movement at the individual level. Of interest in this study are oxygen isotopes in dental tissues. Oxygen isotopes are incorporated when developing tissues are in equilibrium

with body water, which is derived from consumed water. The oxygen isotopic composition of the tissue is ultimately determined by the source of the water (Longinelli, 1984). This association was first used in palaeoclimatological and palaeohydrological research, but was later applied to archaeological migrations studies (Evans, Chenery, & Fitzpatrick, 2006).

Other isotopes have also been used in studies of geographical origin or human movement. An early study used carbon isotopes to glean information about geographical origin (Nakamura et al., 1982). Strontium and lead isotopes have also been used to reconstruct human movements (Montgomery, Budd, & Evans, 2000). More recently, strontium and oxygen isotopes have been used to determine human migration patterns (Evans, Chenery, et al., 2006; Evans, Chenery, & Montgomery, 2012; Price, Burton, & Bentley, 2002; Price et al., 1994, 2004). Strontium is initially sourced from a region's underlying geology, and isotope ratios vary across a region, providing similar interpretive opportunities as oxygen isotopes.

Metabolic fractionation is integral to the incorporation of isotopes into body tissues. Fractionation occurs between consumed water and body water, and again between body water and tissue incorporation. In mammals with consistent body temperature, these fractionations have been found to be consistent, and the relationship between the origin water and final isotope values in tissues has been quantified (Levinson, Luz, & Kolodny, 1987; Longinelli, 1984). Although this comparison is not without conflict and potential error (Amiot et al., 2007; Daux et al., 2008; Lecuyer, Grandjean, & Emig, 1996; Pucéat et al., 2010), calibrations and conversions have permitted tissue values to be converted to water values. This has introduced the potential

to incorporate geographic locales into interpretations of human movement (eg. Evans, Chenery, & Fitzpatrick, 2006) without the use of modern reference samples.

Despite advancements in precision and interpretive aids, the interpretation of isotopic data is not without uncertainty. Oxygen isotopes from biological tissues reflect both physiological and environmental factors. Although the physiological input is similar from individual to individual within a species, potential shifting factors must be considered. In mammals, the activity of feeding through lactation produces a fractionation in oxygen isotopes from mother to offspring, which produces a trophic level effect (L. E. Wright & Schwarcz, 1998). This can complicate the interpretation of teeth formed prior to weaning, and thus, the use of post-weaning teeth is preferred in most studies. Alternately, biological tissues that are suspected to have formed around the age at which weaning occurred should show a shift in isotopic values due to the cessation of the trophic level effect, and oxygen isotope research has become a tool for solving this aspect of life history reconstruction (Richards et al., 2002; L. E. Wright & Schwarcz, 1998).

Preparation and analysis of stable isotopes from human remains

Stable isotope geochemistry, with the variety of mass spectrometers available, can accommodate four basic sample types: solids, powders, liquids and gases. Each bioapatite can be subject to a range of preparations allowing researchers to make best use of the combination of samples and analytical instruments available to them, increasing the likelihood of obtaining a reliable signal that might serve as a proxy for life activities. Most commonly used in archaeological studies are solid samples, either in powder or crystal form.

Sample preparation is an integral part of any isotopic analysis, serving to ensure that the desired isotope is isolated and quantified. Although oxygen isotopes are more readily accessible through the study of carbonates, phosphate emerged as an oxygen isotope analyte in the 1960s following the development of methods to isolate PO^{3-}_4 (phosphate) as $BiPO_4$ (Firsching, 1961; Tudge, 1960). Soon after, phosphate was isolated from apatites as the more stable Ag_3PO_4 (silver phosphate) (Baxter & Jones, 1910; Firsching, 1961). Eventually, methods were developed to analyze oxygen isotopes from silver phosphate (Crowson, Showers, Wright, & Hoering, 1991; O'Neil, Roe, Reinhard, & Blake, 1994; E. K. Wright & Hoering, 1990). The motivation behind phosphate methods was to compliment carbonate as a palaeothermometer as well as to find a more diagenetically resistant alternative to carbonate analysis (Epstein et al., 1953; Sharp, Atudorei, & Furrer, 2000; Urey et al., 1951).

The methods for converting and measuring oxygen isotopes from prepared phosphate samples accompanied the developments in phosphate isolation in the last decades (Crowson et al., 1991; O'Neil et al., 1994), and have been further developed to include on-line methods, such as high temperature conversion and pyrolysis (for full review see: Werner, 2003). The method presented by this work reduces the time required to isolate phosphate from dental tissues, and extends its use beyond enamel and dentine, to cementum, a tissue less often exploited in archaeometric studies.

Diagenetic considerations

When working with archaeological remains, diagenesis is always a concern. Diagenetic alteration of dental tissues has been extensively investigated and debated in

the archaeological literature (Kohn, Schoeninger, & Barker, 1999; Lee-Thorp, 2002; Shin & Hedges, 2012; Tütken, Vennemann, & Pfretzschner, 2008; Wang & Cerling, 1994). As such, the type and condition of remains must be used to inform choices regarding the most appropriate analytical tool and preparation technique. Enamel is widely held to be the most diagenetically resistant dental tissue (Sharp et al., 2000; Wang & Cerling, 1994; Zazzo, Lécuyer, Sheppard, Grandjean, & Mariotti, 2004); however, a number of studies demonstrate that biogenic isotope values can be altered in this mineralized, tightly packed tissue (Wang & Cerling, 1994; Zazzo, Lécuyer, et al., 2004). Dentin and cementum are more highly organic than enamel, with smaller crystals and greater porosity. These characteristics allow space for diagenetic fluids, increase surface area access to mineral crystals, and make these materials more susceptible to diagenetic alteration in the burial environment (Wang & Cerling, 1994). Two main types of diagenetic processes that impact archaeological bones and teeth have been identified (Lee-Thorp, 2000). The first is the incorporation of non-apatitic material in natural tissue pore spaces. Recrystallization and growth of secondary apatite crystals is the other form of diagenesis that could affect the oxygen isotopic composition of dental tissue.

As discussed, oxygen isotopes can be measured from either the carbonate or phosphate components of apatites. Phosphate boasts a greater resistance to diagenetic processes, such as oxygen exchange through dissolution and precipitation. Many authors have argued that isotopic signals derived from phosphate are "nearly perfectly preserved after death" (Kolodny et al., 1983, p. 398). This assessment was largely forwarded by Tudge (1960) based on experiments by others demonstrating that exchange between the oxygen of water and that of phosphate ions, once bonded, is minimal or non-existent at

surface temperatures. These experiments showed that it is phosphate metabolism reactions that cause equilibration with surrounding water, encouraging the view that phosphate is a resistant biological component after the death of the organism (Winter, Carlton, & Briscoe, 1940). More recently, however, the effect of microbially mediated diagenesis, or isotopic exchange has been detailed, demonstrating that phosphates are not immune to diagenetic effects (Ayliffe, Lister, & Chivas, 1992; Blake, O'Neil, & Garcia, 1997; Zazzo, Lecuyer, & Mariotti, 2004). The growth of many microorganisms depends on phosphate and the preferred form is inorganic, the greatest source of which is often the apatites of organic materials, such as those found in the biological hard tissues of deceased organisms (Blake et al., 1997). Recrystallization of bioapatites as a result of enzymatic activity occurs in early diagenesis in association with organic degradation and is likely to be incompletely equilibrated, meaning that the measured isotope abundance ratios may reflect a combined biogenic-depositional/diagenetic signal (Blake et al., 1997; Zazzo, Lécuyer, et al., 2004). It may be possible to identify postmortem activity of bacteria through microscopic textural analysis (Kohn et al., 1999; Zazzo, Lécuyer, et al., 2004); however this is not always possible. Overall, it is important to be aware of the possible diagenetic alteration of the materials being examined, and whether this diagenesis extends to the tissues, elements, and components of concern.

Various imaging techniques have been employed to characterize the biological integrity of archaeological human remains. Burton (2008) lists optical microscopy, infrared spectroscopy, x-ray diffraction, thin section analysis, and the use of a microprobe as methods for identifying contamination in bone. These analyses are equally valid in the assessment of dental tissue integrity, and could apply to other tissues and materials as

well (LeGeros & Tung, 1983). In addition to material imaging, van Klinken (1999) identifies chemical indicators, elemental data, and stable isotope ratios as means for identifying the quality of materials. Values not in line with those characteristic of comparable modern materials, may suggest poor sample integrity. The author recognizes the circular nature of this consideration, but argues that there is some value in this consideration (van Klinken, 1999).

Diagenetically altered materials are best avoided; however, there are ways to make the best of altered materials. The first type of diagenesis, the incorporation of non-apatitic minerals, is commonly mitigated by a simple weak acid washing. This serves to dissolve more soluble materials, ideally those that are post-depositional (Lee-Thorp, 2000). Another means of obtaining a usable sample from a previously contaminated one is to isolate the uncontaminated portion directly. This has been referred to as sample-specific component extraction or 'S-methods' and has been used in the extraction of specific amino acids, specific tripeptides and carboxylic acid (2002). This strategy could also apply to phosphate isolation when microbial activity is not suspected. The S-methods are advantageous because they can be carried out without specifically identifying the contaminants present. S-methods are typically more costly with respect to time and resources, but may add value when diagenesis is suspected. Care must be taken to ensure that data obtained from an isolated portion of a given bioapatite is not directly compared to isotope values from another, as it has been demonstrated that different components are isotopically related, but not equal (Cerling & Sharp, 1996; Longinelli, 1966). The relationship between oxygen isotope ratios in carbonate and phosphate has been extensively studied and has been considered as a potential means of identifying

diagenesis in bioapatites (Iacumin, Cominotto, & Longinelli, 1996) as well as a means of reconstruction biogenic values (Zazzo, Lécuyer, et al., 2004). In this study, phosphate is used with the intention of mitigating diagenetic issues with the samples available, both identified and unidentified.

Chapter 3: Materials and Methods

The Study

The study reported here investigates human movement in two medieval Danish cemetery populations through the isotopic analysis of dental material. Oxygen isotopes are used as a proxy for human movement. Social, political, and economic life in Denmark underwent significant change from the beginning to the end of the medieval period (Corsi, 2008; Yoder, 2006). As discussed, any change in push or pull factors may provide incentive for human movement. This incentive may influence an individual or a group to relocate and it is this that may be reflected in the dental chemistry of medieval populations. Human remains from Sejet and Ole Worms Gade provide an excellent opportunity to investigate the socioeconomic shift that occurred during the medieval period. Together the sites span the majority of the medieval period, with Sejet in use during most of the Middle Ages, and Ole Worms Gade primarily in use during the late medieval period. In addition, Sejet is a rural cemetery while Ole Worms Gade is urban. These attributes will permit the comparison of human movement in urban and rural contexts during a period of increasing urbanization and will examine human movement on both the individual and population scales. Overall, this analysis will contribute to other questions being asked regarding the impact of the shifting socio-economic conditions on other aspects of medieval life in Denmark and in Northern Europe in general.

The sites

A section of Ole Worms Gade, a street located in the Danish city of Horsens, was excavated by the Horsens museum from 2007 to 2009. The site, named after the street (Ole Worms Gade; HOM 1649), consists of a large cemetery associated with a medieval city. During the Middle Ages, the cemetery was located outside of the city walls. Excavations uncovered approximately 450m^2 of the estimated 9,400m^2 original cemetery footprint (Pedersen, 2010). Most of the 650 excavated graves contained preserved skeletal material and 400 skeletons have been analyzed by Pedersen (Pedersen, 2010). Skeletal preservation at the site was largely affected by subsequent burial and other activity, meaning that the majority of graves with skeletal material were disturbed and had less than one third of the skeleton present at the time of excavation (Pedersen, 2010).

Mortality profiles can be used to reveal demographic trends of a population. These do not always reflect living population profiles; however, some interpretations can and have been made. Children (under age 16) represent approximately 33% of the primary burials (Pedersen, 2010). This number is considered to be an underrepresentation due to the likelihood that many did not preserve, and is consistent with other medieval cemetery populations. Women are underrepresented as compared to men, possibly a consequence of burial custom. Men also outlived women by approximately two and a half years, which could be interpreted as evidence for increased maternal mortality. High mortality in infants, a common characteristic of medieval populations, is not reflected in the excavated material at Ole Worms Gade; however, this is likely due to the above-mentioned taphonomic factors (Pedersen, 2010). Finally, both men and women between the ages of 20 and 25 are greatly underrepresented. This is unlikely to be as a result of

reduced risk of mortality at this age, and as such, is open to other interpretations, many of which hint at human movement at this age.

The condition of dental material at Ole Worms Gade was recorded by Pedersen for the 400 primary burials selected (Pedersen, 2010). Enamel hypoplasia, an indicator of general stress at the time of enamel formation, was present in 51% of the maxillary canines observed, a proportion not unlike other medieval populations (Boldsen, 2007; Hillson, 1996; Pedersen, 2010). The population also exhibited heavy tooth wear, in particular, after the age of 30. Approximately 45% of individuals had at least one carious lesion present in their dentition. Pathological conditions reflected in the skeleton have been recorded and discussed elsewhere, and will be discussed later only insofar as they relate to the individuals analyzed.

Sejet is a rural archaeological cemetery located southeast of Horsens. This site was excavated in 2006, also by the Horsens museum. An area of approximately 400m^2 was excavated, comprising the southwest corner of the medieval cemetery, with 632 individual skeletons recovered, 400 of which were found in single graves (Pedersen, 2008). Material preservation was assessed based on the quality of the material preserved, not quantity. Overall, the *in situ* skeletons were approximately half poorly preserved. Construction of more recent graves is evidenced in the preservation status of older materials, as is modern land use and construction. Of the individuals over 16 years of age for whom sufficient preservation permitted confident sex determination, 23% were men, while 18% were women. Children made up 39% of the burial population, some of which were recovered in multiple burials, suggesting that these may be victims of epidemic disease. Life expectancy for adults at Sejet is similar to that at Ole Worms Gade, both

lying near 37 years of age. The discrepancy between men and women's life expectancies is greater at Sejet, nearing seven years, also attributable to higher maternal mortality (Pedersen, 2008). At Sejet, the skeletal population is greatest under the age of 15 and over the age of 30; however, unlike Ole Worms Gade, the number of 20-25 year olds is not as low, and is similar to that of 25-30 year olds in the same cemetery (Pedersen, 2008, 2010). The implications of this are unknown; however, an understanding of human movement at the sites may help to identify the root of these demographic differences.

Once again, the frequency of enamel hypoplasia in this medieval population is high at over 60% for both children and adults. The presence of caries was slightly lower at 32%, and a great frequency of tartar was observed (Pedersen, 2008). Though other bony pathologies will not be discussed here, it is notable that bony changes in the joints related to osteoarthritis are observed in greater frequencies in the population buried at Sejet, than those at Ole Worms Gade (Pedersen, 2008, 2010). This is likely due to the demanding physical labour that would have characterized daily life in rural areas in medieval Denmark.

Neither site has yielded specific dates, and while the medieval period extends from 1050 A.D. to the mid 16th century, findings suggest that Ole Worms Gade cemetery was primarily in use during the late medieval period (Pedersen, 2010; Yoder, 2006). Excavations at Sejet demonstrate use at this site for most of the medieval period, but only sporadically through the later period, likely ending well prior to 1574, the year in which the associated church was demolished, and the parish merged with neighbouring Uth Kirke (Kjærgård, 2006; "Sejet Kirker," 2006). Coins found in excavations at Sejet have been approximately dated to 1400-1500 AD (Kjærgård, 2006), though elsewhere it is

reported that four Danish coins date to the 1200s while one German coin dates to some time before 1379-81 ("Sejet Kirker," 2006). Individual burials may be categorized temporally based on burial position as outlined by Kieffer-Olsen (1993). Observed differences in arm position at burial are used to divide burial populations in medieval Denmark into three periods. Period 1, from the beginning of the medieval to 1300, is characterized by arms positioned alongside the body, or position A (Kieffer-Olsen, 1993; Yoder, 2010). From 1300-1375, position B, with arms crossed over the waist, dominated burial practices, making up Period 2. Finally, Period 3, from 1375 to the close of the medieval period is characterized by arms over the chest (Kieffer-Olsen, 1993; Yoder, 2010). This avenue will be considered when interpreting individual isotopic data.

Materials

Dental samples

Three sets of dental samples were used for the research reported here. The primary research sample was analyzed to address archaeological questions regarding human movement in medieval Denmark. The two standard dental materials were used to develop the methods and preparations to which the research sample was later subjected, and as standards or as comparative materials for a variety of analyses.

Archaeological dental materials

The main research sample used in this study is a collection of archaeological human teeth. The teeth are from individuals excavated from two medieval Danish

cemeteries, Ole Worms Gade (HOM 1649) and Sejet (HOM 1046). The material from both excavations is curated at the University of Southern Denmark in Odense. One hundred and seventy-four teeth were collected from the remains of 167 individuals and are now curated in the Bioanthropology Digital Image Analysis Laboratory (BDIAL) in the Department of Anthropology at the University of Manitoba. This sample contains 99 teeth from 95 individuals excavated from Sejet, and 75 teeth from 72 individuals excavated from Ole Worms Gade. Left mandibular canines were collected when sufficiently preserved, and its antimere selected second. Other teeth were collected, including maxillary canines and incisors, if neither mandibular canine was available, and sometimes in addition if preferred teeth were heavily worn. A sample of 26 teeth was analyzed in this study, with an equal number from each Sejet and Ole Wormsgade. The teeth were selected from the greater sample based on preservation condition and the presence of sufficient material for the analyses chosen.

The teeth are numbered according to grave and individual, following the system employed at the time of excavation and cataloguing. Grave numbers are preceded by an 'A' while individual numbers are preceded by an 'X'. Individual identification numbers alone will be used in this report, as grave and individual numbers are identical for all individuals in this study. Identification numbers for individuals excavated from Sejet fall between 1 and 999 (e.g. X106), while those from Ole Worms Gade fall between 1000 and 2000 (e.g. X1433).

Standard dental materials

Cow

Maxillary teeth were collected from three dead and decomposed cows (*B.p. taurus*) at a farm in Saskatchewan, Canada. Mandibular teeth were not collected, as an unequivocal association of mandible to individual could not be made due to prior scattering of remains by scavengers. The three individuals had been dead and exposed on the ground surface for a period of three to ten years. None of the individuals is known to have suffered any prolonged disease in life. All three individuals were born at the farm from which they were collected, as were their mothers. The cows spent most of their time grazing near their birthplace, and some time in other pastures, approximately 40 and 90 kilometers away.

Human

Four third molars from one living individual were collected shortly after being extracted by an oral surgeon. The teeth were extracted for reasons unrelated to this research, and collected with appropriate ethics review and approval (Protocol #J2012:036). The individual did not change geographic location for any prolonged period of time or to any significant extent during the formation of the teeth collected.

Methods

Sample recording and general sample preparation

Much progress has been made in developing and adapting scientific analyses for their application to bones and teeth. Analytical methods continue to be refined in order to obtain the greatest amount of information regarding past life, while preserving as much of

the material as possible. Striking a balance between these two factors can be difficult and unclear; however, it is essential to consider the destructive nature of each analytical process from start to finish to ensure that maximum value is being gained when destroying biological and other sensitive archaeological materials. Some developments, such as those in digital imaging, may eliminate the need to alter or destroy these finite resources altogether, while methods that are inherently destructive are benefitting from improved sampling resolution and less destructive sample preparation. Progress in refining sample resolution has also opened up new threads of research in many areas, permitting the investigation of new questions. The general sample preparation reported here was designed to facilitate a wider array of analyses than are reported here, and to preserve some material for future research.

The archaeological dental specimens were subject to cleaning and cataloguing as well as photography, 3D scanning, and casting prior to further sample preparation. The teeth were gently cleaned with water, ethanol and acetone using a cotton-tipped stick. Care was taken not to damage the teeth, which were visibly fragile, in particular near to the cement-enamel junction. The presence and location of enamel defects, caries, and calculus was recorded following the standards laid out by Buikstra and Ubelaker (1994). The Smith and Knight stages of occlusal wear were recorded and used to identify teeth suitable for measurement (1984). Detailed crown measurements were taken of the physical specimens based on a combination of two methods (Buikstra & Ubelaker, 1994; Fitzgerald & Saunders, 2006). Calculus was gently removed prior to casting so as to permit the full recording of enamel surface topography; method and results reported elsewhere (Gamble, 2013).

In order to stabilize the teeth for sectioning, all teeth were embedded in epoxy resin. Teeth were then sectioned using a Beuhler Isomet 1000 variable speed saw using a diamond edged blade at 100 RPM. Midline sagittal thin sections were made for analyses reported elsewhere (Gamble, 2013), and the remaining portions (medial and distal sides) were retained for the analyses reported here. Similar embedding and sectioning methods were employed for the cow and modern samples.

Thermal Combustion Elemental Analysis (TC/EA) mass spectrometry

A significant amount of sample preparation is required to isolate the phosphate from other oxygen sources in both geological and biological samples to facilitate the mass spectrometric analysis of phosphate oxygen. Although oxygen isotope data is more easily obtained through the analysis of carbonate oxygen, phosphate oxygen is preferred in many cases where any degree of diagenetic alteration is suspected. These issues have been discussed, however, it is worth reiterating that the phosphate-bound oxygen is less likely to be replaced post mortem than the oxygen bound to carbonate (Kolodny et al., 1983; Tudge, 1960; Winter et al., 1940).

