Putting Stress to the Test: A Critical Evaluation of the Biological Response and Physical Manifestation of Stress in the Human Skeleton

by

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ABSTRACT

Using traditional osteological methods of stress analysis, specifically skeletal lesions and growth and development patterns, this research focuses on the timing and manifestation of stress in the hard tissues of the body and the interaction between these various indicators of poor health. The Danish Black Friars cemetery population spanning the medieval and post-medieval periods (13^{th}-17^{th} centuries) is used for this research to explore changing health trends during a period of socioeconomic disruption and expanding urban dwelling. The results of this study show a distinct trend between the late and early post-medieval periods where stress was elevated after the Reformation (AD 1536). Additionally, females generally show higher levels of stress than males. The poorer health experienced in the post-medieval period was likely influenced by the changing living conditions of the late 16^{th} century where urban dwelling increased in Denmark introducing new pathogens, poorer living conditions and new environmental and working stressors, all contributing to poorer health. While the overall level of stress appears to increase into the post-medieval period, the average age-at-death is higher after the Reformation suggesting that while these individuals may have been exposed to more prolonged periods of stress, they were able to adapt and survive in spite of these hardships. This research also examines the ability to analyse stress through the extraction of ancient proteins from bone. Using MircoBCA and enzyme-linked immunoassay (ELISA) analysis, it is shown that protein preservation is good in the Black Friars population and the level of protein reflects the expected fluctuations associated with the demography of the population (i.e. age, sex) and not necessarily the influence of stressors. Overall this study provides a comprehensive examination of stress from its initial biological signal through to skeletal disruption in a climate of expanding urban development and changing socioeconomics in Denmark.

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For Ulla

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CHAPTER 1: INTRODUCTION

As a dynamic living tissue, the human skeleton provides a unique opportunity to explore the human condition at a biological level and more specifically how the skeleton adapts, evolves and changes in response to its surrounding environment. As a discipline created between the boundaries of archaeology and osteology, bioarchaeology promotes an exploration of the relationship between cultural influences and biological processes, and the impact of this relationship on the skeletal tissues of archaeological populations (Clark 1972; Buikstra 1977). This "context-embedded approach" (Palkovich 1996) is a key element of bioarchaeological research where skeletal variability is critically analyzed beyond just the genetic framework of the human body. From a bioarchaeological perspective then, "human skeletons represent answers and the goal of osteology is to frame the questions" (Armelagos and VanGerven 2003:53).

Studies of health, more specifically skeletal stress, have been foundational in bioarchaeological research where the ability to identify episodes of poor health are based on gross macroscopic physical changes such as lesion formation or linear growth disruption. Identified as any perceived or actual threat (Selye 1936; Selye 1973; Charmandari et al. 2005; Chrousos 2009), stress activates multiple biological systems that work in tandem to promote survival and maintain homeostasis (Chrousos and Gold 1992; Habib et al. 2001). While the stress response is an intricate process with multiple particularities, stress in an osteological context largely remains non-specific, as multiple confounding factors may influence the skeletal manifestation of stress over different periods of the life course (Goodman et al. 1988; Armelagos and VanGerven 2003). As such, when analyzing physical indicators of stress that affect skeletal tissues, bioarchaeologists must be aware that any visible changes reflect a timeline of health and not merely the conditions present at the time of death (Goodman 1993). While identified as a

primary challenge of bioarchaeological research, the overlapping nature of intrinsic and extrinsic stressors have prompted the development of collaborative and innovative research that integrates multiple methods of analyses (e.g. Ribot and Roberts 1996), employs new technologies and theories (e.g. Scott and Hoppa 2015), and re-evaluates the common assumptions associated with skeletal research and studies of health (e.g. Wood et al. 1992). It is because of this shift in bioarchaeological research design that current analyses of skeletal stress are better equipped to address questions of individual and population health and the relationship between culture, the environment and human biology.

1.1 PURPOSE AND SIGNIFICANCE

This research will focus on how indicators of skeletal stress can be measured and analyzed in archaeological populations to inform our understanding of health and well-being in the past. By situating this research within a clinical framework of the biological stress response, the relationship between well-established markers of skeletal stress can be better explored and compared across different demographic groups highlighting individual and population trends.