The archaeological teeth analyzed in this study were poorly preserved, many exhibiting severe antemortem wear, leaving them susceptible to further degradation post burial. Dark staining was observed on the outer layer of enamel in some samples, which did not come free through regular cleaning procedures. Finally, when the teeth were sectioned, many exhibited an opaque and crystalline appearance across one or all of the internal tissues, though primarily centered in the dentine. It is as of yet unknown what the

cause of this visual alteration is, though chemical characterization of these areas was carried out and will be discussed later.

Sample preparation

Powdered samples are required for phosphate isolation. Cow tooth samples were prepared as required, providing experimental material for the preparation of the phosphate isolation method. First, the tooth was cut in half in order to make internal structures visible permitting more confident sampling. The exterior and cut surfaces of the tooth that were to be sampled were then cleaned by abrasion with fine sand paper and/or a gritted rotary tool bit. Fine powder was cleaned off, and a clean diamond-gritted rotary tool bit was used to sample each tissue. Equipment was thoroughly cleaned with ethanol between use on different tissues and samples. Each sampled tissue was homogenized and stored in a glass vial until further processing.

Archaeological samples were prepared similarly, however, having been embedded, tissue sampling was carried out using a micromill (New Wave Research). With the aid of a camera (Olympus SZ61) 5 mg of enamel was carefully sampled from each tooth, preferably from only the mesial or distal half, leaving the other half available for other analyses. The enamel was ground and homogenized using an agate mortar and pestle. This was necessary to ensure that sufficient surface area would be exposed to organic-removing chemicals.

Variations on the phosphate sample preparation reported below date back to the 1960s when phosphate (PO^{3-}_4) was isolated first as bismuth phosphate ($BiPO_4$) and then as the more stable (tri-)silver phosphate (Ag_3PO_4) (Firsching, 1961; Tudge, 1960). Ag_3PO_4 precipitation quickly became the preferred analyte for phosphate oxygen work, and its isolation has undergone many alterations by researchers optimizing the method to

best suit their samples and laboratories (Dettman et al., 2001a). Overall, sample preparation is an integral part of any isotope analysis, ensuring that the desired isotopes can be isolated and quantified. For this study, a revised method is used for isolation of the phosphate (PO_4) component of bioapatite from dental tissues in the form of Ag_3PO_4 with a particular focus on enabling more rapid sample preparation, and ensuring isotopic fidelity of samples through effective removal of organic material. Several published methods were attempted and an optimized hybrid method using methods by O'Neil, Roe, Reinhard, and Blake (1994), Stephan (2000), and Wiedemann-Bidlack, Colman and Fogel as guides was established (2008). Method optimization was carried out using cow enamel and cementum samples.

All chemicals used were reagent grade and deionized distilled (DI) water was used in the preparation of solutions and for rinsing. Initial attempts using approximately one mg of sample and the O'Neil et al. (1994) method were found to be unsuccessful, producing very little precipitate, none of which appeared to be Ag_3PO_4. Subsequent attempts following the Wiedemann-Bidlack et al. method were also unsuccessful, resulting in the precipitation of NH_4NO_3 as opposed to Ag_3PO_4. Next, a combination of the O'Neil et al., Stephan, and Wiedemann–Bidlack et al. (1994; 2000; 2008) methods was derived. This method, which successfully produced Ag_3PO_4 was carried out as follows:

- Weigh 5 mg sample into plastic centrifuge tube
- Immerse sample in 2.0 ml 2.5% NaOCl for oxidative removal of organics

  24 hours (covered)
- Centrifuge and remove liquid with dissolved organics

- Centrifuge and rinse with DI water, removing rinse solution ×4
- Immerse in 2.0 ml 0.125 M NaOH to remove humic substances and remaining organics

24-48 hours (covered)

- Centrifuge and remove liquid with dissolved organics
- Centrifuge and rinse with DI water, removing rinse solution ×4
- Immerse sample in 200 µl HF (hydrofluoric acid) to dissolve apatite

24 hours (covered)

- Centrifuge and transfer supernatant into beakers, leaving behind any precipitated CaF_2
- Immediately, at 50˚C on a hot plate, add 1.8 ml silver ammine solution using a 100 µl pipette Silver ammine solution: 0.2M $AgNO_3$; 0.3M NH_4NO_3; 0.74M NH_4OH. (O'Neil et al., 1994)
- Filter crystals from remaining liquid using nitrocellulose filter paper (Millipore) on a vacuum filter. Rinse any residue from beaker onto filter with DI water, and rinse crystals on paper with DI water 4-5 times.
- Transfer filter paper to oven held at 70˚C to dry overnight

Dry samples were collected and transferred into glass vials. In order to ensure that the precipitate resulting from this modified method is pure Ag_3PO_4 and free of contaminating organics, smear mounts were made of precipitate from one cementum and one enamel Ag_3PO_4 sample and analyzed by x-ray powder diffraction using a Bruker D8 microdiffractometer. The resulting spectra reflected appropriate intensities of all elements

making up Ag_3PO_4 with no contaminants appearing at detectable levels (Figure 4). Further to this, an array of Ag_3PO_4 samples produced using variants of the finalized method was subjected to visualization using scanning electron microscope. The method adopted for use on the archaeological sample, and to produce the data presented here produced pure samples of small euhedral crystals, measuring between 0.7 and 1.7 μm across (Figure 5). This is in contrast with samples produced following less effective organic removal methods.

The amount of silver ammine solution required for maximum precipitation of Ag_3PO_4 from enamel was optimized through experimentation and yield calculation. Yield was calculated as a ratio of precipitate weight to original sample weight (mg/mg). Adding 1.8 ml silver ammine solution resulted in approximately 80% yield, and was used in this method. This is lower than some similarly published yields (120%, 210%) while higher than others (50%) (Bera et al., 2010; Tütken, Vennemann, Janz, & Heizmann, 2006; White, Spence, Longstaffe, Stuart-Williams, & Law, 2002). Elsewhere, Ag_3PO_4 yields are report simply in percent yield, without elaborating on how this is calculated. This is problematic, since the exact composition of biological samples is unknown. As such, an exact yield cannot be calculated stoichiometrically, and thus, these yield data will not be compared to that presented here.

Analysis.

The Ag_3PO_4 was analyzed using a Thermal Combustion Elemental Analyzer (TC/EA) paired with a Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) via a ConFlo III universal interface (all components Thermo Scientific, Thermo Finnigan), at the University of Manitoba stable isotope laboratory. This setup permits online

conversion of solid phosphate oxygen to CO gas in an oxygen-free environment. Samples are weighed into silver cups (3.5 × 5mm), sealed, and loaded into an auto-sampler. The sampler is then flushed with helium and sealed. The TC/EA pyrolyzes the samples, one at a time, in a reactor held at 1450˚C. A glassy carbon tube located inside a ceramic outer tube inside the reactor is packed with silver wool and glassy carbon granulates followed by a carbon crucible that catches the samples as they are dropped into the reactor. Oxygen in the sample is converted to CO gas, by reaction with carbon from the crucible. The gas is then entrained in He carrier gas, travelling from the reactor, through a gas chromatography (GC) column held at 90˚C and into the elemental analyzer. The GC column functions to separate gases from one another so that any gases other than the desired CO are eluted at a different time, forming discrete peaks. The gases produced are emitted to the mass spectrometer through the Conflo III interface, which also serves as the input point for the reference CO gas analyzed before each sample. Standards peaks are flat-topped because they do not pass through the GC column (Sharp & Cerling, 1996) on the way to the spectrometer. Analysis sequences each included three 30-second long reference gas analyses, used as a benchmark for gas yield. This was followed by analysis of the sample, dropped at 170 seconds, lasting to 600 seconds.

Each set of analyses was accompanied by several standardizations. Thermo Benzoic Acid was analyzed regularly, serving to inform the user of general performance quality, and of any daily drift that may occur throughout a series of analyses, permitting correction when necessary and reasonable. This standard produced a $\delta^{18}O$ value of 25.0±0.2‰ over 48 analyses. Also analyzed regularly were two Ag_3PO_4 standards, Acros Ag_3PO_4 and B2207 Ag_3PO_4, with values of 12.1±0.4‰ and 21.7±0.3‰ respectively. The

known values of the Acros and Benzoic standards were determined in-house using externally calibrated Ag_3PO_4 reference materials provided by the United States Geological Survey (USGS MR-3 and MR-5) with values of 10.9‰ and 29.3‰. The B2207 standard is an Elemental Microanalysis certified reference material, with an externally calibrated known value. Daily calibration was carried out by creating a calibration line using least squares linear regression of known to measured values of the two Ag_3PO_4 standards. A sample size of 300 μg was assessed to produce 5.6 V CO peaks, which were matched to the standard CO mass 28 peaks. This sample size also compliments the silver phosphate precipitation method as one 5 mg enamel sample produces enough analyte to run each sample in triplicate.

Secondary Ion Mass Spectrometry (SIMS)

Sample preparation

Secondary ion mass spectrometry is a micro-bulk stable isotope technique. The *in situ* sampling in SIMS is unlike other spectrometry sampling systems that analyze gaseous samples isolated externally, or with specialized integrated equipment (Katzenberg, 2008). As such, sample preparation is greatly simplified, though it must be done precisely so as to prevent complications that can result in erroneous data.

Sectioned samples were prepared by embedding one section in a phenyl ring using a matrix of epoxy-resin. Once dried embedded sections were polished using a progression of finer polishing media, ending with 1μm Buehler diamond paste on a polishing pad, until all saw marks and other scratches were removed. Any discontinuities in the flat surface introduce inconsistencies in how the beam will contact the sample in

that area and may produce sample charging and other undesired phenomenon. These areas can be avoided if detected in the optical, but are best eliminated when possible. Following polishing, samples were carefully cleaned and photographed so as to facilitate orientation once inside the sample chamber of the instrument. Finally, the samples were gold coated using an Ernest F. Fullham 18930 Effacoater to prevent charging.

Analysis

SIMS sampling employs a primary ion beam to ionize atoms at the surface of a flat sample, and then sputtering these secondary ions which facilitates their transport through the ion detection system. This method of sample introduction benefits from very high spatial resolution, making it minimally destructive. Samples were analyzed using a Cameca ms 7F secondary ion mass spectrometer at the University of Manitoba, using a protocol similar to that of Hervig et al. (1992). Prepared samples were loaded into the sample chamber and held at high vacuum. A 7 nA primary Cs^+ ion beam, accelerated at 10 kV, was used to sputter and ionize particles from the sample surface. The beam was focused to a spot of approximately 30 μm and a normal incidence electron gun was used to mitigate potential surfaced charging. Mass resolving power was 347 and a 200-volt offset was employed. Secondary oxygen ions (species ^{16}O and ^{18}O) were detected using a Balzers SEV 1217 electron multiplier coupled with an ion counting system with an overall dead time was 52ns. Each analysis lasted 8-10 minutes, comprising 70-cycles. Repeated analyses were made across each tissue to test heterogeneity.

Despite relatively simple sample preparation requirements and high sample preservation, this method is not without its challenges. SIMS introduces a fractionation between the isotopic values present in the sample and those measured, commonly termed

instrumental mass fractionation, or instrumental bias (Eiler & Valley, 1997; Valley & Kita, 2009). Ionization potential is a property specific to each atom, however, this potential is altered in relation to the surrounding matrix (Eiler & Valley, 1997; Slodzian, 2004). As such, the bias for each material is dependent on its chemistry and crystal structure (Valley & Kita, 2009). This 'matrix effect', necessitates the use of a standard of very similar or identical composition for each unknown sample. This is problematic because the composition of many samples is not known and the development of such a grand body of standards, such as what would be necessary for biological samples, is impractical. As a result, the comparison of oxygen isotopes measured using SIMS is unreliable for materials for which an appropriate standard has not been established. In this study, no suitable standard was available, and this method was used to obtain preliminary results from material that could be used to develop a biological standard for bioapatites.

Isotopic data reporting

The relative isotope abundances being measured in mass spectrometry are extremely small, as the natural occurrence of ^{18}O is much less than that of ^{16}O, with ^{18}O making up only 0.2% of naturally occurring oxygen, whereas ^{16}O accounts for approximately 99.8% (Hillson, 2005; Schoeninger, 1995). These ratios are compared to the defined value for Standard Mean Ocean Water (SMOW) that was initially defined in relation to the National Bureau of Standards reference sample 1 (Craig, 1961). These isotope value comparisons are expressed in the form of per mil (‰) enrichments

following the equation: $\delta=[(R_{sample}/R_{SMOW})-1] \times 1000$, where R is the isotope abundance ratio $^{18}O/^{16}O$ (Craig, 1961; McKinney, McCrea, Epstein, Allen, & Urey, 1950).

Chemical characterization methods

Electron microprobe analysis (EMP)

Electron microprobe data was collected using a Cameca SX100 on polished and coated samples. Backscatter electron X-Ray maps of Ca, P, F, and Mg were produced using a 15 keV acceleration voltage and a current of 50nA. Spot analyses were conducted with an acceleration voltage of 15 keV, a 10μm beam and beam currents of 20 nA and 50 nA for major (Al, P, Ca, Mg, S, K, Sr, Pb, Mn, Si, Cu, Ce) and minor/trace (F, Na, Cl, Fe, Ba) elements, respectively. Longer count times were also employed for minor elements at 120 seconds, while 20 second counts were used for major elements. Detection limits for each element are different, and are higher for lighter elements such as fluorine. Sixteen standard geological materials were used to standardize the results.

Scanning electron microscopy (SEM)

SEM was employed on two varieties of materials. First, sectioned teeth were examined to characterize visual abnormalities. Second, precipitated silver phosphate powders were visualized to assess their purity as well as the shape and size of the crystals. Samples were carbon coated, and analyzed using a Cambridge Stereoscan 120 scanning electron microscope fitted with a tungsten filament. Once under vacuum, a 20.0 kV beam was used to produce images at various magnifications to best visualize the

samples. A Kevex 7000 EDS spectrometer was employed for initial basic chemical characterization through energy dispersion.

X-ray powder diffraction analysis (XRD)

XRD analysis was carried out to make initial assessments regarding the quality and purity of the Ag_3PO_4 that was precipitated while developing the preparation method. Smear mount slides were prepared from precipitated crystals using ethanol and analyzed using a Bruker D5000 powder diffractometer scanning at a speed of 1 second per 0.02° step over a total of 60°, resulting in an analysis lasting approximately 50 minutes. The system uses a K710H 2.7kW sealed-tube type X-Ray generator using Cu radiation.

Raman

Raman analysis is a laser-based spectrometry designed to measure vibrational spectra resulting from inelastic scattering of light. Vibrations communicate information regarding the crystal structure, bonding, phase, and composition of a material and is commonly used in a wide variety of investigative fields (G. D. Smith & Clark, 2004). In this project raman analysis was employed in an attempt to identify any chemical dissimilarities between samples that produced consistent and reliable data and those that did not. The problematic data produced by some samples will be discussed in detail later, and it suffices here to describe that it was suspected that chemical differences present in the samples prior to preparation might have produced isotopic anomalies and heterogeneity. Raman analysis, carried out over two days, was performed on embedded thick sections using a Jobin-Yvon Horiba LabRam Aramis Raman microspectrometer employing the near Infra-Red (785 nm) and Green (532 nm) lasers. Data presented was

collected with $10^{-0.6}$ laser filtration. The spectral resolution is better than 1 cm^{-1} with a lateral spatial resolution better than 1 μm.

Chapter 3: Results

Method Testing

In order to assess the viability of the methods chosen for the samples selected, trial analyses were conducted. These method assessments were made using the modern human and cow standard teeth described above. The results are presented and discussed below.

Secondary Ion Mass Spectrometry (SIMS)

One barrier to using SIMS on biogenic tissues is the lack of a suitable standard. Modern samples, cow and human, were tested in this study as potential standards for the analysis of archaeological material. The establishment of a SIMS biogenic tissue standard requires an isotopically homogeneous tissue independently calibrated using another technique, such as oxygen isotopic analysis of silver phosphate using continuous-flow isotope ratio mass spectrometry (CF-IRMS).

The modern human enamel tested produced a mean oxygen isotope value of 9.9±1.5‰ (uncalibrated values), revealing a range of 4.9‰ (Figure 1). Even greater intra-tissue variability was observed in some cow samples. In both cases, the variation appears to be related to the growth axis of the tissue being examined (Figures 1 and 2). Isotopic heterogeneity in animal dental tissues has been extensively examined by others to assess seasonality of diets, migrations, and water sources, most recently using a variety of *in situ* sampling methods (Aubert et al., 2012; Balasse et al., 2001; Fricke, Clyde, & O'Neil,

1998). Given the substantial isotopic range within the intended standard materials, these materials were deemed unsuitable as standards for this method, and efforts were shifted to thermal pyrolysis of phosphate oxygen as an alternate method of isotopic assessment for sample materials.

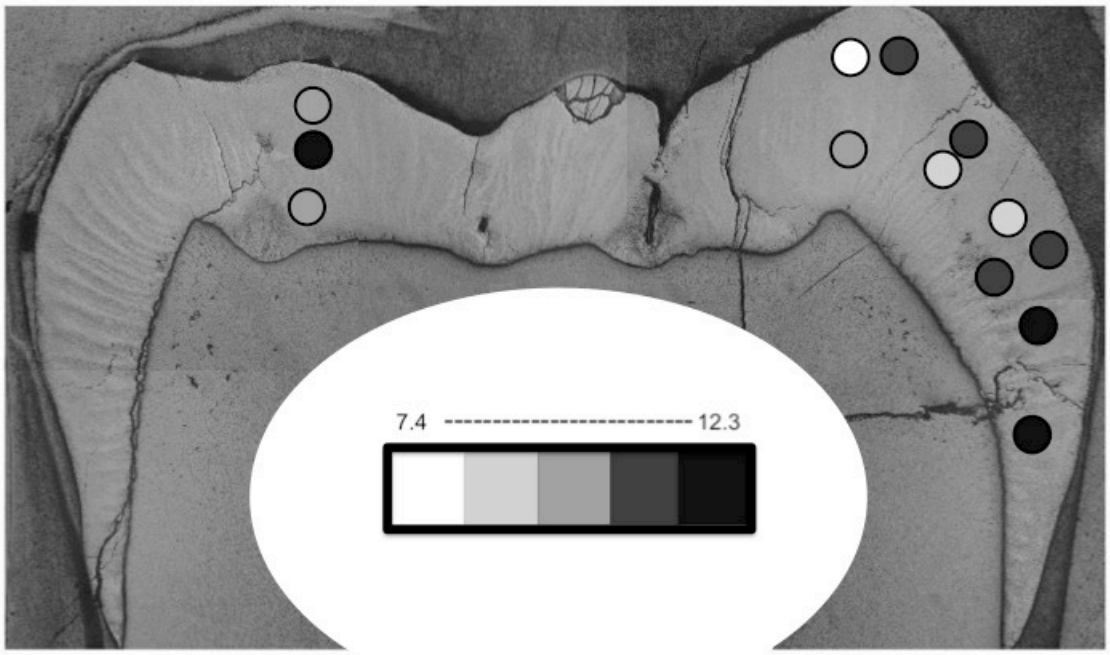

Figure 1. Natural $\delta^{18}O$ variation in modern human third molar, detected using secondary ion mass spectrometry.

Data uncalibrated, reported in ‰. Approximate sampling locations, with oversized points for visualization.

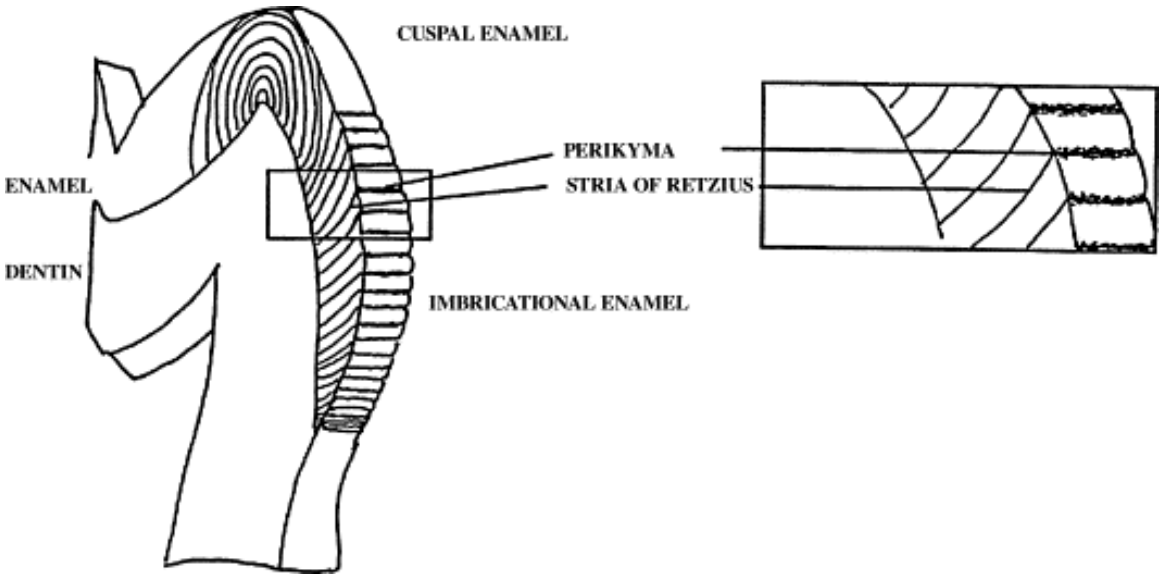

Figure 2. Illustration of the natural growth structures in dental enamel.

Reprinted from Journal of Human Evolution, 47, 1-2, Debbie Guatelli-Steinberg, Clark Spencer Larsen, Dale L Hutchinson, Prevalence and the duration of linear enamel hypoplasia: a comparative study of Neandertals and Inuit foragers, p.67, Copyright (2004), with permission from Elsevier.

Silver phosphate (Ag_3PO_4) precipitation and analysis

Researchers have employed a variety of methods for the precipitation of silver phosphate as preparation for oxygen isotope analysis. Experimentation using cow enamel and cementum was conducted to develop a method with an optimal balance between enabling the use of small sample sizes, ensuring effective organic removal, and facilitating rapid preparation.

Organic removal

The quality of prepared Ag_3PO_4 is, in part, dependent on effective organic removal. Most organic removal methods involve soaking and rinsing powdered samples prior to chemical preparation, while additional efforts have been made in post-

precipitation heat application. Organic removal is of heightened concern for the samples in this study because of the presence of visible diagenesis, and the desire to investigate the isotopic composition of cementum, an organic-rich tissue (Hillson, 2005).

Initial precipitation attempts in this study employed a soak in NaOCl, following well-tested methods for organic content removal (Wiedemann-Bidlack et al., 2008). This was tested on small samples of approximately 1 mg, and Ag_3PO_4 extraction produced very little precipitate of inconsistently coloured crystals, including dark grey and black (Table 2). Despite success in experimenting with larger sample sizes, which produced products of greater yield and brighter colour, initial SEM analyses showed that organic removal was incomplete (Figure 3). The organics present are likely to make up a small portion of the material, as they were not detected by XRD, which produced no reflections outside of the Ag_3PO_4 spectra (Figure 4). XRD typically does not detect components making up less than 10% of the material in question. Crystals with a clean appearance using the SEM were obtained by adding an organic removal soak in NaOH designed to remove humic substances in bone samples (Stephan, 2000), in addition to the more common NaOCl treatment.

Given the success of this combination, the other most commonly employed soak in hydrogen peroxide (H_2O_2) was not tested (eg. Lamb, Melikian, Ives, & Evans, 2012; O'Neil, Roe, Reinhard, & Blake, 1994). SEM images show that crystals precipitated following our method complete with dual organic removal typically range from 0.7-1.7 μm in diameter, are predominately euhedral in shape, and visibly free of amorphous organics (Figure 5). A recent review of bulk sample pretreatments prior to Ag_3PO_4 preparation for phosphate $\delta^{18}O$ supports these experiments, finding that NaOCl is more

effective at organic removal than H_2O_2 as determined by nitrogen content (Grimes & Pellegrini, 2013). The combination of NaOCl and NaOH was also reviewed, though not as extensively as other treatments. The combination was found to be equally effective in organic removal as NaOCl alone, as measured by the same standard (Grimes & Pellegrini, 2013). The effect of the dual pretreatment on $\delta^{18}O$ was not compared against NaOCl alone on enamel material in the review by Grimes and Pellegrini (2013); therefore, a detailed analysis of pretreatment effects on $\delta^{18}O$ is not available.

The relationship between crystal colour and purity has been questioned, and remains somewhat ambiguous. It has been argued that any crystal colour ranging darker than bright golden yellow is indicative of poor sample quality, namely residual organics (Wiedemann-Bidlack et al., 2008). Stephan (2000) describes precipitating green crystals despite two organic removal pretreatments as being a result of organics adhered to the crystal surface, and emphasizes that the organics will potentially compete with the desired analyte during analysis. Elsewhere, the precipitation of brownish Ag_3PO_4 crystals free of XRD or SEM-detectable contaminants has been described (Lecuyer, Grandjean, O'Neil, Cappetta, & Martineau, 1993), and a study of pretreatment methods for organic removal suggests that crystal colour and shape have no import on $\delta^{18}O$ (Grimes & Pellegrini, 2013). Others argue that Ag_3PO_4 is photoreactive, darkening with exposure to light. Some standard materials suppliers, provide Ag_3PO_4 in darkened glass containers to prevent such degradation and safety data sheets indicate that it is light sensitive (eg. Acros Organics, n.d.). Despite this, some researchers report having made yellow material that remains unchanged when exposed to light in storage over a period of years, while other material from less pure sources, while initially yellow, has darkened over time

(Roe, 1999). The importance of producing bright yellow crystals continues to be disputed because the dark colouration might be due to excess silver oxidizing over time rather than the presence of organic matter (Stuart-Williams, 1999). This is supported by very early work on the atomic weight of phosphorus suggesting that supposed photoreactive degradation of Ag_3PO_4 involves mostly residual silver, warranting little concern (Baxter & Jones, 1910). Crystals produced using the paired organic removal and precipitation methods reported here produced samples in shades of yellow. Some darkening may have occurred in storage, though colour change was not monitored closely.

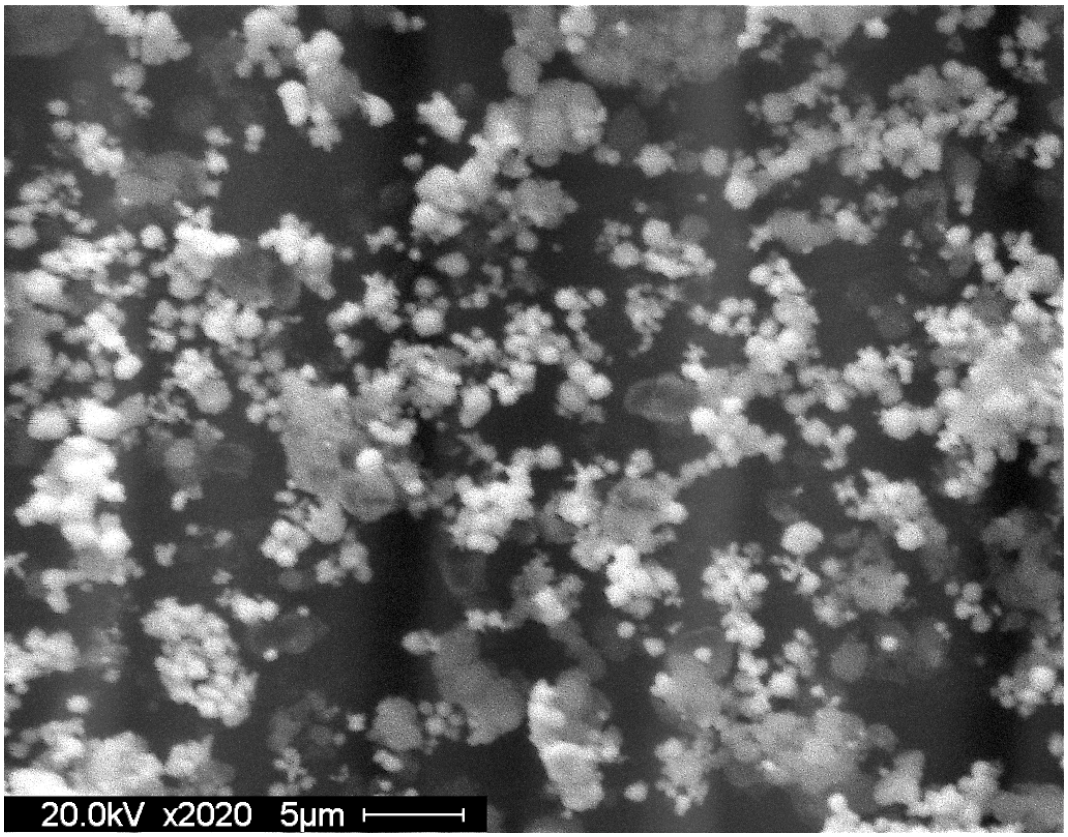

Figure 3. SEM image of Ag_3PO_4 with residual organics (amorphous) precipitated from cow enamel prior to optimization of organic removal.

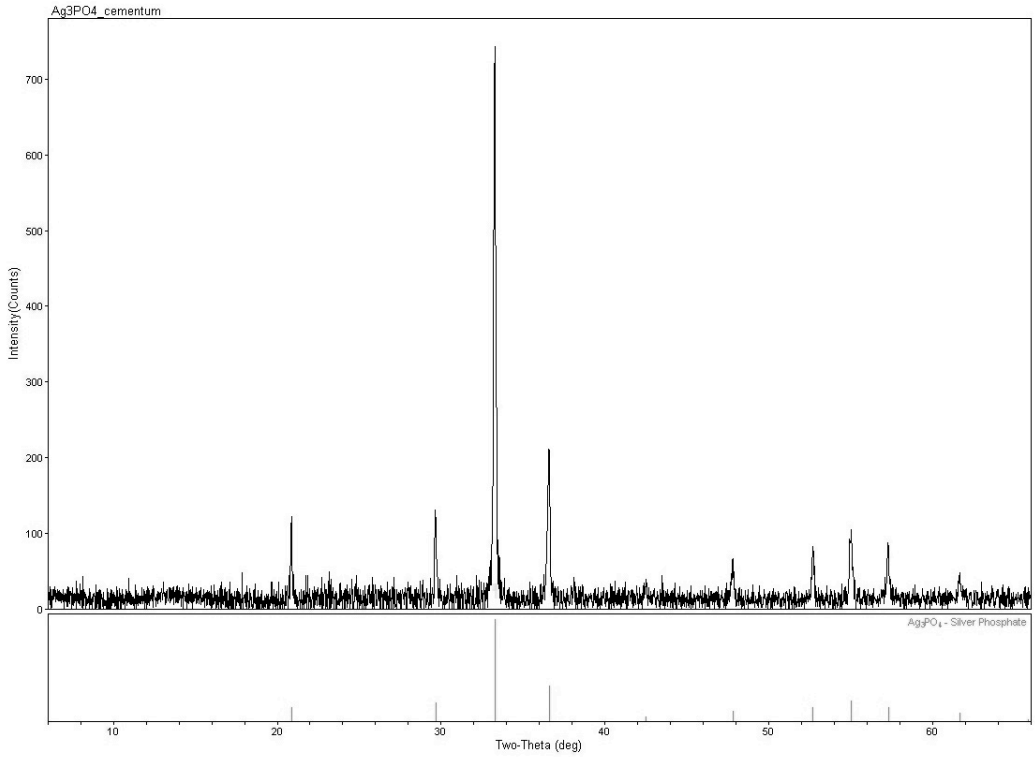

Figure 4. XRD spectrum of Ag_3PO_4 produced from cow cementum.

Spectrum shows reflections at all angles expected of Ag_3PO_4 as shown in reference peaks below.

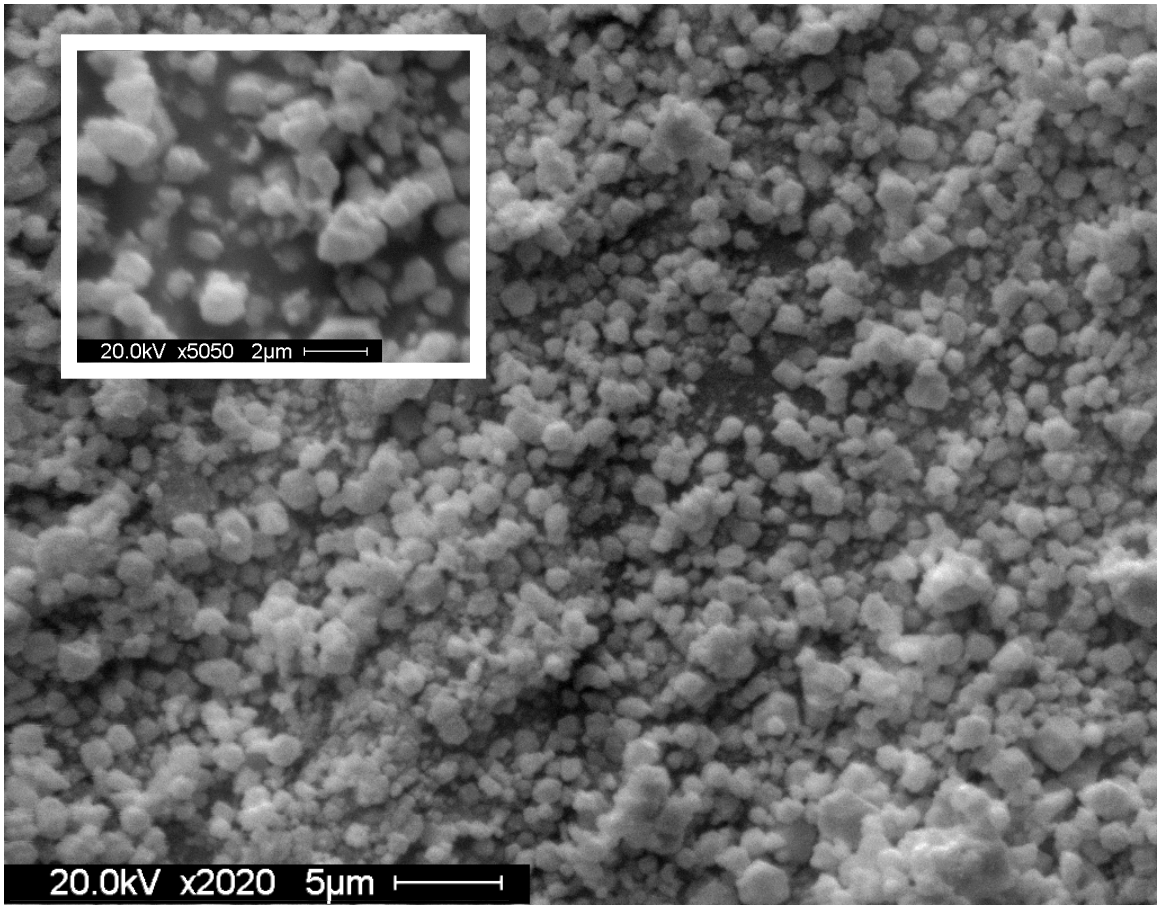

Figure 5. SEM images of Ag_3PO_4 clean of organics.
Magnification: x2020; Inset: higher magnification (x5050).

Heat has also been used as a means of eliminating organics from samples. While heating or ashing samples as a pretreatment has been established for use in many chemical analyses on biological samples, including in preparation for oxygen isotope work on bulk samples using *in situ* techniques, it is not regularly applied in the preparation of silver phosphate for oxygen isotope analysis (Lindars et al., 2001; Steadman, Brudevold, Smith, Gardner, & Little, 1959; Zanchetta, Leone, Fallick, & Bonadonna, 2005). Heating silver phosphate samples post-preparation has been

employed for the purposes of removing organics or adsorbed water from samples. O'Neil et al. (1994) heated silver phosphate samples at 550°C to remove water. This directive has been followed by some, with Stephan (2000) citing that this treatment aids in the removal of labile organics. Others have experimented with degassing at lower temperatures. One study showed that degassing at 400°C produced no observable isotopic difference from degassing at 150°C (Lecuyer et al., 1993). Elsewhere, degassing techniques ranging from 80°C to 400°C have been variably applied, with treatment times ranging from minutes to hours (Daux et al., 2008; Dettman et al., 2001b; Lecuyer et al., 2007).

Experiments with high temperature degassing, or organic removal, were carried out in this study. Sample and standard Ag_3PO_4 was baked at 500°C in a vacuum-sealed muffle furnace for periods of five minutes. Colour change was observed in most samples, with samples ranging from darker shades of yellow to green turning brighter yellow, and standard samples, originally yellow, shading to orange, and back to yellow upon cooling. Gravimetric loss was apparent, though not precisely measured. SEM imaging of baked material showed that the crystals, previously separate and euhedral, were fused together to varying degrees (Figure 6). Isotope data from baked material, obtained using the TC/EA protocol previously described, reported in Table 1, produced values lower than those previously obtained from non-baked materials of the same standards. The offset was inconsistent between samples.

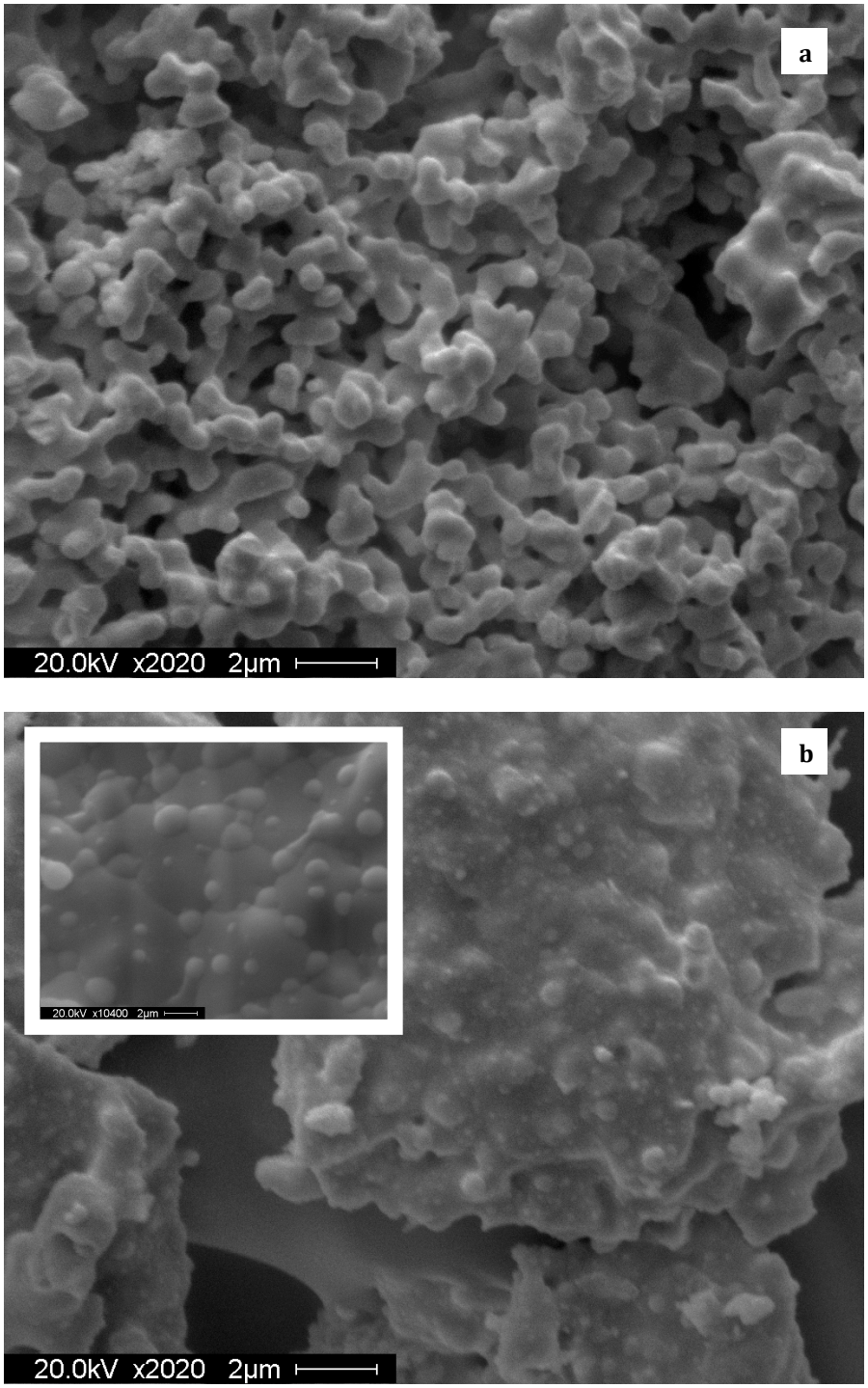

Figure 6. SEM images of baked samples.

(a)Ag_3PO_4 crystals made using our method fused after baking at 500˚C for 5 minutes (same sample as Image 2, after baking). Magnification: x2020; (b) Ag_3PO_4 crystals made using the Dettman method (see below) fused after baking at 500˚C for 5 minutes. Magnification: x2020; inset: higher magnification: x10400.

Table 1. $\delta^{18}O$ offset of operating values vs. 'baked' values

Ag_3PO_4 Standard material	Operating $\delta^{18}O$	"Baked" $\delta^{18}O$	Offset (‰)
Acros Ag_3PO_4	12.1	5.6	6.5
	12.1	5.2	6.9
	12.1	5.6	6.5
	12.1	4.9	7.2
	12.1	4.8	7.3
	12.1	4.6	7.5
Isomass Ag_3PO_4	21.7	8.3	13.4
	21.7	8.4	13.3
	21.7	8.1	13.6

Ag_3PO_4 yield optimization

Low product yields were a characteristic of the precipitate made in initial attempts for this study. Incomplete precipitation of phosphatic oxygen leaves potential for fractionation between the apatitic phosphate and the precipitated phosphate, resulting from one isotope preferentially reacting over the other. As noted above, the use of larger sample sizes produced greater relative yields. Beyond this, experiments with precipitation methods were conducted to optimize yield and ensure an effective preparation. The finalized method reflects yield optimization with respect to volume of silver ammine solution, with 1.8 ml producing the highest, most consistent yield at 0.85 mg product per 1 mg sample.