This research will also explore the biochemical evidence of stress and how chemical signatures extracted from ancient bone may be compared and contrasted to the well-established macroscopic indicators of poor health. This relationship is a key component of this research as this biochemical data may effectively close the analytical gap that has been present in bioarchaeological research until now, providing new insight into the chemical changes associated with stress and the consequences of this on the hard tissues of the body. By exploring and identifying the threshold at which stress affects the skeletal tissues of the body and which regions may be more susceptible to these physical changes, there is an opportunity to expand the analytical framework of archaeological studies of stress and health.

This research is significant in that it will provide the most up-to-date analysis of stress with a specific focus on the biological stress response and how this impacts the manifestation and distribution of stress lesions and linear growth disruption. With this data there is an opportunity to explore the hierarchical nature of stress and which skeletal tissues may be most vulnerable to disruption during periods of poor health. In establishing a better timeline of how stress effectively spreads across the skeletal tissues, bioarchaeologists will be better equipped to identify individual and population differences in health. This timeline approach for the study of stress will also benefit from the integration of biochemical data where it may be possible to identify stress from the onset, increasing the interpretive possibilities for this type of bioarchaeological research.

While this research will emphasize the methodological and analytical dimensions of stress research in archaeological populations, the results will focus on the specific stress patterns identified in the Black Friars cemetery sample. Spanning the medieval and early post-medieval periods (13^{th}-17^{th} centuries), the Black Friars cemetery sample represents the urban population of Odense, Denmark. Situated within the Danish medieval and post-medieval periods, the Black Friars cemetery population was no doubt exposed to many cultural, environmental and social insults during this period, all of which would have influenced the overall health of this population. While the historical and archaeological texts can enlighten our knowledge of the Danish experience between the late medieval and early post-medieval periods, it is only through an in-depth analysis of the skeletal remains that a better understanding of the human condition can be achieved. In creating an in-depth health profile of the Black Friars population, this research will contribute to the knowledge of livelihood, mortality and morbidity during the 13^{th}-

17th centuries in Denmark, all of which influence our understanding of history, culture and society in the past.

This research is situated within the well-established bioarchaeological methods of stress assessment (e.g. Angel 1966; Park 1964; Garn et al. 1968; Sweeney et al. 1969; Pindborg 1970; Steinbock 1976; El-Najjar et al. 1978; Nikiforuk and Fraser 1981; Huss-Ashmore et al. 1982; Goodman and Armelagos 1985; Goodman and Armelagos 1988; Stuart-Macadam 1989; Goodman and Rose 1990; Mays 1995; Lewis and Roberts 1997; Scott 2009; Walker et al. 2009; Scott and Hoppa 2015). In combining these various methods that have been explored by multiple researchers in different contexts, the best possible suite of osteological changes have been compiled for this analysis. Further, the integration of both lesion- and growth-based methods allows for an exploration of stress from different biological perspectives where the underlying mechanisms that mediate their formation provide a more nuanced understanding of how the stress response, once activated, targets specific skeletal tissues. In addition to these well-established methods of stress examination, the use of biochemical protein to address questions of health will also be explored. As a new method of osteological interpretation, this biochemical focus is an innovative approach to skeletal health, as chemical changes are the precursor to the physical manifestation of stress in bone. Until now, stress research has been confined to the analysis of skeletal changes associated with chronic periods of prolonged stress, whereas the integration of biochemical methods may shorten the timeline of interpretation arguably providing an opportunity for bioarchaeologists to explore skeletal stress before it has even begun to visibly manifest in the skeletal tissues. Using established archaeological methods of protein extraction (e.g. Collins et al. 2002; Smith et al. 2005; Cleland et al. 2012), the corroboration of skeletal

lesions and growth disruptions with biochemical data provides an innovative approach to the well-established and relied upon methods of health assessment in archaeological populations.

1.2 RESEARCH OBJECTIVES

The main research question for this project is how can different indicators of skeletal stress better inform our understanding of health and well-being in archaeological populations? Within this broad framework are more specific research objectives that identify the gaps in the stress literature and will be explored throughout this dissertation.

Based on a clinical understanding of the stress response, one of the objectives of this research will be to further elaborate on the severity and duration of stress in archaeological populations using macroscopic indicators of disruption. Further, this research also seeks to clarify the biological relationship between lesion- and growth-based skeletal manifestations of stress, specifically how might the presence of one affect the manifestation of the other. In exploring the relationship between different indicators of stress, this research will also seek to identify the role biochemistry has in the bioarchaeological analysis of stress, particularly the accuracy in which ancient proteins can be used to explore health during life. Additionally, this research aims to explore how this analysis of stress can better inform our understanding of the Black Friars cemetery population and what factors contribute to observable fluctuations in health between the medieval and post-medieval periods.