A streamlined version of the O'Neil et al. (1994) Ag_3PO_4 precipitation method, similar to that developed in this study, was also assessed for potential efficacy in producing higher yields and reducing precipitation time. Dettman et al. (2001a, 2001b) outline a 3-step precipitation involving dissolution in HF, neutralization with NH_3OH and immediate precipitation upon addition of concentrated $AgNO_3$. This method was

compared to that developed in this study by pre-treating two sets of samples for organics and then precipitating one set according to each method. Both methods make use of hydrofluoric acid to fulfill two methodological requirements: sample dissolution and calcium removal through CaF_2 precipitation and removal (Table 2). The Dettman et al. (2001b) method then adjusts pH before precipitating, while our method uses a silver ammine solution in a single step. Testing revealed some differences in the end products of each method. Crystals precipitated using the Dettman et al. (2001b) appear darker in colour, ranging into dark greens and browns, despite having been equally pre-treated for organics. This method also produced more material, weighing in at over 1 mg product per mg sample for both enamel and cementum, while our method, later optimized, yields less. The causes of each difference are not easily discernible. It is possible that excess silver is present in the Dettman et al. method (2001b), playing a role in both factors by artificially enhancing the yield, and causing dark colouration. Dettman et al. (2001b) report that products made using their method that are brown in colour produce XRD spectra identical to commercial Ag_3PO_4 except for splitting in one minor peak. Experimentations carried out in this study following Dettman et al.'s method produced an XRD spectrum with the reflections and splitting as described (Figure 7). SEM images show that the products made using the method are less uniform with some oddly shaped crystals, and a much wider range in crystal size (Figure 8).

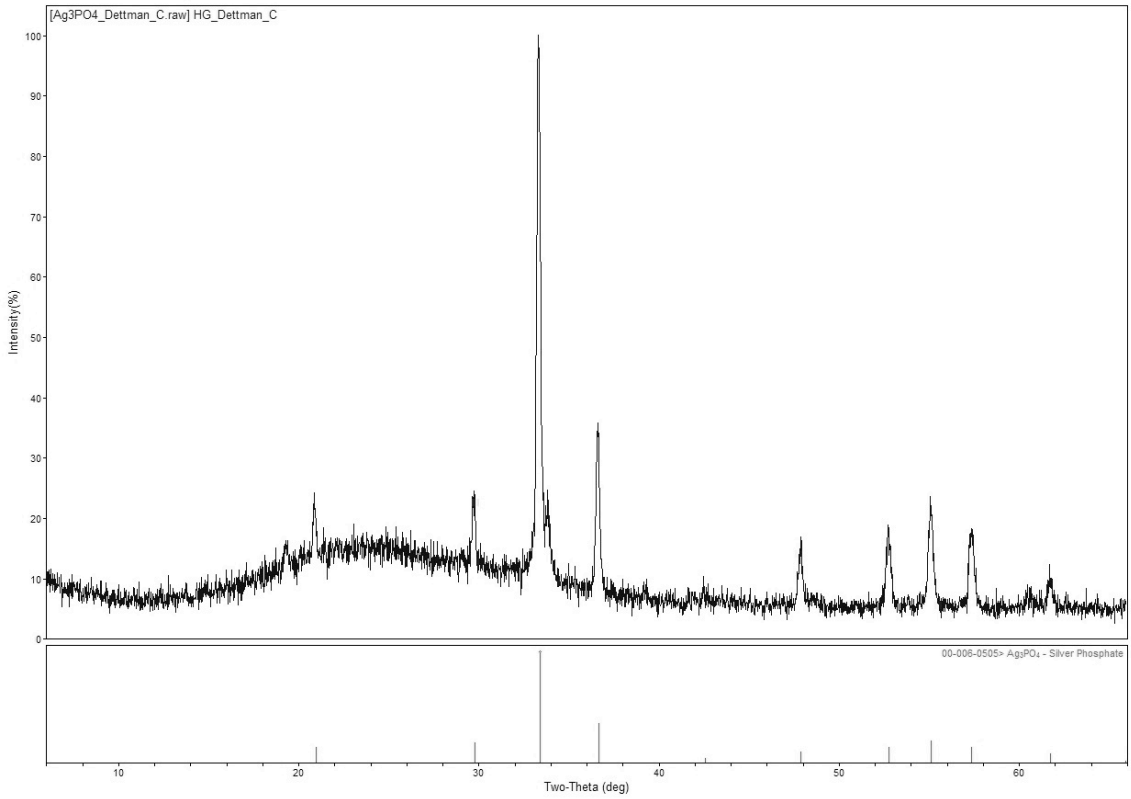

Figure 7. XRD spectrum of Ag_3PO_4 produced using the method by Dettman and colleagues (2001a).

Spectrum shows reflections at all angles expected of Ag_3PO_4 as shown in reference peaks below, with some minor splitting.

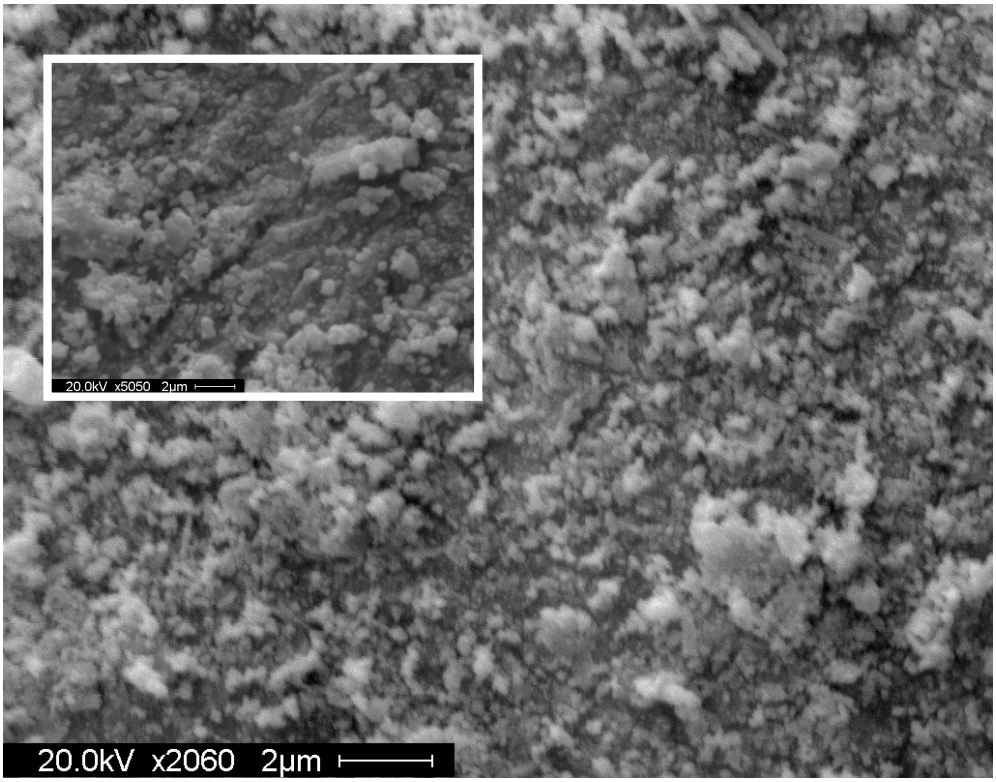

Figure 8. SEM images of Ag_3PO_4 crystals made using the Dettman et al. method (2001b);

Magnification: x2060; inset: higher magnification: x5050.

Table 2. Published methods tested in this study.

Method	O'Neil et al. (1994)	Dettman et al. (2001)	Wiedeman-Bidlack et al. (2008)	Present Study
Organic removal step(s)	30% H_2O_2	\	2.5% NaOCl	2.5% NaOCl 0.125M NaOH
Dissolution chemical	2M HNO_3	2M HF	2M HNO3	2M HF
pH adjust	2M KOH	\	\	\
CaF_2 precipitaiton & removal	2M HF	HF*	2M HF	HF*
pH adjust	\	20% NH_3OH	\	\
Precipitation chemicals	Silver ammine solution**	2M $AgNO_3$	Silver ammine solution***	Silver ammine solution**
Precipitation time	>1hr.	Rapid (immediate)	12 hrs.	Rapid (< 5 min.)
Ag_3PO_4 characteristics (as published)	Light coloured/yellow-green to brown; Large and small crystals;	Colour variable; High yield;	Bright yellow colour; Large crystals;	\
Ag_3PO_4 characteristics (as observed in trials in this study)	Yellow-green crystals	Dark colour; High yield	Dark crystals; Low yield	Yellow colour; Medium crystals;

*= HF is the agent serving to precipitate and remove CaF_2, but is not a separate step from the dissolution. ** = 0.2M $AgNO_3$; 0.35M NH_4NO_3; 0.74M NH_4OH; ***= 0.22M $AgNO_3$; 0.37M NH_4NO_3; 0.85M NH_4OH.

Analysis: accuracy and reproducibility

Accuracy of this method was tested using the standard NBS120c (Florida phosphate rock). Although this material is not intended as an isotope standard, it has become one of the primary standards used for this purpose. The appropriateness of this standard has been questioned (Dettman et al., 2001b); however a great number of publications have reported $\delta^{18}O$ values for it, and it remains in use. Upon preparation of the phosphate rock as Ag_3PO_4, it was noted that the sample did not dissolve as readily as the other samples prepared. Dissolution problems with this material have been noted elsewhere, and contribute to the concern surrounding this material as a standard (eg: O'Neil et al., 1994). The mean $\delta^{18}O$ value obtained in this analysis for NBS 120c was 21.5±0.5‰ (n=7). This value and error are comparable to published values for this standard (Figure 9).

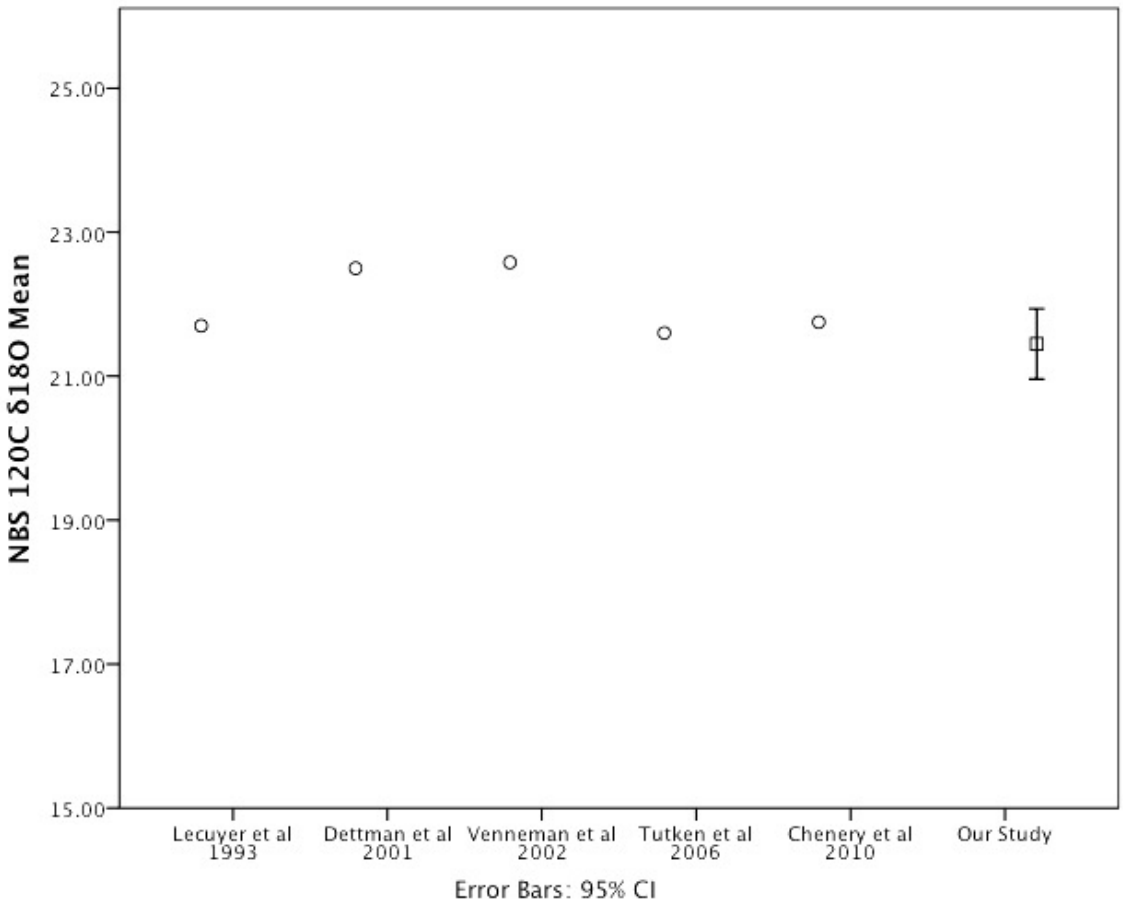

Figure 9. Mean $\delta^{18}O$ values for NBS 120c phosphate rock – published mean values and mean value obtained in this study.

Error bars: 95% CI.

The reproducibility of the finalized method was established using both cementum and enamel from cow teeth. Analyses of cementum from one tooth were carried out on three separate samples of Ag_3PO_4 crystals, over two days. The mean $\delta^{18}O$ value was 9.1±0.4‰ (1σ, n=10) (Figure 10). Five enamel samples from the same tooth, prepared in two Ag_3PO_4 batches, and analyzed over the same two days, produced a mean value of 9.6±0.3‰ (1σ) (Figure 10). Finally, Ag_3PO_4 samples produced in three batches from the enamel of another tooth, different from the tooth providing enamel and cementum for the

analyses above, were analyzed in a single day, producing a mean $\delta^{18}O$ value of 11.7±0.5‰ (1σ, n=14) (Figure 10).

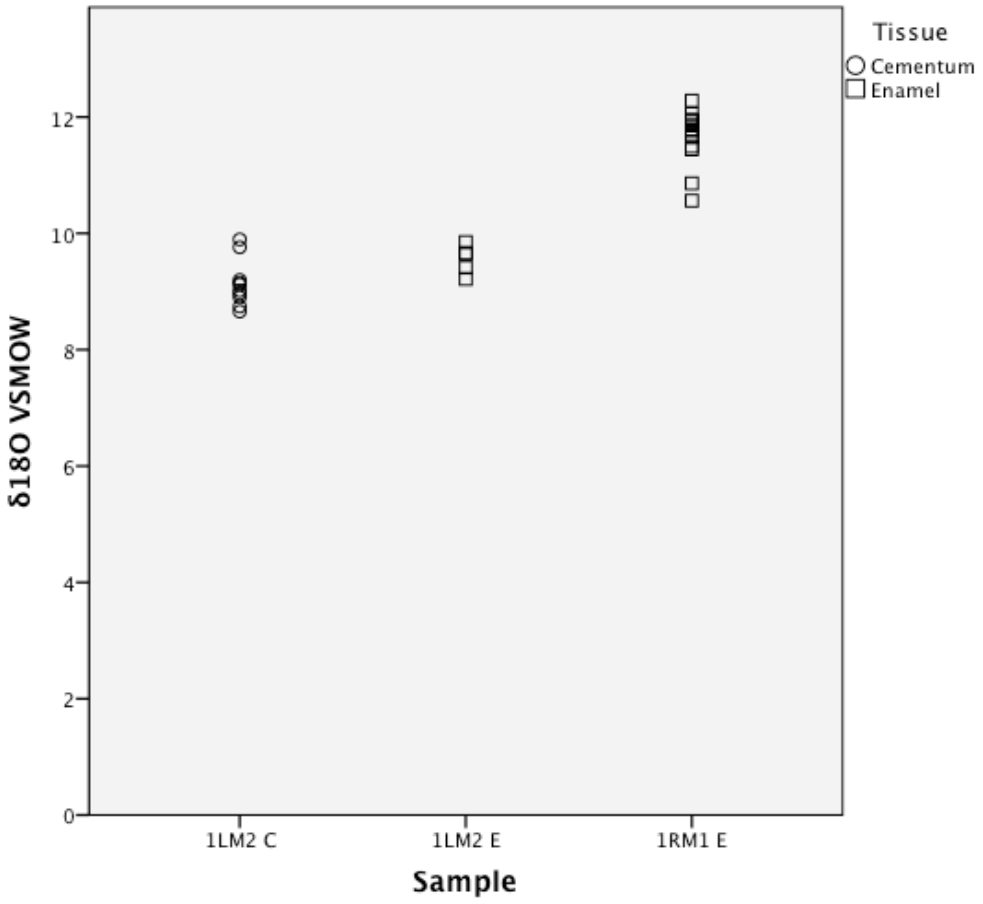

Figure 10. $\delta^{18}O$ values for three cow dental samples, using the method presented here. All samples derived from the same individual.

CO yield

Oxygen yield resulting from pyrolysis is difficult to establish. Yield has been assessed in the past by using the calculated CO (mass 28) peak area (Vs) resulting from pyrolysis of a single μmol of oxygen in materials with demonstrated 100% pyrolysis. This is then compared to the peak area of the analysis, and the μmols of oxygen in the analyzed sample (Kornexl, Gehre, Hofling, & Werner, 1999). In this study, oxygen yield

was calculated using a commercial inorganic Ag_3PO_4 sample as the standard for 100% yield. A linearity analysis was conducted, to determine the relationship between CO peak area (Vs) and the μg of oxygen in the analyzed sample. Oxygen was calculated as 15.29% of the total mass of Ag_3PO_4. The linear relationship established was applied to the mass 28 peak area of each analysis, and the resulting mass of measured oxygen was compared to the theoretical oxygen content, producing a percentage yield for each analysis. Although this method of yield assessment can not be compared to those used by others, it is worth noting that reports of method explorations in the pyrolysis of Ag_3PO_4 report 87±2% yield, while others report yields of 90-100% (Kornexl et al., 1999; Wiedemann-Bidlack et al., 2008).

Method trials in this study produced consistently high yields of 103±9%, with the exception of analyses that produced nitrogen peaks. Data associated with nitrogen peaks were removed from this and further analyses, and the issue will be considered in further discussions. High and consistent yield is desired to ensure that fractionation through incomplete pyrolysis of oxygen in the sample is not occurring. Observations of the correlation of $\delta^{18}O$ values to the CO yield of the individual analysis can also provide information regarding possible fractionation. Method testing using cow tissues produced a small positive Pearson correlation coefficient of r=0.227 between $\delta^{18}O$ values and CO yields. The samples analyzed using the finalized method produced CO yields of 102±9%. The final method samples exhibit a greater correlation between isotope ratio and CO yield than the overall experimental data (r=0.562), however, assessed individually, the sample sources (enamel from one individual, or cementum from another) are not all similarly correlated, with some exhibiting positive correlations, and others negative. As

such, the relationship between CO yield and $\delta^{18}O$ is unclear, even in conditions of high and relatively consistent yield.

Chemical Characterization

The materials collected for this study were visibly degraded from the burial environment. Given this, the best-preserved specimens, as determined by a visual examination of the exterior, were selected for analysis. Despite this effort, the interior of some samples exhibited macroscopic alteration apparent upon sectioning. Nine of the 26 teeth belonging to the research sample exhibited macroscopically visible opacity in the enamel tissue. Twenty-two of the 26 teeth have visibly altered dentine. Dentine in these teeth appears matte creamy white in areas and or has spots or areas that appear shiny and crystalline. In some teeth these discolourations are diffuse and extend into the cementum.

Chemical characterization of one tooth with visibly altered dentine was investigated by electron-dispersive spectrometry. This basic investigation revealed variation across the tissue in Ca/P ratios. Relative Ca/P ratios were elevated in patches within the dentine. Electron microprobe analysis (EMPA) was conducted on one modern human sample and two medieval teeth in an attempt to elucidate the chemical nature of the visible inconsistencies in all tissues. EMPA x-ray maps of P, Ca, Mg, and F illustrate some basic differences between the archaeological and modern samples. The archaeological samples exhibit lower overall values of Mg in dentine, while Ca and F measured from enamel in these samples is also lower than in the modern sample. Higher levels of Ca are evident in the medieval dentine samples, and supposedly diagenetic spots, which appear white and crystalline, exhibit observably higher levels of both P and

Ca. The archaeological samples of dentine also contain many more areas exhibiting low counts in all elements, consistent with voids in the tissue. These are both separate from and adjacent to 'crystalline' spots and areas. Backscatter electron images illustrate overall atomic differences between the samples, as well as the presence of the diagenetically altered areas (Figure 11).

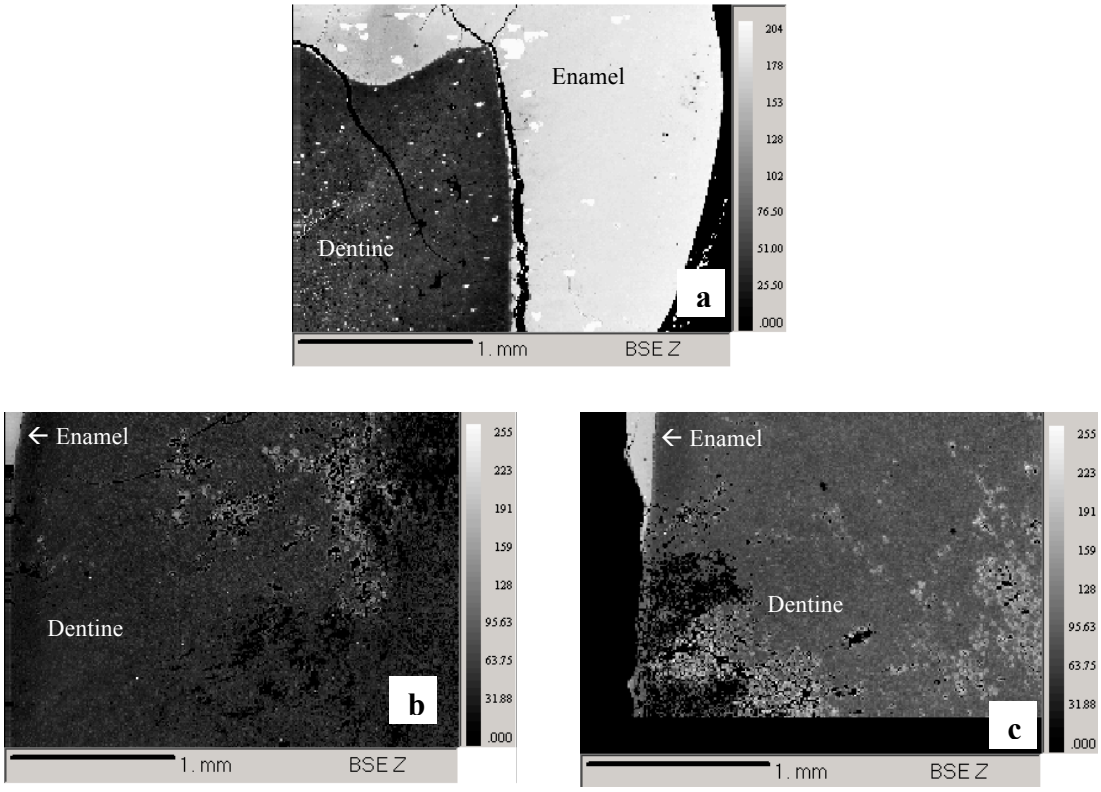

Figure 11. Backscatter electron images of modern and archaeological dentine and enamel.

(a) Modern sample showing an abundance of high-reflectance areas in enamel, and low-reflectance areas in dentine; (b) Sample 2 (X487) has similar overall reflectance as modern the sample; (c) Sample 3 (X1187) shows overall higher but variable reflectance (brighter colour) in dentine, suggesting a higher degree of alteration.

Spot analysis was conducted to further investigate differences between the modern (sample 1) and archaeological (samples 2 and 3: X487 and X1187 respectively) samples. Analyses were made of white 'crystalline' spots observed in dentine of samples

2 and 3, of opaque enamel in sample 2 and of normal-appearing areas of each tissue. A notable difference was observed in the quantity of MnO in each sample. A single analysis of 'crystalline' dentine in sample 3 exhibited exceptionally high MnO contents (7.18 wt. %), with an overall average for MnO in altered dentine tissue of 1.6 wt. %. This average is 1-3 orders of magnitude greater than unaltered dentine. One analysis of enamel from sample 2 also exhibited high MnO contents of at 5.98 wt. %, which is distinct from adjacent analyses. Less variation exists in the contents of FeO observed in 'crystalline' dentine from sample 3, which has an average of 1.47 wt. %. Sample 2 exhibits Al_2O_3 contents that are higher than other samples. The highest Al_2O_3 contents are found in opaque enamel at 0.07wt. %, while cementum and altered dentine contain 0.04 wt. % and 0.01 wt. %, respectively. This compares to an average of 0.002 wt. % for the other samples overall. This is considerable as Al_2O_3 was not detectable in enamel from the modern tooth. Also present in the enamel of sample 2 is BaO, averaging 0.01 wt. %.

Sample 2 was chosen for EMPA analysis in order to investigate the opaque enamel observed in this and 9 of the sample teeth chosen for isotopic analysis. When mean data obtained from all analyses from the enamel of sample 2 are compared against averaged enamel values of samples 1 (modern) and 3, some chemical differences can be observed. Mn, Si, F, Ba, K, and S are found in higher concentrations (Table 3). Ce and Al are detectable at low levels whereas they are undetectable in samples 1 and 3. Sample 2 is depleted in Fe, Sr, and Cu. Although F was found in greater concentrations in the sample with some visibly opaque enamel than in other enamel samples, F was found in highest concentrations in the cementum of Sample 3 at 1.9% ppm. Trends in composition between opaque enamel and visibly normal enamel are reported in Table 3.

Table 3. Trace element concentrations of enamel from archaeological (samples 2 and 3) and modern (sample 1) samples as measured by electron microprobe.

Element	Sample 2 Enamel Weight % Average	Samples 1&3 Enamel Weight % Average
Sulfur (S)	0.029	0.011
Potassium (K)	0.027	0.019
Strontium (Sr)	0.009	0.021
Manganese (Mn)	0.365	0.010
Silicon (Si)	0.024	0.002
Copper (Cu)	0.012	0.027
Celenium (Ce)	0.010	0.000
Fluorine (F)	0.078	0.033
Iron (Fe)	0.007	0.021
Barium (Ba)	0.012	0.006
Aluminum (Al)	0.035	0.000

Some elemental patterns in the enamel of sample 2 are apparent (Figure 12, Table 4). Elements whose concentrations increase toward the exterior of the tissue where the opacity is visible are Al, Mn, Si, Cl, F and Cu. The opposite trend is discernable in Mg, and Na. Although these trends are not strict, they are worth consideration in comparison to natural variations in enamel chemical composition.

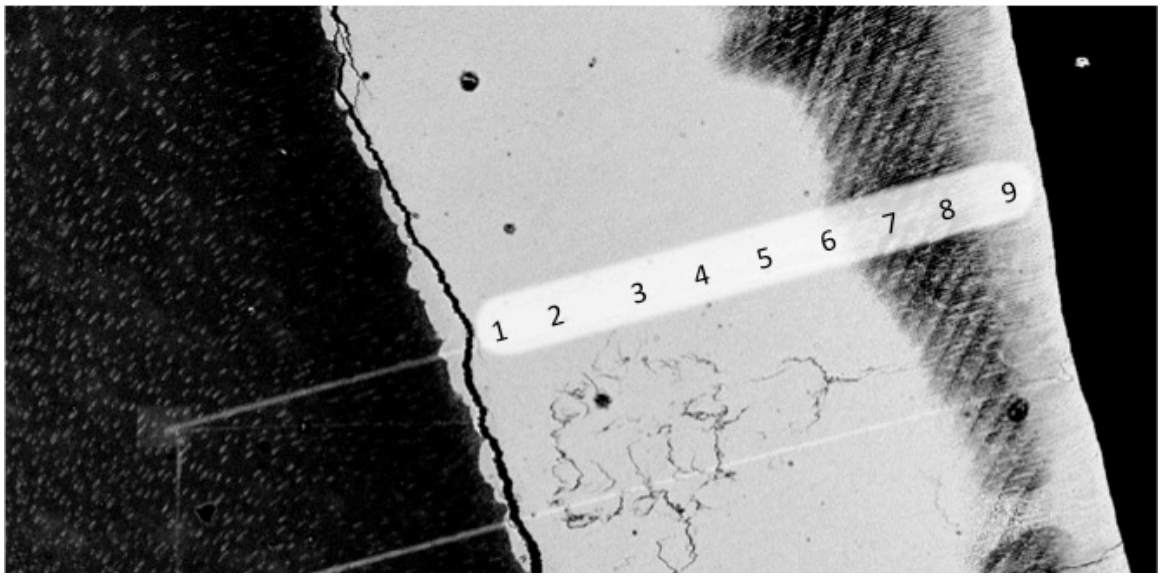

Figure 12. Distribution of points sampled across the opaque enamel of Sample 2.

Table 4. Trace and minor element concentrations measured across the opaque enamel of Sample 2 in weight %.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Elemental concentrations increasing toward the exterior of the tissue									
Al	0	0.0031	0	0	0.0209	0.0220	0.0423	0.0361	0.0424
Mn	0	0.0071	0	0	0.0102	0.0075	0.0064	0.0313	0.0401
Si	0.0017	0.0009	0.0033	0	0.0449	0.056	0.0839	0.0438	0.0529
Cl	0.0910	0.1099	0.1432	0.1703	0.3649	0.4930	0.6219	0.6361	0.6386
F	0	0	0	0	0.0269	0.0323	0	0.0278	0.0600
Cu	0	0.0148	0	0	0	0.0031	0	0.0288	0.0449
Elemental concentrations decreasing toward the exterior of the tissue									
Mg	0.2919	0.2778	0.2758	0.2581	0.1490	0.1020	0.0578	0.0590	0.0283
Na	0.6537	0.6789	0.6833	0.6224	0.5176	0.3826	0.2587	0.2876	0.2741

Following isotope analysis four teeth from the research sample and one modern human specimen were analyzed using Raman spectroscopy. Raman analysis was carried out over two days. No significant difference in the chemical composition of samples that produced inconsistent isotopic data from those producing consistent data was observed in the Raman spectra.

Isotope Results: Danish Sample

Sample preparation and analytical yields

Some analyses were excluded from the results reported below due to low yields. In this study yield inconsistencies relate to either the Ag_3PO_4 precipitation yield measured as a sample to product weight ratio, or to oxygen conversion to CO yield during pyrolysis. Some correlation between $\delta^{18}O$ values and yield is evident. Overall Ag_3PO_4 precipitation yield for the study sample was 91.6±4% (95%CI; SD=29%). No correlation between precipitation yield and $\delta^{18}O$ values is evident (Pearson's r=−0.08). There is a positive correlation between CO yield and $\delta^{18}O$ values (Pearson's r= 0.84). Mean CO yield for the entire sample is 78.7±2% (95%CI; SD=13%). For the purposes of the analysis reported below, single $\delta^{18}O$ analyses exhibiting CO yields below 70% were removed. Three individuals have fewer than three analyses exhibiting yields over 70%, leaving no data to examine or resulting in exceptionally large confidence intervals. These individuals, X360 (N=1), X1350 (N=2), and X1638 (N=0) are excluded from group analyses and will be discussed separately. The remaining analyses exhibit an overall CO yield of 84.5±1% (95%CI; SD=8%). The Pearson correlation coefficient is reduced between $\delta^{18}O$ and CO yield through the removal of low yielding analyses to r=0.64.

Inconsistencies in yield can be indicative of any number of potential issues relating to sample preparation, analysis or intrinsic sample properties. Attempts were made to determine the source of the inconsistency in yields and the presence of N2 peaks observed in this study. Re-analyses producing more consistent data were used for group

and individual analyses when obtainable. The specifics of re-analysis and data quality are discussed below.

Oxygen isotope composition

The range in $\delta^{18}O$ values in enamel phosphate measured across the study sample is narrow. The overall study sample, excluding analyses producing low Ag_3PO_4 or CO yields, or N2 peaks, exhibits individual $\delta^{18}O$ mean values ranging from approximately 15–18‰ (Table 5). This set of values can be divided into three narrow groups delineated at 16.9‰ and at 15.7‰, using Average Linkage Cluster Analysis with squared Euclidean distances. In Average Linkage analysis, the distance between the groups is the average of the distance for all pair combinations between the groups. Distance between data points is measured by squared Euclidean distance, which adds the squared lengths of each of the lines making a right angle between the points. (See Johnson & Wichern, 2002 for full discussion). The first group, with means above 16.9‰, is made up of individuals X291, X1076, X1186, and X1416. Another eight individuals, X15, X157, X506, X106, X435, X1086, X1180, and X1269, have means in this range, but exhibit 95% confidence intervals (CI) that extend into the next group. Individuals X113, X126, and X381 comprise the second group of means, which ranges between 15.7‰ and 16.9‰. Individuals X487 and X1360 have 95% CIs that reach into group 1, with means of 16.7‰ and 16.8‰ respectively. Individuals X577 and X1587.1 also have means in group two, but within a 95% CI extend into the third group. The third group exhibits means below 15.7‰ and includes individuals X1114, X1292, and X1433, with the last having a 95% CI extending into group 2. Finally individual X77 spans all three groups when confidence

intervals are plotted, with its raw mean value in the second group at 16.3‰. All individual mean values with grouping delineations are illustrated in Figure 13.

Groupings based on means become more meaningful when each site is considered separately. Although the difference between overall means from each site is not statistically significant (ρ=0.725), there are nevertheless observable differences. Sejet individuals are equally divided between groups one and two, and are absent from group three. A total range of 1.6‰ characterizes the Sejet individual mean values (Table 5), whereas the mean values from Ole Worms Gade (OWG) have a greater overall range of 3.0‰. The means obtained for half of the individuals from Ole Worms Gade put them in group one, while the rest are equally distributed between groups two and three (Table 5).

Table 5. $\delta^{18}O$ mean values and deviations excluding low yielding data and samples. Groups based on $\delta^{18}O$ means.

Site	Individual	Mean $\delta^{18}O$	σ	N	95% CI	Group
Sejet	15	17.0	0.2	6	0.2	1
Sejet	77	16.3	0.5	4	0.8	2
Sejet	106	17.1	0.4	5	0.5	1
Sejet	113	16.5	0.1	5	0.1	2
Sejet	126	16.2	0.1	5	0.1	2
Sejet	157	17.3	0.2	3	0.5	1
Sejet	291	17.6	0.2	5	0.2	1
Sejet	381	16.4	0.3	5	0.4	2
Sejet	435	17.1	0.5	5	0.6	1
Sejet	487	16.7	0.2	3	0.5	2
Sejet	506*	17.5	0.3	3	0.7	1
Sejet	577	16.0	0.3	4	0.5	2
OWG	1076	17.2	0.2	6	0.2	1
OWG	1086	17.0	0.4	4	0.6	1
OWG	1114	15.3	0.3	5	0.4	3
OWG	1180	18.0	0.5	3	1.2	1
OWG	1186*	17.8	0.7	5	0.9	1
OWG	1269	17.4	0.6	7	0.6	1
OWG	1292	15.0	0.1	3	0.2	3
OWG	1360	16.8	0.2	6	0.2	2
OWG	1416	17.9	0.5	6	0.5	1
OWG	1433	15.4	0.3	3	0.7	3
OWG	1587.1	16.2	0.3	4	0.5	2

* = Data reported from this individual were obtained from re-analysis using a slightly altered method detailed below.

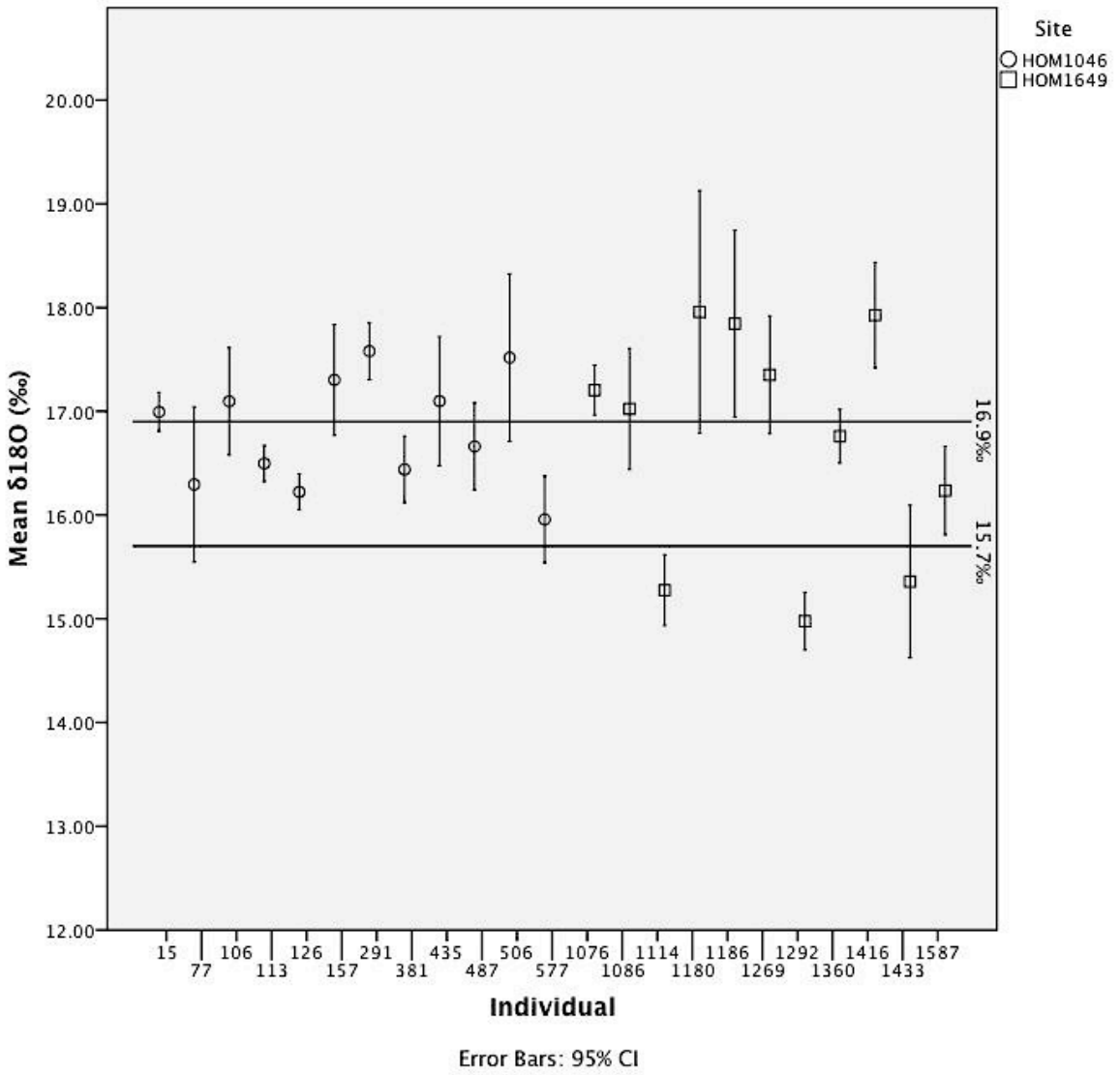

Figure 13. Mean $\delta^{18}O$ values for individuals from Sejet and Ole Worms Gade with 95% confidence intervals.

When $\delta^{18}O$ is plotted against time period, some trends can be observed. The 1st period, dating to before 1300 A.D, is characterized by arm position A. The individual mean $\delta^{18}O$ values from Ole Worms Gade form two discrete groups dating to this period, one group ranging between 17‰ and 18‰, while the other clusters around 15‰ (Figure 14). These two clusters of individuals, considered at a 95% confidence level are discretely separated by a 0.7‰ gap. This clustering is apparent with or without one

individual whose arm positioning is inconclusive, but suggestive of the 1st period. Mean $\delta^{18}O$ values from Sejet individuals dating to Period 1 span the difference between the two Ole Worms Gade groups in this period, ranging from 16-17‰, with confidence intervals overlapping into both clusters. In the second period, values from Ole Worms Gade lie between the two groupings observed in Period 1, though the lower cluster continues to maintain its separation. Sejet means are slightly higher, but largely overlap with Ole Worms Gade's in the 2nd period, with all individuals clustering around 17‰. Only three individuals in the research sample can be confidently placed in the 3rd period based on arm position. The Period 3 individual excavated from Ole Worms Gade has the highest measured mean $\delta^{18}O$ at 18.0±1.2‰ while the two individuals from Sejet range between approximately 15.5‰ and 17‰ (Figure 14). The Period 3 individual from Ole Worms Gade exhibits similar values to those from the same site dated to Period 1; however, a large confidence interval makes this comparison tenuous. Finally, individual X126 could not be assigned to any burial period, and exhibits a mean $\delta^{18}O$ value of 16.2±0.1‰ (Table 5).

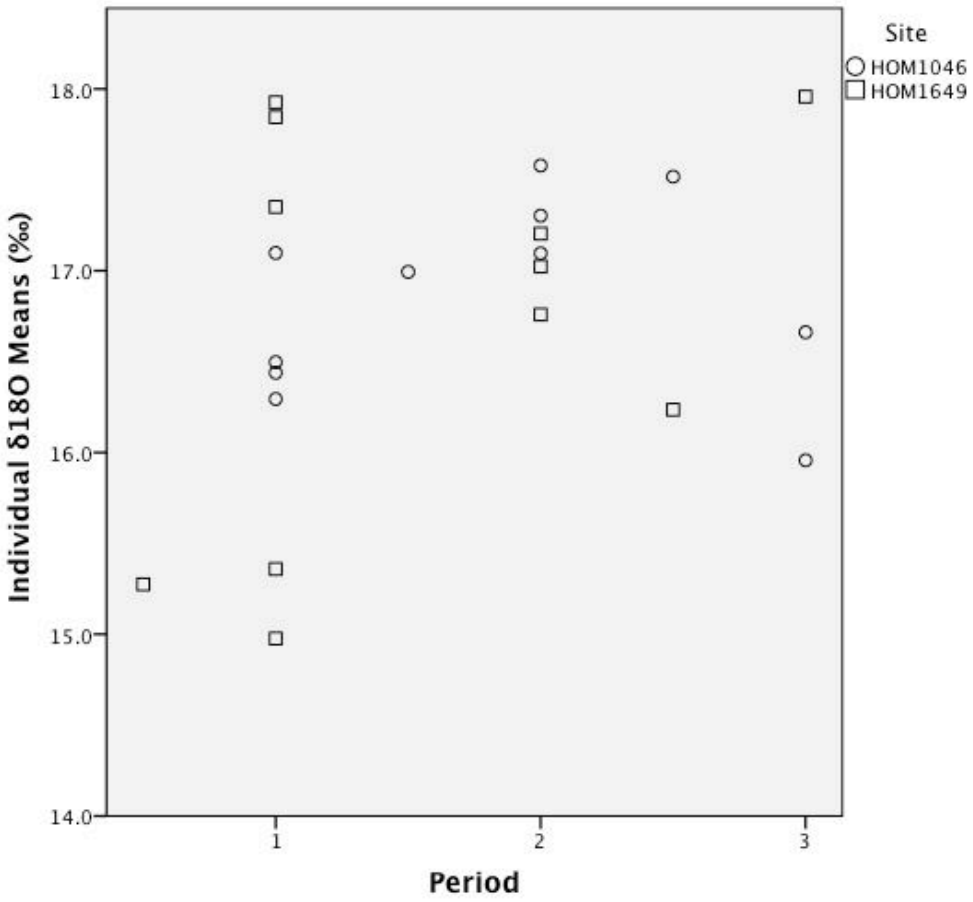

Figure 14. Mean $\delta^{18}O$ values for individuals from Sejet and Ole Worms Gade by period. Individual X126 not included. Data points between period labels indicate individuals whose burial position is suggestive of the periods to which the data points are adjacent.