Building on these research objectives, four hypotheses are proposed with respect to the observed impact of stress in the skeleton. Using well-established indicators of stress (i.e. lesion formation and growth disruption) in addition to biochemical changes, these four hypotheses will be addressed in this dissertation. It is assumed that the stress observed is prolonged in nature, as individuals must survive beyond the onset of the disturbance to register physical manifestation in

the skeleton (Wood et al. 1992). It is based on this assumption that chronic stress produces skeletal changes that the following four hypotheses are outlined and will be discussed in further detail in Chapter 4:

- *Hypothesis 1* states if morbidity (occurrence of illness) and mortality (occurrence of death) are elevated within a population, it is expected that skeletal evidence should indicate stress, or that stress indicators should be present;
- *Hypothesis 2* states that the severity of that stress and how it is manifest in the skeleton will be reduced in successive age cohorts (i.e. after a certain age, those affected by the increased stress will have likely succumbed to these insults);
- *Hypothesis 3* states that the severity of stress may be higher in children who represent the non-survivors of their community (i.e. individuals affected early in life by high levels of physiological stress do not survive to adulthood). As such, it is expected that adult survivors will not demonstrate the same severity of stress as observed in the subadults;
- *Hypothesis 4* states that a small proportion of individuals, as a result of differential frailty (Wood et al. 1992), might live beyond what is expected despite showing an elevated severity of stress.

The dissertation is structured as follows. Chapters 2 and 3 provide an overview of the relevant literature associated with this project. Chapter 2 focuses on the historical context of medieval and post-medieval Denmark outlining specific environmental (e.g. The Late Medieval Agrarian Crisis; The Little Ice Age), political (e.g. The Count's War) and health events (e.g. the Black Death) that effectively shaped the Danish Middle Ages influencing the daily lives of the Black Friars population. Chapter 3 discusses the osteological context of this research and situates the outlined research questions and hypotheses within the current bioarchaeological literature.

Beginning with an overview of skeletal metabolism and the growth process, this chapter presents an in-depth analysis of the stress response from a clinical perspective and unites these data with the traditional osteological indictors of stress including: cribra orbitalia, porotic hyperostosis, enamel hypoplastic lesions, Harris lines, body size indicators and cortical thickness. This chapter also outlines the challenges of analyzing stress in archaeological remains and the primary limitations within this field of study. Chapter 4 outlines the materials and methods of this project discussing the criteria of skeletal selection, the dating methods of the Black Friars cemetery population and the age and sex demographics of the population. Additionally, Chapter 4 outlines the specific stress analysis methods used in this study (lesion- and growth-based) and discusses the protocol for ancient protein extraction and analysis. Chapter 5 presents the results and statistical analyses of this research with emphasis placed on the differences observed between time period, sex and age. Focusing on how the various stress indicators interact with one another there is overlap between each section as different indicators are compared and contrasted. A discussion of the results is presented in Chapter 6 along with an in-depth summary for each aspect of this project including pathological and stress-lesion data, growth patterns, biochemical analysis and mortality patterns. Comparative archaeological data is also used in this discussion to assess how the visible patterns of stress in the Black Friars population compare to other contemporaneous populations. The main focus of the discussion is on the potential stressors that may have influenced the visible heath trends in the Black Friars population including: environmental stressors from urban dwelling, daily labour requirements, under nutrition and malnutrition, psychological stress and biological demands (i.e. pregnancy, menarche). This chapter concludes with a discussion of the interaction between the lesion- and growth-based stress indicators as well as the biochemical data and how these interactions can be used to

address the four outlined hypotheses. Chapter 7 presents the conclusions of this research with a final summary of the Black Friars population and future directions for the study of skeletal stress. These future directions outline the need to continue with analyses of stress duration and severity based on a clinical understanding of the stress response and the biological systems that mediate these changes.