When $\delta^{18}O$ values are plotted against individual mean age at death, some trends are discernible. Age was divided into three groups: under 25 years of age, between 25 and 45 years of age, and over 45 years of age. At Sejet, there is a statistically significant decrease in $\delta^{18}O$ with increasing age at death (ρ=0.003). Interestingly, this trend is reversed at Ole Worms Gade; however, the differences in $\delta^{18}O$ values between age categories at the urban site are not statistically significant (ρ=0.190). There is no

statistically significant difference between the mean $\delta^{18}O$ values by sex in either site or in the study sample as a whole.

Data quality

In an attempt to determine whether good quality isotope data could be obtained from samples previously producing low peak sizes or otherwise erroneous data, a number of experiments were conducted. First two samples, X1076 and X1360, were prepared a second time following the same procedure as the first. The initial sample from X1076 had been run five times, with four analyses producing low CO yield (<70%), and three producing small N2 peaks. X1360 had also been run five times, and all five analyses produced low CO yields. Enamel powder was drilled a second time from X1076 and X1360. Sample from both the first drill and the second drill of X1076 were prepared as Ag_3PO_4 following the established protocol. No material from the initial sampling of X1360 remained, and material was prepared from this individual a second time using the newly drilled material. The later preparations of X1076 material produced varying results. The initially drilled material, re-prepared, produced good yields (81% CO), and was analyzed six times, producing a mean $\delta^{18}O$ value of 17.2±0.2‰. The run using newly drilled material produced low Ag_3PO_4 yield (29%), and could only be analyzed three times, producing a lower mean value (16.6‰). Despite this, the re-analyses, taken together, exhibit an overall standard deviation of only 0.4‰. Given the low sample preparation yield of the new material, the first re-preparation of X1076 material was used for individual and group analyses above. Re-preparation from individual X1360 produced all around good yields. It was analyzed over two days, and produced a mean value of

16.8±0.2‰. It is this data that is included in discussion of isotope data from this sample and the research sample as a whole.

Next, prepared material from three previously analyzed samples as well as some standard Ag_3PO_4 was baked under vacuum for four hours at 150°C. While the sample data produced slightly more consistent values, none were sufficient to provide usable data for analysis. Re-analysis of X360 after baking produced values with slightly higher yields (72%), and greater consistency (0.2‰ SD), however they did not match the initial value from the first analysis. Individual X1638 had initially produced very low yielding analyses, and although yield was higher after baking, it remained below 70%, and was also excluded from data analysis. Data derived after baking from individual X1186 showed improvements over the original data, exhibiting less variation (0‰ SD), and no detectable N2 peaks as had been present in one of the original three analyses. Despite this slight improvement, none of the data obtained from baked material is included in data analysis, in part due to an overall $\delta^{18}O$ shift observed in the Acros standard Ag_3PO_4 that was treated to the same low temperature baking. The standard value shifted by approximately 0.8‰. This offset is miniscule as compared to the ~7‰ shift observed when baking at 500°C (Table 1); however, the shift in the standard is greater than the overall error for this material (0.4‰).

Finally, two previously analyzed samples were prepared without any organic removal steps. Half of each sample product was baked for four hours at 150°C before analysis and the remainder was analyzed after regular drying in an oven at 70°C. These samples produced similar values to the previous preparation for each sample. X506, when re-prepared produced more consistent values (0.3‰ SD), and no N2 peaks. Once baked,

the values were more consistent yet (0‰ SD), with a slightly higher mean (17.8‰). Re-preparation of X1186 produced lower values (17.4‰) than the original set, with the baked samples once again producing slightly higher values (17.9‰). As mentioned, data derived from baked samples have been excluded from the results above. Reporting of X506 is based on the re-prepared sample, excluding organic removal steps. X1186 data is derived from the initial analysis as well as the second preparation excluding organic removal.

As noted, some inconsistencies are observable in the data collected from the research sample. Potential causes of erroneous data will be discussed. Overall, while low yields and N2 peaks are of concern, day to day reproducibility from samples not exhibiting these inconsistencies is good, as demonstrated by low standard deviations and confidence intervals (Table 5).

Chapter 4: Discussion

Isotope studies can provide great insight into archaeological activities. Direct analysis of human remains can circumvent many of the problems encountered when studying human movement from a material archaeological perspective. For instance, isotope data will not permit the interpretation of trade or other means of cultural diffusion for interaction through migration if none took place. Isotopic analysis comes with its own potential sources of error and misinterpretation, which include post-mortem diagenesis, potential sample contamination, and instrumental error, among others. The results of the analyses conducted in this study will be evaluated, taking note of potential sources of variation within the research sample, and assessing the likelihood that trends observed are indicative of *in vivo* activities. First, however, a number of methodological challenges will be addressed.

Secondary Ion Mass Spectrometry of biological tissues

The results obtained from both varieties of standard dental material using secondary ion mass spectrometry (SIMS) reveal great intra-tissue heterogeneity. SIMS analysis was carried out in order to assess the potential of available materials as calibration standards for bioapatites in secondary ion spectrometry. As noted, matrix specific standards must be established in order to account for 'matrix effects' unique to each material, relevant to *in situ* spectrometric method (Eiler & Valley, 1997; Slodzian,

2004; Valley & Kita, 2009). As such, the heterogeneity observed is problematic for tissue standardization. It is likely that this heterogeneity reflects natural variation in the tooth, present despite the absence of any great movement or weaning signal, with both factors having been ruled out in sample selection. The range introduced from the natural heterogeneity exceeds the range introduced by sources of error, resulting in an unacceptably large standardization error. A standard exhibiting the heterogeneity observed in the samples available in this study, even if a small area were isolated, would greatly reduce the interpretive value of any data calibrated to it.

The isotope heterogeneity observed in the samples examined using SIMS has implications for the isotope data being considered as a whole. The observed $\delta^{18}O$ range in the human tooth is significant and demonstrates natural heterogeneity in human enamel similar to that observed in faunal studies. In traditional oxygen isotope studies this heterogeneity is lost in sampling and homogenization of tissues for sample preparation. As such, it has been written elsewhere that human enamel is homogeneous, and that each tooth represents a singular snapshot of an individual's isotopic history, suggesting that homogenization occurs in a reservoir prior to incorporation into dental tissues (e.g. Evans, Stoodley, & Chenery, 2006). This is not reflected in the data obtained in this study. Laser ablation, another *in situ* spectrometric technique (gas chromatographic isotope ratio mass spectrometry) has also emerged as a means of capturing heterogeneity in dental samples of many sizes and significant intra-tooth variation has been observed in several species (Passey & Cerling, 2006; Sharp & Cerling, 1996).

Although it is believed that the heterogeneity observed in the modern sample using SIMS is natural, factors potentially specific to the sample in question may be at

play and should be considered in contrast to the Danish research materials. The modern human sample is a 3rd molar. This tooth is the last to develop, mineralize and erupt, with enamel formation completing at approximately 11 years of age (Reid & Dean, 2006). Despite being large teeth, the enamel formation time of third molars is approximated at 1.9 years, whereas mandibular canine enamel forms over approximately 4.7 years (Reid & Dean, 2006). The majority of the Danish research sample in this study consists of mandibular canines, with only two maxillary canines, the enamel development time of which is nearly identical to its lower antimere. Thus, the canines take longer to mineralize leaving the isotopic record in the enamel open to natural variation for longer. Finally, given that the third molar specimen is modern, it is possible that small movements during the period of enamel development, or consumption of non-local water, whether from food or drinking water, are reflected in the observed heterogeneity. The diversity of water sources practically available to modern populations is also likely greater than the sources available to historic populations living more sedentary lifestyles, with stronger ties to local water and food. All considered, natural intra-tissue variation in the medieval Danish sample is expected to be similar to that observed in a modern human tooth using SIMS.

Other factors further hindered the use of the materials available as SIMS standards, and consequently the use of SIMS on the study sample. As noted, a proper standard must be independently calibrated. In order to calibrate the proposed SIMS analysis of the Danish materials, it was required that an effective cementum standard be established. Cow cementum, which is substantial in some areas of the root, facilitating physical sampling for TC/EA-IRMS calibration, is highly porous, complicating SIMS analysis with greater than normal surface charging. Human cementum, while less

problematic in SIMS analysis, is present only in very thin layers on the root surface, making it very difficult to sample for TC/EA-IRMS calibration. Given the challenges with Ag_3PO_4 isolation on sample sizes of less than 5 mg, human cementum is largely ruled out due to this sampling difficulty.

Recently, researchers have conducted tests to assess the quality of secondary ion spectrometric data as it compares to more conventional spectrometric data. Aubert and colleagues (2012) standardized their Sensitive High Resolution Ion Microprobe (SHRIMP) analyses with the standard NBS-19. One experiment examining the $\delta^{18}O$ of fish otoliths reveals a 0.8‰ offset between SHRIMP values and those obtained by conventional means, suggesting a matrix related fractionation difference between the aragonite and calcite of the sample and standard materials, respectively (Aubert et al., 2012). Another study found no offset between secondary ion data and more conventional analysis of otoliths, using an internal calcite reference material (Hanson, Wurster, Edinburgh Ion Microprobe Facility, & Todd, 2010). The offset observed between methods in otolith $\delta^{18}O$ was not observed in the experiments examining dental enamel by Aubert and colleagues (2012), though some discrepancies, likely relating to spatial resolution differences were observed. The dental enamel experiments were once again compared to results reflecting carbonate-phase oxygen of conventional analysis, and were subject to a 9‰ offset correction because secondary ion analysis largely reflects phosphate- phase oxygen (Aubert et al., 2012). The offset between phosphate- and carbonate- phase oxygen has been established (Iacumin, Bocherens, Mariotti, & Longinelli, 1996). However, some uncertainty exists regarding a possible attenuation in carbonate oxygen as compared to phosphate oxygen (Pellegrini, Lee-Thorp, & Donahue,

2011). As such, the inappropriate application of this offset could mask other fractionations or offsets at play such as diagenetic alteration, or offsets relating to *in situ* methods. The study by Aubert and colleagues (2012) illustrates the many benefits of secondary ion spectrometry in oxygen isotope analysis of biological tissues, while outlining the need for matrix-specific biological standards.

Sample Quality

The spectrometric method ultimately employed in this study, Thermal Combustion Elemental Analyzer (TC/EA) coupled with an isotope ratio gas source mass spectrometer is preceded by a much more involved sample preparation than those required for *in situ* analyses. Phosphate is chemically isolated from dental material through precipitation of Ag_3PO_4. The Ag_3PO_4 isolation method refined in this study and the analytical protocol for the prepared samples are reported above. Method testing on cow enamel and cementum produced largely consistent oxygen isotope results. Differences in $\delta^{18}O$ are observed where expected: inter-individually as well as intra-individually between different teeth and tissues. The minor differences observed between cementum and enamel (Figure 10) from the same sample are to be expected, as these tissues form at slightly different times, and likely reflect natural variation in consumed water during life due to geographic and seasonal factors. This variation has been demonstrated by many researchers, and has been used to study seasonality among other things (Cerling and Sharp, 1996). The variability observed in the TC/EA experimental data reported here reflects an averaging of seasonal and other natural variation. Given this, the inter-tissue and inter-tooth differences observed in this data must be interpreted as reflections of natural variation provided by the method employed.

Other factors tested in the development of the methods used in this study provide some insight into the issues of organic removal and diagenesis. As noted, Ag_3PO_4 samples clear of visible organics were obtained via a dual chemical pre-treatment. Heat treatment of the precipitated Ag_3PO_4 was also tested as a means of removing organics, and driving water from samples, or degassing. The effects of high temperature heat treatment observed in this study are consistent with experiments carried out in the early 1990s. These experiments demonstrated that silver phosphate does not measurably adsorb water, as gravimetric loss resulting from heat treating silver phosphate occurred only at temperatures higher than 500˚C, and not at lower temperatures when water would be driven off (Figure 15) (Crowson et al., 1991).

Despite early gravimetric evidence for the non-hygroscopic nature of Ag_3PO_4, it remains unclear whether adsorbed or other atmospheric water is present in prepared Ag_3PO_4, and thus, whether degassing is necessary. It is also unclear whether heat treatment at any temperature is an effective means of organic removal. Evidence for its effectiveness in bulk samples has been demonstrated by Lindars et al. (2001) though attraction of atmospheric water is observed following heating at very high temperatures, and cautioned against. Extensive testing of heat treatment on prepared samples of Ag_3PO_4 is not available. The physical and isotopic effects of heat treatment at 500˚C observed in this study (Figure 6; Table 1) demonstrate that this procedure goes beyond any supposed organic or water removal and should not be maintained as a purification practice for Ag_3PO_4 samples, at least not when the sample must then be transported outside of vacuum-sealed environments. As noted, experiments at lower temperatures have shown no isotopic change between samples degassed at 150˚C to those degassed up

to 400˚C by Lecuyer et al. (1993); however, the need for degassing at any temperature remains in question, and its consistency with samples prepared using chemical pretreatments for organic removal remain untested. Lower temperature (150-200˚C) degassing experiments carried out on Danish and standard Ag_3PO_4 in this study produced desirably consistent results, but a small isotopic shift was not only observed in the sample materials, but also in the standard materials. While Lecuyer et al. (1993) report no isotopic shift in degassing experiments up to 400˚C, there is no clear report of these experiments on synthetic Ag_3PO_4 standards. Given the uncertainty associated with the need for degassing in general, and the isotopic shifts observed in this study as a result of various degassing temperatures, samples subject to heat treatment at any temperature were excluded from analysis in this study.

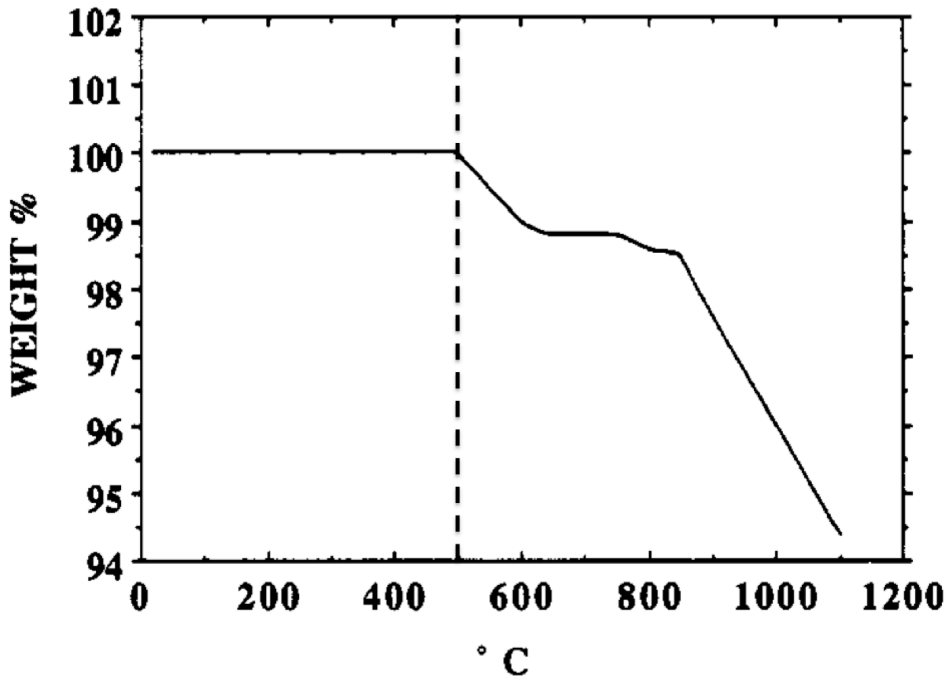

Figure 15. Gravimetric loss of Ag_3PO_4 at temperatures above 500˚C.

Adapted with permission from Crowson, R. A., Showers, W. J., Wright, E. K., & Hoering, T. C. (1991). Preparation of phosphate samples for oxygen isotope analysis. Analytical Chemistry, 63, 2397–2400. Copyright (1991) American Chemical Society.

High temperature conversion elemental analysis works to convert an element of desire into a gas for isotopic quantification. In the case of oxygen analysis, glassy carbon is used to convert the oxygen in the sample to CO. In any mass spectrometric method, the interference of isobaric compounds must be considered, such as N_2 in the case of CO analysis. This potential interference is mitigated by separating N_2 peaks from CO peaks in the gas chromatography column that separates gases from one another. While this helps to ensure that the major peak at mass 28/30 is not reflecting N_2 as well as CO, there should not be any nitrogen present.

In the early stages of method development in this study, N_2 peaks were observed with some regularity. This phenomenon was never observed in the analysis of standards, and was not necessarily present with samples analyzed one after another, but was found associated with samples from the same source. Given these observations, N_2 peaks were considered to be reflective of lingering organics in the prepared samples. Modifying the preparation method mostly eliminated these peaks. Nitrogen peaks were observed in all of the analyzed samples prepared following the method of Dettman and colleagues (2001b), suggesting that the N may not be originating from organics, but from excess $AgNO_3$ from the precipitation chemicals. The absence of N_2 peaks in samples prepared employing the method developed in this study, with the same organic removal steps as were used for the Dettman samples, supports this interpretation.

The presence of N_2 peaks resurfaced in the analysis of the Danish samples in ten of the 168 analyses, originating from six of the 26 individuals. In some cases these corresponded with low CO yields of the major peak or erroneous data but never presented

in all of the analyses from any one individual. The N_2 peaks reached amplitudes of 50 to 80 mV, which is small compared to the major sample CO peaks averaging approximately 4.5 V. Nitrogen blanks in similar pyrolysis systems have been measured at five to 20 mV (Kornexl et al., 1999). In the interest of eliminating potentially compromised data, all analyses with measurable N_2 peaks were excluded from group and individual results, and interpretations.

Sample quality has been discussed at length here, as the data obtained is only as robust as the samples analyzed and methods used. Efforts were made to assess the quality of the archaeological samples to determine whether removing organics, both diagenetic and biogenic, would lead to the isolation of strictly biogenic phosphatic oxygen as intended. As Schoeninger and colleagues have aptly noted,

> there has been the temptation to do chemical or isotopic analyses on all bone irrespective of the appropriateness of the sample in terms of preservation. When the results of the analysis match expectation, there has been the inclination to accept them as meaningful. Otherwise, the results are rejected from consideration. Put in these terms, a circularity is obvious (1989, p. 282)

There has been significant advancement in methods developed to determine the appropriateness of archaeological samples, bone and otherwise. Concern over whether non-biogenic forms of stable oxygen had been incorporated post-mortem is addressed using some of these advancements. Selected samples were subjected to Scanning Electron Microscopy (SEM), Ion Microprobe Analysis, and Raman Spectroscopy to investigate inconsistencies in the internal tissues of some samples. Initial characterization

using SEM revealed elevated calcium to phosphate ratios in patches in the dentine of concern, suggesting that crystalline-looking areas may have a higher calcium content. This alludes to the presence of a diagenetic calcite or calcite polymorph. Other visibly opaque or crystalline-looking areas were found to contain elevated levels of manganese and iron consistent with diagenetic deposition of Mn- and Fe- oxyhydroxides as reported by Kohn and colleagues (1999). Samples were variably altered, and in some areas both biogenic and diagenetically altered tissues were analyzed.

In summary, diagenetic alteration appears to have been a factor in the post-mortem history of some samples; however the impact seems to have been largely limited to the dentine and cementum tissues. The issue of enamel opacity remains a concern, though there is insufficient information to link it to microbial activity, or potential phosphate oxygen alteration. Kohn and colleagues observed that although their chemical analysis cannot illustrate the likelihood of microbially related re-precipitation in fossil enamel in their study, the secondary depositions provide some insight. They suggest that the oxyhydroxides found in dentine and cementum are in areas that bacteria are unlikely to be capable of accessing, suggesting that the alteration minerals are not directly precipitated by microbes (1999). Observed secondary precipitation of minerals in the research sample is similar to the precipitation reported by Kohn and colleagues. All considered, the samples were believed to be appropriate for phosphate oxygen isotope analysis of the enamel only.

Interpretation of Results

Local Proxies

Oxygen isotope studies designed to identify human movement in past populations can gain much insight into the origins of individuals when local $\delta^{18}O$ values, provided by an outside source, can be identified. This can take the form of a modern reference sample, or a burial sample identified by archaeological or historical means to have been a strictly local population. In this study, no modern or historic local reference sample is available from which to determine a natural local range. As such, alternate proxies are considered.

International bodies have organized a global effort to create an isotopic record of rain (meteoric) water. This has led to a more comprehensive understanding of oxygen isotope variation worldwide. The International Atomic Energy Agency (IAEA) is responsible for the Global Network of Isotopes in Precipitation (GNIP), a network with collections from of over 800 meteorological stations in 101 countries since 1961. The precipitation and isotope data from this network is available in multiple formats, including world maps of isotopes in precipitation. These maps illustrating general trends indicate that mean annual $\delta^{18}O$ values for precipitated water in central and eastern Denmark are in the range of −8‰ to −11‰ (IAEA, 2001). Although this data provides a promising start to establishing a local signal, this insight is limited by the data available, and data from Danish stations is far from complete. There are five Danish sampling stations in the GNIP database. The nearest station to the archaeological sites in this study is the Odense station, located approximately 50 km from Horsens. The GNIP provides only six $\delta^{18}O$ values from samples at this station over 21 years. No samples have been

analyzed for $\delta^{18}O$ from three of the other Danish stations. The final station, Taastrup, located near Copenhagen, produced monthly samples over six years, nearly all of which were analyzed for $\delta^{18}O$, providing the most comprehensive set of $\delta^{18}O$ data from modern Denmark (IAEA/WMO, 2006).

The Online Isotopes in Precipitation Calculator (OIPC) employs the GNIP database to estimate annual and monthly mean hydrogen and oxygen isotope values for any site given geographic coordinates (Bowen & Revenaugh, 2003; Bowen, Wassenaar, & Hobson, 2005). The coordinates 55.817N 9.9E 23m and 55.816N 9.858E 9m were established and employed for estimates of Sejet and Ole Worms Gade, respectively. A mean $\delta^{18}O$ value was estimated at −9.6±0.8‰ (95% CI) for Sejet and −9.5±0.8‰ (95% CI) for Ole Worms Gade (Bowen, 2013). These estimates are consistent with global and regional mapped data for annual averages in the same area as reported above. More detailed observation of the GNIP data from Odense and Taastrup reveals that the OIPC estimates are heavily weighted to the more representative data from Taastrup, an appropriate computation given the very limited data available from samples collected at Odense. The quantity of data available in a given area acts as the greatest limitation to $\delta^{18}O$ estimation of a given site (Bowen & Revenaugh, 2003). As such, it is possible that greater local variation exists that is missed by the data available. Despite this, these estimates suggest that inter-site variation is unlikely. Monthly averages from both Sejet and Ole Worms Gade reveal that there are two ranges that make up the annual variation in $\delta^{18}O$: the period from May to September is characterized by heavier $\delta^{18}O$ means from −9‰ to −7‰ while September to April experiences precipitation depleted in $\delta^{18}O$ with means ranging from approximately −12‰ to −10‰ (Bowen et al., 2005; Bowen, 2013).

In order to use the isotopic composition of meteoric water as a comparable local range, the human tissue values must first be converted to water values. Several conversion calculations between $\delta^{18}O$ values derived from phosphate ($\delta^{18}O_p$) and the $\delta^{18}O$ of oxygen in water ($\delta^{18}O_w$) are available (Daux et al., 2008; Levinson et al., 1987; Longinelli, 1984; Luz, Kolodny, & Horowitz, 1984). These are established using linear regression between the measured values from collected water samples and modern tissue samples (or historical samples for which there is a confidently identified provenance) from the same locality. Some have argued that the error introduced in these conversions renders them unreliable (Pollard, Pellegrini, & Lee-Thorp, 2011). The limitations of this comparative method are recognized; however, this method remains useful in cases where no modern or historic comparative sample of phosphate isotope values is available.

It has been recognized that factors aside from drinking water contribute to phosphate $\delta^{18}O$ values. Body temperature, size, and respiration all play a role (Luz et al., 1984). In addition, food sources also contain water and contribute to an overall consumed water $\delta^{18}O$ value that is slightly enriched as compared to drinking water (Daux et al., 2008; White et al., 1998). It is more accurate to consider phosphate oxygen as being derived from body water as opposed to drinking water, as this concept takes into account some of the physiological factors listed. Conversions from $\delta^{18}O_p$ to $\delta^{18}O_w$ reflect the relationship between environmental water and body tissue, which necessarily includes any individual level fractionations and other sources of consumed water. These will be considered in the context of the research sample.

Converted water $\delta^{18}O$ values were calculated for each individual included in the initial analysis using the combined calculation published by Daux and colleagues (2008).

Mean values for the entire sample range from −11‰ to −6‰ with an overall sample mean of −7.9‰ (Table 6). This range is in near perfect agreement with the monthly mean range of −12‰ to −7‰ provided by the OIPC data (Bowen et al., 2005; Bowen, 2013); however, the overall mean, and the majority of the individuals exhibit $\delta^{18}O$ values higher than those recorded from modern precipitation interpolations.

Table 6. $\delta^{18}O$ mean values and deviations excluding low yielding data and samples. Groups based on $\delta^{18}O$ means.

Individual	Mean $\delta^{18}O_p$	Mean $\delta^{18}O_w$	N	Burial Period
15	17.0	−7.5	6	1/2
77	16.3	−8.6	4	1
106	17.1	−7.4	5	2
113	16.5	−8.3	5	1
126	16.2	−8.7	5	n/a
157	17.3	−7.1	3	2
291	17.6	-6.6	5	2
381	16.4	−8.4	5	1
435	17.1	−7.4	5	1
487	16.7	−8.1	3	3
506*	17.5	−6.7	3	2/3
577	16.0	−9.1	4	3
1076	17.2	−7.2	6	2
1086	17.0	−7.5	4	2
1114	15.3	−10.2	5	~1
1180	18.0	−6.1	3	3
1186*	17.8	−6.2	5	1
1269	17.4	−7.0	7	1
1292	15.0	−10.7	3	1
1360	16.8	−7.9	6	2
1416	17.9	−6.1	6	1
1433	15.4	−10.1	3	1
1587.1	16.2	−8.7	4	2/3
	16.8	−7.9		

* = Data reported from this individual was obtained from re-analysis using a slightly altered method. See results for details.

Challenges to using the estimated values established using OIPC as a local range for the historic data in this study include possible shifts in local precipitation $\delta^{18}O$ since historic times and the suitability of precipitation as a proxy for drinking water. One major factor in the $\delta^{18}O$ of local precipitation anywhere is temperature. Early estimates suggest that isotopic values in precipitation shift by 0.7‰ per ˚C (Dansgaard, 1964). This positive

correlation remains supported, though continental and seasonal factors are also at play (Siegenthaler & Oeschger, 1980). Surface temperature and amount of precipitation appear to account for approximately 45% of long-term variation in the $\delta^{18}O$ of precipitation (Rozanski, Araguas-Araguas, & Gonfiantini, 1993).

Shifts between present temperatures and temperatures during the medieval period in Europe have been recorded (Daux, Lécuyer, Adam, Martineau, & Vimeux, 2005). Climatic shifts during the medieval period are suspected to be more geographically complex than current data can reflect. Events such as the Medieval Warm Period and the Little Ice Age surely impacted oxygen isotopic signatures in various regions, but a continent-wide picture of these shifts is not agreed upon, as temperature changes were not uniformly severe (Fagan, 2000; Hybel & Poulsen, 2007). Some temporal trends can be considered in the sample with respect to climate change. Individuals who lived and were buried during Period 1 experienced favourable climate, up to 1°C warmer than 20th century averages in summer (Fagan, 2000; Yoder, 2006). However, the modern OIPC data on which estimates are based dates to 1965-1970, prior to the latest climatic shift to current temperatures (IAEA/WMO, 2006; National Research Council, 2006). Data compiled by the National Research Council Committee on Surface Temperature Reconstructions for the Last 2,000 Years demonstrates that average Northern Hemisphere temperatures in the mid 20th century are similar to recorded values for the Medieval Warm Period (Fagan, 2008; National Research Council, 2006). Temperatures calculated for the Little Ice Age are observed to be cooler than this modern period by approximately 0.5°C. Given this, the converted values for individuals in the burial Periods 2 and 3 may be depleted as compared to modern OIPC estimated values by as much as 0.35‰ as per

estimates by Dansgaard (1964). Individuals living during the second and third periods experienced less predictable climates than in the first period, with great seasonal variation and overall cooler winters (Fagan, 2000). As such, 0.2‰ will conservatively correct mean values from individuals whose burials are dated confidently to the second and third burial periods.

The appropriateness of precipitation as a proxy for drinking water is largely dependent on the local source and collection of drinking water. Most modern drinking water is sourced from near-surface aquifers, though some populations source water from aquifers fed by precipitation falling at altitudes distinct from the consumer community. The heavy isotope content of tap water tested in a variety of locations agrees well with OIPC predictions based on isotopes in precipitation reported by Daux and colleagues (Bowen, 2013; Daux et al., 2008). Therefore, precipitation will be considered comparable to modern drinking water as outlined in $\delta^{18}O_p$ to $\delta^{18}O_w$ conversion calculations. This does not account for the sourcing and treatment of water historically. When comparing converted historic values with modern OIPC values, reservoir effects must be considered. Historic populations were more likely to source drinking water from nearby surface reservoirs such as lakes and streams. Surface bodies are heavily reflective of local precipitation; however moving bodies such as rivers and streams can provide water with upstream isotopic signatures, while the isotopic signature of standing bodies may be affected by evaporative fractionations, known as reservoir effects (Dansgaard, 1964; Daux et al., 2008). Cultural practices such as water collection and storage, or beverage preparation, can augment these effects, further enriching drinking water over naturally occurring sources of water (Knudson, 2009).

Upstream effects are of relatively little concern for the sites in question, as Denmark does not exhibit great natural elevation. The highest point in modern Denmark lies at approximately 170m above sea level while average elevation is only approximately 30m above sea level. Elevations of 9 m and 23 m above sea level were used for the local estimates for the two research sites, demonstrating relatively little difference in elevation between the sites and in the area in general. The elevation of the nearest OIPC recording site at Odense is at an elevation of 17 m, while the collection site that produced most of the isotope data available in Denmark lies at 28 m above sea level (IAEA/WMO, 2006). Altitude effects calculated by Poage and Chamberlain indicate that natural $\delta^{18}O$ variation in precipitation in Europe is 0.21‰/100m of elevation (2001). Given the mixing of water sources reflected by streams and rivers, and the limited topographical variation in the region, the possible altitude effect in nearby drinking water sources at the sites is minimal. This is supported by regional isotopic maps which illustrate a relative uniformity in the precipitation isotopes across central and eastern Denmark, the area containing both archaeological sites and both GNIP sampling sites listed above (IAEA, 2001).

Fractionation due to reservoir effects is more likely to have played a role in the $\delta^{18}O$ signature of drinking water at the sites. Well water was a likely source of drinking water for both urban and rural populations, especially prior to the use of public pipelines, not found in Europe until the 13th century (Kristensen, 2004). Though well water sourcing is not necessarily linked with reservoir effects, water storage, being of particular importance in years of drought, can be. Storage and treatment of water in some capacity likely led to slight enrichment in $\delta^{18}O$ over local modern OPIC precipitation estimates.

This enrichment would not be reflected in the $\delta^{18}O_p$ to $\delta^{18}O_w$ calculation and could be corrected for; however, direct information about water sources at the sites of interest are not established, and specific treatment of water cannot be known at this time.

Beyond drinking water, another component of body water, the water derived from food, can be examined. Naturally hydrated foods contain water that is enriched as compared to environmental water, and cooked foods also incorporate enriched water through fractionation of water during cooking, and incorporation of that enriched water into the food (Daux et al., 2008). Modern $\delta^{18}O_p$ to $\delta^{18}O_w$ calibrations necessarily subsumes this oxygen source. Isotope differences arising from diets distinct from the modern study populations have been identified in some modern and historic populations. Modern diets largely consist of cooked food, enriching total consumed water by approximately 1.05-1.2‰ over drinking water values (Daux et al., 2008). Maximum $\delta^{18}O$ enrichment of consumed water over drinking water is estimated at 2.0‰, possible through a diet of raw vegetables and large quantities of meat and fish, or a diet consisting mostly of cooked vegetables (Daux et al., 2008).

Diet in medieval Denmark underwent a series of shifts. The Medieval Warm Period was favourable to grain cultivation, and diets were comprised mainly of barley and rye with regional access to fish, and limited access to meat, legumes and vegetables or fruit (Yoder, 2010). Later droughts and cooling led to intensification of existing animal husbandry. Wheat became the major grain crop consumed, and prepared ales were consumed more regularly (Yoder, 2010). In summary, medieval diet consisted of components enriched in $\delta^{18}O$ as compared to water, as do modern diets. Without specific quantities and extensive knowledge of preparation techniques, a quantification of the

relative enrichment is impossible; however, given that cooked grains in malt and porridge were a significant component of medieval diets, it is likely that water incorporated through food consumption was not drastically enriched or depleted than the food water incorporated in the samples that the $\delta^{18}O_p$ to $\delta^{18}O_w$ conversion calculations are comprised of.

Converted $\delta^{18}O_w$ means from individuals dated to Periods 2 and 3 have been corrected by 0.2‰ on the basis of the difference in surface temperatures experienced between this burial period and the modern period from which the OIPC data was derived (Table 7). Other potential corrections were not made due to a lack of direct evidence available to quantify the appropriate correction. The correction alters the overall sample mean by approximately 0.1‰.

Table 7. $\delta^{18}O$ mean values and deviations excluding low yield data and samples. Groups based on $\delta^{18}O$ means.

Individual	Mean $\delta^{18}O_p$	2σ Mean $\delta^{18}O_p$	Mean $\delta^{18}O_w$	Corrected $\delta^{18}O_w$ Mean	2σ Mean $\delta^{18}O_w$	OIPC Site $\delta^{18}O_w$	Burial Period
15	17.0	0.4	−7.5	-7.5	1.1	−9.6	1/2
77	16.3	0.6	−8.6	-8.6	1.2	−9.6	1
106	17.1	0.5	−7.4	-7.2	1.1	−9.6	2
113	16.5	0.3	−8.3	-8.3	1.0	−9.6	1
126	16.2	0.3	−8.7	-8.7	1.0	−9.6	n/a
157	17.3	0.4	−7.1	-6.9	1.1	−9.6	2
291	17.6	0.4	-6.6	-6.4	1.1	−9.6	2
381	16.4	0.4	−8.4	-8.4	1.1	−9.6	1
435	17.1	0.6	−7.4	-7.4	1.2	−9.6	1
487	16.7	0.4	−8.1	-7.9	1.1	−9.6	3
506*	17.5	0.4	−6.7	-6.5	1.1	−9.6	2/3
577	16.0	0.4	−9.1	-8.9	1.1	−9.6	3
1076	17.2	0.4	−7.2	-7.0	1.1	−9.5	2
1086	17.0	0.5	−7.5	-7.3	1.1	−9.5	2
1114	15.3	0.4	−10.2	-10.2	1.1	−9.5	~1
1180	18.0	0.6	−6.1	-5.9	1.2	−9.5	3
1186*	17.8	0.8	−6.2	-6.2	1.3	−9.5	1
1269	17.4	0.7	−7.0	-7.0	1.2	−9.5	1
1292	15.0	0.3	−10.7	-10.7	1.0	−9.5	1
1360	16.8	0.4	−7.9	-7.7	1.1	−9.5	2
1416	17.9	0.6	−6.1	-6.1	1.2	−9.5	1
1433	15.4	0.4	−10.1	-10.1	1.1	−9.5	1
1587.1	16.2	0.4	−8.7	-8.5	1.1	−9.5	2/3
Mean	16.8	0.5	−7.9	-7.8	1.1		

* = Data reported from this individual was obtained from re-analysis using a slightly altered method. See results for details. Corrected $\delta^{18}O_w$ values reflect corrections based on temperature offsets between the Little Ice Age and the modern period reflected by OIPC data for Denmark.

Potential migrants

Global variation in modern $\delta^{18}O_w$ illustrates some divide within Denmark; however the region in which the two sites are located is characterized by one $\delta^{18}O_w$ range in distribution maps (IAEA, 2001). Given this lack of zonation, and the near identical site means, one local value will be considered, representing the area in which both sites are located. If the monthly means are employed as a conservative estimate of the local $\delta^{18}O$ range for central Jutland to eastern Denmark, nearly all of the samples from this study fall within that range of −7 to −11‰. It should be noted that this range is likely broad given that most studies find that a δ18O range of approximately 2‰ characterizes a site (Evans, Chenery, et al., 2006; Price et al., 2013; Prowse et al., 2007). Three individuals produced corrected $\delta^{18}O_w$ means at the low end of this range, while five samples with relatively enriched values lie outside of the local seasonal ranges. Individuals X1114, X1433, and X1292 produced corrected $\delta^{18}O_w$ means of −10.2±1.1‰ (2σ), −10.1±1.1‰, and −10.7±1.0‰ respectively. These individuals constitute Group 3 as determined by cluster analysis. Individual X1180 has a recorded mean $\delta^{18}O_w$ value of −5.9±1.2‰. This individual's mean $\delta^{18}O$ value is higher than four others, X291, X1416, X506, and X1186 that also produced mean values outside of the monthly range, at −6.4±1.1‰, −6.1±1.2‰, −6.5±1.1‰, and −6.2±1.3‰ respectively. Given the magnitude of the error associated with the conversion from $\delta^{18}O_p$ to $\delta^{18}O_w$ values, the distinction of the individuals with higher values from the remainder of the study sample and as distinctly outside of the local range is not clear.

In order to determine whether the potential outliers are likely to be migrants, the normality of the observed values was tested using a normal probability quantile plot.

Figure 16 illustrates that only the individual with highest $\delta^{18}O_p$ value (which corresponds to high $\delta^{18}O_w$ values) deviates from the normality line at the high end of the spectrum, whereas all three individuals at the lower end of the spectrum more clearly deviate from normal. The 2σ ($\delta^{18}O_p$) error associated with each of these values is lower than the error associated with the conversion to $\delta^{18}O_w$. Despite this, the upper five individuals do not diverge from the group, and only the three lower individuals will be considered as probable migrants. The highest individual (X1180) will be considered in discussions of possible shorter-distance movement or incomplete separation of origin and destination $\delta^{18}O$ values.

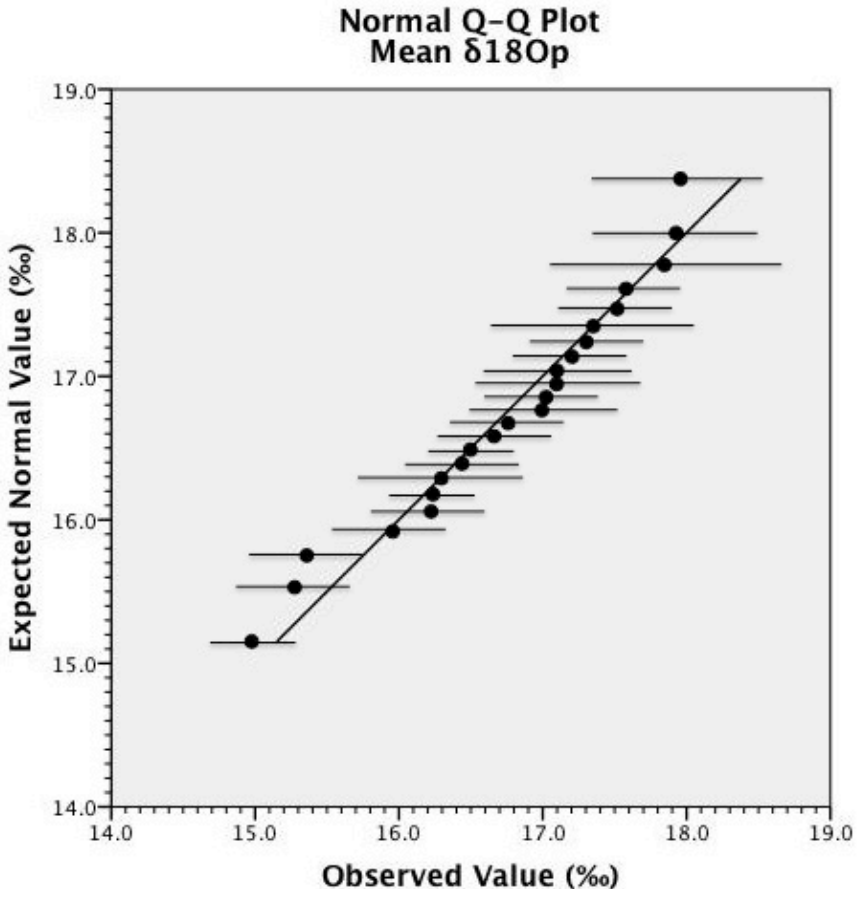

Figure 16. Normal probability plot of $\delta^{18}O_p$ means (2σ error).

In order to gain insight into any trends relating to potential migrants, demographic information gleaned from skeletal analysis by others is outlined (Gamble, 2013; Pedersen, 2008, 2010). The three individuals demonstrating low $\delta^{18}O_w$ are X1114, X1292, and X1433, all excavated from Ole Worms Gade, most likely dating to the first burial period. Individual X1114 died between the ages of 40 and 50, and was likely male, though the sex of this individual is uncertain. Individuals X1292 and X1433 are both females. Individual X1292 likely died between the ages of 28 and 38, while individual X1433 lived to 30 or 35 years of age (Gamble, 2013). The individual producing the highest $\delta^{18}O_w$ value, individual X1180, was a woman buried at Ole Worms Gade in Period 3, having died between the ages of 42 and 54 (Gamble, 2013).

The 13 individuals analyzed from Sejet range in age from 19-60 years, while the age at death of analyzed individuals from Ole Worms Gade span the range of 18-55 years (Gamble, 2013). The scarcity of individuals between the ages of 20 and 25 observed in the full Ole Worms Gade sample is reflected in the study population. In the age profile context, the potential migrants in this sample are relatively representative of the general sample, and fairly representative of the overall adult population excavated from each of the sites, as outlined earlier. Not represented in the profiles of observed potential migrants are individuals below the age of 25. The young adult population below 25 years of age makes up only 8-15% of the overall adult study population from both sites, making this unsurprising.