CHAPTER 2: LITERATURE REVIEW – HISTORICAL CONTEXT

2.1 INTRODUCTION

This chapter will focus on the broad historical context of the skeletal population used in this research detailing the history of the Black Friar Dominican Order in Odense and the archaeological excavation of the Black Friars monastery. Situated within this specific historical context, a broad discussion of the medieval to post-medieval period will be provided with specific focus on climate, economy, subsistence, living conditions, and disease during these centuries.

2.2 THE DOMINICAN ORDER

Founded by St. Dominic, the Dominican Order or Preach Friars were more colloquially known as the Black Friars, so named for the black cappa worn over their white habit (Hinnebusch 1973; Ashley 1990; Becher 1999). The Dominican Friars, similar to other Orders, were known for their commitment to poverty through a lack of worldly possessions, but more widely recognized for their commitment to knowledge and learning (Hinnebusch 1973; Ashley 1990; Orrman 2003b). As a 'learned Order' the Dominican Friars were mobile and intended to enlighten the largely uneducated masses and combat heresy through their preaching (Hinnebusch 1973). As the Dominican Order grew in the early part of the 13th century, monasteries were established in university towns throughout Europe so that all brothers could enroll, effectively contributing to a rise in the education of the Scandinavian clergy (Orrman 2003b). Further, during the creation of the Order's legislation in AD 1220, it was decreed that all monasteries must employ at least one professor so that it could also function as an educational institution (Hinnebusch 1973; Becher 1999). This emphasis on learning was employed by the Dominican

Friars for both cultural gain and to protect the church from opposition by expanding the reach of these Friars across the known world (Bennett 1937:52).

The founder of the Order, Dominic de Guzman was born in the latter half of the 12th century to Spanish nobility and was known for his scholastic aptitude and dedication to learning (Ashley 1990). It has been argued that Dominic's early life as a successful student was integral to the formation of the Dominican Order and its intellectual character (Hinnebusch 1973). After his completion of university, Dominic became an ordained priest and accompanied Bishop Diego on a journey to Denmark in AD 1203. It was during this journey into Scandinavia that Dominic recognized the need to preach and spread knowledge into the untamed Pagan territories of the northeast (Ashley 1990). The Dominican Order was officially recognized by the Catholic Church in AD 1216 and the first Scandinavian monastery was established in AD 1223 in the now Swedish city of Lund (Becher 1999). After AD 1238 and the formation of the 'Dacia' province, the Dominican Order had control of 15 monasteries throughout Scandinavia (Jakobsen 2008), with further expansion up until AD 1250 (Orrman 2003b).

2.3 THE ODENSE BLACK FRIARS MONASTERY AND CEMETERY

The city name Odense is derived from Old Danish "Odens Vi" meaning the shrine of the Norse God, Odin (Lauring 1976; Christensen 1988). During the Viking Age, Odense was likely an important centralized ceremonial site on Fyn as one of the seven ring fortresses (Nonnebakken) was built during the era of Harald "Bluetooth" Gormsson before the turn of the millennia (Kaufmann and Kaufmann2004; Randsborg 2009). However, written records of the city are absent until AD 988, after which time Odense became an important Christian city when it was named the fourth diocese of the Catholic Church in Denmark (Christensen 1988). In addition to this centralized power of the Church during the early medieval period, Odense was

also the parliamentary seat for the central region of the country leading to increased settlement and expansion of the city (Hybel and Poulsen 2007). In AD 1086 Odense became infamous when King Knud was murdered by the people in St. Alban's Priory, which was eventually converted into a royal shrine in AD 1101 (Oakley 1972). King Knud's murder was spurned by the displeasure of the masses to pay the demanded tithe for ecclesiastic development, as Christianity was still relatively new in Scandinavia during this period (Lauring 1976). In AD 1247 Odense experienced further disruption as part of the city was burned by an invading army under the command of Abel of Denmark, the Duke of Schleswig against King Erik the 4th (Erslev 1889). As the younger brother of Erik, Abel mounted an attack to gain independence for the Duchy of Schleswig (southern Jylland) in AD 1246. Abel invaded much of Jylland and eventually moved into Fyn where the city of Odense fell victim to this dispute. Eventually, a truce was reached in AD 1250 at which time Erik was murdered and Abel claimed the Danish throne (Lauring 1976). Despite the civil unrest during the early medieval period, Odense remained an important centralized power, but arguably reached its culmination in the post-Reformation period as the commercial center of Fyn (Lauring 1976).