No trends in demographic information are evident with respect to the potential migrants from the research sample. Most interestingly, all of the potential migrants with

low isotope ratio values (i.e. Group 3) were excavated from Ole Worms Gade and are most confidently dated to burial Period 1. The individual exhibiting the highest isotope ratio value was also recovered from the same site; however this individual was likely buried during the third period.

Though it is not impossible that factors aside from geographic variability contributed to variation in the $\delta^{18}O$ of the study sample, they are unlikely to be significant. As noted, dietary intake contributes to the $\delta^{18}O$ of body water and thus, forming tissues. Isotope and trace element studies investigating diet often credit potential status differences as the cause of some intra-population variation. The materials examined here represent childhood tissue formation, and while status differences could be considered to be less prevalent in children than adults in many populations, examples of status differentiation in childhood diet exist elsewhere in the medieval period as indicated by trace element study (Shutkowski, 2002). This is less likely to be a major factor in $\delta^{18}O$ given that dietary intake is only one contributing factor to body water, and that enrichment in food is largely due to cooking practices, and resulting water enrichment, which are less likely to have diverged between high- and low- status familial units than the types of foods consumed.

Geographic origins

If the individuals identified to have potentially non-local childhood $\delta^{18}O_w$ signals are reflective of human movement in the medieval period, it is of interest to investigate the possible geographic origins of these individuals. Regions exhibiting mean annual $\delta^{18}O_w$ values depleted as compared to the local study signal include Sweden, much of Finland, and the remainder of the Baltic coastline as well as the Baltic islands (IAEA,

2001). It should be noted that the depleted values observed in the study sample are not outside of the local range as determined by modern proxies. These individuals are being considered as potential migrants due to the separation and relative depletion as compared to the remainder of the sample, and their deviation from a normal distribution (Figure 16).

Estimated values for several nearby locations were determined using the OIPC (Bowen, 2013) to get a more detailed view of potential origins. Odense, located approximately 50 km southeast of Horsens on the island of Fyn, at 55.47N, 10.33E, 33m, has a modern annual mean of approximately −9.5‰. Parchim, Germany is thought to be where one of the coins excavated from Sejet was minted, and (53.43N, 11.85, 50m) has an approximate mean annual $\delta^{18}O$ of −9.1‰. Looking farther eastward, lower annual mean $\delta^{18}O$ values have been measured; the annual $\delta^{18}O$ of Copenhagen is −9.7‰ while even further, Pärnu, Estonia has a mean annual $\delta^{18}O$ of −11.0‰. Mean values of −10.1 and −10.3‰ characterize the annual precipitation on the coasts of Lithuania and Latvia. Gothenberg and Kalmar, on the West and East coasts of Sweden, exhibit mean annual precipitation $\delta^{18}O$ values of −10.1‰ and −10.2‰ respectively, while even more negative values can be found in Northern Sweden (−10.6‰) (Bowen, 2013).

Mean annual $\delta^{18}O_w$ values higher than the local signal are present within modern Denmark. Western Jutland is characterized by mean $\delta^{18}O_w$ of −5‰ to −8‰ in regional isotope maps available (IAEA, 2001). However, western Jutland locations investigated using the OIPC reveal annual means of −9.3‰ (Ribe) or lower, while few higher values can be found closer to the research sites than Amsterdam (−8.2‰). If there were individuals who moved to the burial area after childhood from areas with higher oxygen

isotope signatures as is suggested by individual X1180 in this sample, it is possible that they came from western Jutland; however, Danish areas west of the sites do not differ significantly enough from the local study area to be identified with the level of precision obtained in this study. While it is possible that one or more individuals exhibiting mean $\delta^{18}O$ values on the high end of the sample range moved from outside of Denmark, it is not possible with any certainty to identify them as migrants, and not possible to identify potential origins given the narrow variation to the west of the country.

Migration at the population level

Prior to considering the potential implications of the possible migrants in the two burial populations, the limited scope on migration in the region that this data presents should be reiterated. Migrants identified using isotopic analysis of enamel represent individuals who lived in a non-local area during their developmental years. Their presence in a mortuary context suggests, but does not demonstrate, that they were residents of the town, rural area, or village surrounding the cemetery in question at the time of death. If local residence prior to death and burial is assumed, the life period at which movement from the non-local origin to the local area remains unknown. It can be assessed that, if a clearly non-local signal is observed, movement into the local area occurred after the dental tissue in question was completely formed, being approximately 5-6 years of age for the research sample (Reid & Dean, 2006). Finally, outmigration from the local area cannot be determined from the data available. If the data is ascertained to be indicative of movement into the local communities, in-migration rates may be discernible, but are unlikely to be accurately reflected in the very limited sample size presented in this study.

Overall, the sample presented is small, and cannot be considered representative of the two cemeteries that contain well over 500 individuals each; however, it is possible that some trend in movement over time exists, being hinted at by the variation over time in this sample. Burial Period 1, which dates from the beginning of the medieval period to 1300 is the period in which all of the migrants, as determined by the analyzed sample, were buried. The potential migrants dated to the first burial period may be partially indicative of the burial period distribution of the analyzed sample; however, clear evidence of migration in Period 1 is not unlikely given the period of growth that populations in Europe and Scandinavia underwent in the early medieval, and the associated push and pull factors related to this growth and the establishment of the town of Horsens. Although the identification of individual X1180 as a potential migrant is uncertain, the individuals with values at the upper end of the oxygen isotope spectrum may be suggestive of continued movement into the research populations from the West.

The population sizes of the communities associated with each of the study cemeteries are unknown. The image available of life surrounding the cemetery of Ole Worms Gade is incomplete at best. Ole Worms Gade is located in Horsens, a town known to have been an urban center in medieval times, expanding significantly in the 13th century along with other European trading centers (Horsens Museum, n.d.). A study of population diversity in six Danish medieval cemetery populations supports the assertion that Danish towns were sufficiently 'urban' to be home to a more diverse population than rural villages or suburban populations (Petersen, Boldsen, & Paine, 2006). As such the dynamic between and within cemeteries and thus burial populations is expected to be quite complex. Excavations in Horsens have been carried out on varying scales at several

locations throughout the modern city. The excavations have all been limited in size, and an understanding of life in this medieval town is far from comprehensive, and the medieval town's population remains unknown. Aside from a few burials identified in the town center near the administrative building, Ole Worms Gade presents the only burial population in Horsens. The cemetery at Ole Worms Gade is associated with an independent parish church bearing the name Vor Frue Kirke, or Church of Our Lady ("† Vor Frue Kirke," n.d.). Very little remains of the structure of this church, which is situated outside the medieval town boundary. Nearby findings suggest that it was located nearer to other town development at some point in its early history, and likely built on royal land ("Kirkerne i horsens," n.d.; Klemensen, 2009). The church was probably built in the 12th century, declined in use in the late 15th century until the early 16th century, and was finally allowed to fall into disrepair with its materials used for repairing a nearby church ("† Vor Frue Kirke," n.d., "Kirkerne i horsens," n.d.).

To date, approximately 450 m^2 have been excavated from the cemetery at Ole Worms Gade. Excavations revealed 650 graves, 578 of which contained skeletal material (Pedersen, 2010). It has been estimated that the cemetery covers a total area of approximately 9400 m^2. Given this estimate, it is possible to reasonably expect that the cemetery may contain 12,500 burials at a rate of 600 burials per 450 m^2. Estimates suggest use at Ole Worms Gade over a 380-year period. If the population turned over every 23.8 years, reflecting life expectancy at birth (Boldsen, 2002), nearly 16 generations would have contributed to the cemetery. If the estimated 12,500 burials are divided by the 16 generations, it is estimated that the average contributing population was approximately 781 persons.

An estimate of required or expected migration at this site is nearly impossible due to the indirect relationship between town and cemetery given that Horsens was home to other churches and cemeteries ("† Kirker I Horsens," 2003). It is impossible to know the demographic relationship between the town of Horsens and the parish of the Church of Our Lady. As such, the segment of the population buried at Ole Worms Gade cannot be assumed to be representative of the overall Horsens population, nor can it be assumed to have been a unique segment of the population. The average population of medieval towns in Denmark during the medieval period has been estimated to be less than 1,000 persons, though larger towns of approximately 4,000 inhabitants have been observed (Petersen et al., 2006). If the estimate of 780 persons is taken as accurate, it is possible that much of the living population was buried at Ole Wormsgade throughout the Middle Ages.

If the burial population is considered representative of the overall Horsens population, and not separate insofar as marriage and demographic traditions are concerned, the required migration rate will be lower than that expected of the contributing population, because the overall reproductive population may reflect the greater overall Horsens population, and not just the parishioners at the Church of Our Lady. As noted, a simulation study by Boldsen explores migration as a requirement for stable population maintenance under the conditions of avoiding familial intermarriage, as forbidden by religious standards (1989). The largest population assessed by the migration simulation is 300 persons. Populations of 300 persons require migration into the population at a rate of 0.3% (Boldsen, 1989). In a population of 12,500 persons this equates to 37.5 persons over the entire time of use. Although the population of Horsens, likely much larger than 300 persons could have maintained its population without

breaking marriage laws with even fewer migrants, it is reasonable to assume that the migration rate of the town would have been much higher given that the medieval period was a time of urbanization, and Horsens was an urban center boasting opportunities for trade and market pursuits (Boldsen, 1989, 1996; Horsens Museum, n.d.).

The observed proportion of individuals potentially indicative of having migrated in the very small sample examined from Ole Worms Gade in this study is 27%. While it is improbable that there was a 27% migration rate throughout the period, or even at any given point in the town's history, it is possible that the migration rate was quite high for a given period. As noted, the potential migrants identified from the Ole Worms Gade sample are all dated to before 1300 based on burial arm position. In this early period the migration requirement for the town would likely have reflected its smaller population as it became an urban center in addition to its establishment during this period. It has been noted that the 13th century saw significant population growth and urbanization in Horsens (Horsens Museum, n.d.), perhaps being reflected in the high rate of migration observed in the sample during this period. High late childhood mortality has also been observed in medieval Scandinavian populations, leading to the possibility that migration may have played a key role in population maintenance and growth (Boldsen, 1996).

The rural cemetery in this study is associated with the medieval and subsequent modern village of Sejet. The earliest available record of the historic village of Sejet is a land registry map created in 1793 (Kjærgård, 2006; "Sejet Kirker," 2006). This map outlines 17-18 farms and a number of houses at a time when the cemetery had largely been forgotten, and the village was considered robust ("Sejet Kirker," 2006). It is not known whether the village layout or size would have been similar when the cemetery was

in use, but the cemetery area remains largely undisturbed by post-medieval land use, suggesting that this map can be used as a starting point. An 8 person per household estimate is employed for the medieval period in rural communities as suggested by Boldsen (2002), based on demographic analysis of the completely excavated cemetery at Tirup and demographic requirements for a reproductive unit. If the number of habitation units is conservatively estimated at 17, the population of Sejet at some point in the medieval period may have been approximately 136 persons. Although this is based on several assumptions, there is little to suggest that this estimate is unreasonable. The structure and population size of medieval villages are likely to have varied from site to site, and go unmentioned in the available written sources dating from this time as documentation was not concerned with the peasant population (Roesdahl, 2004).

If the Sejet cemetery was in use for 450 years in the medieval period, and a population of 136 persons was maintained and reproduced every 23.8 years, based on average life expectancy (Boldsen, 2002), 19 generations of people would be expected to be buried in the cemetery, totaling 2,584 persons. If the cemetery is assumed to be the main burial location for residents of the medieval village of Sejet, and not routinely used by other populations, an estimate of the burial population size should be comparable to the overall medieval population based on above estimates. The excavations at Sejet were located in the southwest corner of the cemetery. An area of approximately 400 m^2 was excavated unearthing 632 skeletons (Pedersen, 2008). The excavated area is estimated to be approximately 12% of the total cemetery size, which is estimated at 3100 m^2 based on present land use and historic maps (Kjærgård, 2006). Kjærgård provides 4,500 individuals as a conservative estimate of the total burial population at the Sejet cemetery

(2006). This estimate is higher than for other medieval village cemeteries, and Sejet is considered a densely used cemetery; however, Sejet was also in use for up to twice as long as other similar medieval cemeteries (Boldsen, 1989; Kjærgård, 2006). As is evident, this does not match the above-derived population estimate of the community of Sejet over the medieval period of 2,584.

The discordance between the estimates of the living population of the community of Sejet and the burial population at the Sejet cemetery could be indicative of several factors, aside from the errors inherent to such estimates. The population in Sejet is likely to have fluctuated though the 450 years that the cemetery is thought to have been in use, and may have been significantly higher than what is suggested by a map created after the end of the medieval period. It is also possible that human movement, whether permanent migration or movement associated with war or other activity may have resulted in non-local burials in the Sejet cemetery. Finally, it is likely that other populations made use of the cemetery at Sejet. Historic documents suggest that Nedergård, a nearby farm, of which little has been recorded, would have fallen under the parish church in Uth when the title to the Sejet church was transferred, and the parishes were merged ("Sejet Kirker," 2006). Seeing that Nedergård was in need of a new parish once the Sejet church was left to degrade and be demolished, it follows that the residents of Nedergård had previously been parishioners at Sejet, for at least the later portion of the medieval, if not since the church's construction, a date for which has not been confirmed. This would suggest that the Sejet cemetery might have been the primary burial ground for the people of Nedergård, and likely those living and working on other nearby farms as well. Despite these possible explanations for the discrepancy between the estimate provided by

Kjærgård and the one provided here, it is also likely that the estimate for the size of the entire cemetery is unrealistically large given the rural status of the community. The true burial population is likely to lie somewhere between the two estimates.

The estimated living population of Sejet matches most closely with Boldsen's model population of 150 persons. In the model, a population of 150 persons is expected to experience immigration at a rate of 5.2% to comply with marriage rules while sustaining sufficient birth rates to match death rates (Boldsen, 1989). Considering the estimated living population of Sejet, this equates to 134 migrants contributing to the population over the whole of the medieval period. This rate is expected to have fluctuated over time as population fluctuated due to catastrophes or other forms of demographic stress. If Nedergård and other nearby farms are assumed to have sustained a smaller population than Sejet, it is likely that each contributed members to the other, helping to diversify the population and avoid inbreeding, reducing the required number of migrants from elsewhere.

The final estimate for Sejet burials considering the above estimates of living populations at Sejet and farms such as Nedergård is over 3,000 persons. This estimate is plagued with many assumptions and estimates based on incomplete information and remains incompatible with the 4,500 burial estimate provided by Kjærgård for the cemetery (2006). Individuals who spent their developmental years in a neighbouring area characterized by the same local $\delta^{18}O$ signal as the destination likely fulfilled a significant portion of the required migration rate, reducing the visibility of movement in this population through oxygen isotope measurement. This relationship, at a broader local

scale may also be reflected in the incomplete separation of individual $\delta^{18}O$ means in the upper end of the spectrum from the presumed local population in this study.

Also of interest in the findings at Sejet are two individuals whose remains suggest that they were beheaded (Pedersen, 2008). This in itself does not suggest much about their likelihood of movement during life; however Pedersen (2008) points out that it is atypical for individuals who were punished so severely to be buried with the general population. As such, their presence in the cemetery may identify them as individuals who travelled away from their local community in war or other circumstances and were returned home to be buried after being killed elsewhere (Pedersen, 2008). This indirect evidence pointing to the return of migrants after death supports the presumption that some individuals buried at Sejet exhibiting local $\delta^{18}O$ signals in their childhood tissues may have spent significant time away before being returned upon death.

In spite of the small size of the research sample, the oxygen isotope data appears to support expected trends in human movement in medieval Denmark. Movement of individuals during the early part of the medieval into Horsens with subsequent burial at Ole Worms Gade is consistent with increasing urbanization during this period. The three individuals identified as having moved to Horsens in the early burial period may have come from elsewhere in the Baltic. Existing trade relations in the region likely acted as a vehicle for building knowledge of the Horsens region abroad, which in turn facilitated more permanent movement. More specific push-pull factors are difficult to identify; however, given its growth in early periods, Horsens was likely viewed as a prosperous destination by other medieval populations, especially other Scandinavian populations that were relatively late to urbanize.

Shorter distance movements, undetected by isotope analysis are reasonably assumed to have occurred in relation to urbanization in Horsens. Migration from western Jutland as part of urbanization trends is likely, given that urban centers throughout western Jutland were few (Andrén, 1985; Petersen et al., 2006). It is likely that many others from within the central and eastern Jutland also moved, undetected by oxygen isotope ratio analysis, from rural areas to urban market centers to take advantage of the growing artisan trades and other opportunities provided by urban towns. These movements may also reflect mutual familiarity and interaction through trade and military pursuits throughout these regions.

Local $\delta^{18}O$ signature proxies have not only helped to revealed potential migrants, but have also highlighted the scale of human movement not detectable with the available proxies. Movement within eastern Jutland and the Danish archipelago is not revealed under the current analysis and proxy ranges (IAEA, 2001). What the proxy comparison does indicate about human movement in medieval Denmark is that people were likely moving throughout the Middle Ages, but that movement over considerable distance was likely focused in the early period. The individuals identified as potential migrants are diverse demographically, with nothing distinct setting them apart from the overall research population. Observations of stature and paleopathological indicators between migrant and local populations have been made elsewhere for medieval Danish populations, yielding interesting results (Petersen et al., 2006). Comparisons of health and stature between potential migrants and local populations is not available at this time, but would be a useful investigation in future, in particular when a larger segment of the population could be analyzed.

Chapter 5: Conclusions

This study was designed to determine whether individuals buried at two medieval cemeteries migrated during their lifetimes based on oxygen isotope data from dental enamel. As part of the data collection process this study investigated isotope isolation methods and potential diagenesis relevant to the materials available. The data collected permits the identification of three potential migrants from a sample of 26 individuals. Though this cannot be considered representative of the cemetery populations, which containing over 500 individuals each, the study supports the notion that human movement was an integral part of life in the medieval period, and that these populations were a part of the demographic shifts taking place at this time.

Two mass spectrometric methods were tested to assess their suitability for the human dental materials available from the research subjects. Secondary Ion Mass Spectrometry (SIMS), an *in situ* method for measuring oxygen isotopes is found to have limited use on dental materials, mainly due to the lack of matrix-appropriate standards for biogenic materials. As such, standards were to be prepared from modern cow samples using Thermal Combustion Elemental Analysis Mass Spectrometry (TC/EA-MS) following isolation of the phosphate through Ag_3PO_4 precipitation. This was largely unsuccessful due to the natural heterogeneity present in the cow enamel samples, and the instrumental complications caused by the natural porosity of the cementum tissue.

Given the challenges with SIMS analysis of dental tissues, TC/EA-MS was used to collect the bulk of the data in this study. Silver phosphate was precipitated to isolate the phosphate-bound oxygen in order to eliminate the less resistant carbonate-bound oxygen present in dental enamel. A method was developed combining components of

several published methods to streamline the isolation process through quicker precipitation. A double organic removal process was employed to ensure that any oxygen that does not reflect the individual from whom the sample was collected would be eliminated. The range of $\delta^{18}O$ values measured in each of the cemetery populations was found to be limited; however, comparison to modern local signatures provided some insight into possible migration.

The isotopic composition of meteoric water was available as a local proxy, provided by the Online Isotopes in Precipitation Calculator based on Global Network of Isotopes in Precipitation data. Limited regional variation within central Denmark did not provide for the identification of movement between Sejet and Ole Worms Gade; however, three potential migrants excavated from Ole Worms Gade, were identified. These individuals exhibiting lower oxygen isotope values may have moved from other Baltic or Scandinavian locations. While it is not possible to narrow down the area of origin with the data available, this study provides evidence that point to relatively long-distance movement of individuals into, and likely out of, medieval Denmark. Migration of this nature is indicative of existing relationships across great distances, such as trade relationships, providing a basis on which to make decisions about movement and travel. Shorter distance migrations, not confidently detectable by the data available were also likely commonplace.

Migration rates are not accessible from the data available due to a limited sample size. However, the human movement identified in this study appears to be in line with demographic predictions, such as required migration rates based on population size, and expected movement to centers exhibiting signs of urbanization, such as Horsens where

the Ole Worms Gade cemetery is located. Specific push-pull factors experienced by the individuals investigated are not known.

Limitations encountered in this work would be better understood, and possibly eliminated, through further study. First, analysis of a greater sample size to permit population-level interpretations is needed. Sample preparation has been somewhat simplified by the altered method above, which should facilitate smoother preparation of a large number of Ag_3PO_4 samples. Second, establishment of a suitable biogenic standard for SIMS analysis of dental materials would contribute greatly to similar studies, and permit intra-individual comparison of isotope values. This standard needs to be relatively homogeneous, and true values must be established using a well-tested method. Reliable standardization of *in situ* methods for biogenic materials would shed light on natural heterogeneity in human teeth, and possibly reveal greater detail about human movement on a finer time scale. Finally, access to additional local proxies for the comparison of oxygen isotope values would increase the ability to identify potential migrants and their origins.

This study has provided an examination of some of the spectrometric methods available for use on dental tissues. A revised method for Ag_3PO_4 precipitation as a means of phosphate-bound oxygen isolation was developed to balance the need for effective organic removal and a streamlined precipitation process. Data collected revealed potential migrants in the small sample analyzed, indicating that human movement was a part of the demographic reality in Denmark during the medieval period. There is much left to learn about life in medieval Denmark, and at Sejet and Ole Worms Gade,

specifically, and much potential to be explored in the application of mass spectrometric methods to human dental tissues.

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