The Dominican Friars in Odense, referred herein as the Black Friars, were located in the north eastern part of the medieval city and likely established their monastery around AD 1239 (Møntegården Odense bys Museer 2010). No written records survive that confirm the foundational date of the Black Friars monastery, but is rather based on similar institutional formations in other Cathedral cities in Denmark at this time (Becher 1999). The earliest written record of the Black Friars monastery is from AD 1252 when the city of Odense became the provincial chapter of the Dominican Order (Christensen 1988; Becher 1999). Who established the Black Friars monastery and decided its location in the medieval city of Odense is unknown

(Christensen 1988). Archaeological excavations on the site have recovered organic remains and ceramics dating to the 12th century, suggesting an earlier occupation before the monastery was established (Christensen 1988). Historical records indicate that the Black Friars did not build the original foundations of the monastery, but rather took over the previous ecclesiastical building and used recycled materials to further expand and repair their newly acquired property (Christensen 1988). It is likely that that some of the building materials used to update and repair the Black Friars monastery was acquired from the vacated Nonnebakken church when the Benedictine nuns moved their convent to the southeast village of Dalum (Christensen 1988). Once established, the Black Friars monastery was located at the corner of the city proper; archaeological excavations suggest that the monastery was walled with a primary gate located along the southern perimeter at Overstræde (Christensen 1988). During the medieval period the Black Friars monastery expanded to include a second church structure to the south of the original construction, making the Black Friars monastery a prominent landmark in the medieval city. While the Black Friars square is still present today in Odense, the western and northern boundaries are undefined. Archaeological excavations were unable to locate the terminus of the Black Friars property due to current residences built on portions of the site (Christensen 1988). However, the western and northern regions of the original property were likely characterized by swampy soils that may have been improved by the Black Friars, as contemporary drilling has revealed soil backfilling that was laid into these swampy areas and may have allowed for further expansion and use of the land (Christensen 1988). In addition to the physical expansion of the monastery, the religious reach of the Black Friars was also advanced in AD 1316 when both the Black and Grey Friars of Odense were granted permission to use St. Knud's Church to hear the confessions of their parishioners (Christensen 1988). While the history of the Black Friars

monastery is somewhat incomplete, successful geographic expansion visible in the archaeological record and written documentation of increased religious responsibilities suggests this monastic order was a significant institution in medieval Odense.

After the Lutheran Reformation in Denmark in AD 1536, the monastery was closed with its church demolished in AD 1542 and the remaining buildings used as residences (Christensen 1988; Boldsen and Mollerup 2006) and part of the Grey Friar hospital (Becher 1999). After the formal close of the Black Friars monastery, the graveyard was turned over to the city in AD 1551 and remained in use until in the early part of the 17th century (Christensen 1988; Becher 1999; Boldsen and Mollerup 2006). Historical records show that in AD 1607 permission was granted to demolish the final stone structure standing on the site, marking the end of the cemetery's use (Christensen 1988). Before the Reformation, the monastery was likely reserved for the interment of the friars and the middle classes of society (Jakobsen 2008). Inside the church and along the cloister walks were locations primarily designated for the brothers with the larger cemetery complex to the east reserved for the middle classes (Jackobsen 2008). During the medieval period in Denmark it was tradition for individuals to be buried in their local church cemeteries; however, those with the financial means were able to secure a burial location in a monastery cemetery and put ones soul closer to God after death (Jakobsen 2008). As such, the Black Friars cemetery during the medieval period was likely represented by the middle class while the post-medieval period was characterized by lower class burials, as the cemetery became publically run and more widely used after AD 1551 (Becher 1999). Despite these traditions in the historical literature, the inclusion of women and children in the church and cloister walk regions of the Black Friars cemetery suggests these strict designations were not necessarily adhered to (Jakobsen 2008). Additionally, the need to accommodate a large density of individuals in an

urban environment likely created a more fluid cemetery composition that included individuals from all socioeconomic statuses. As Mollerup and Boldsen (2010) discuss, the closing of three other city cemeteries in the post-medieval period prompted the prolonged use of the Black Friars cemetery after the Reformation and as such, likely incorporated all echelons of society based on need over personal preference of burial location.

The time period of the Black Friars cemetery is comprised of two main periods of interment: the monastic phase (AD 1240 – 1536), and the public phase (AD 1536 – 1600) (Mollerup and Boldsen 2010). There were an increased number of post-medieval interments compared to the medieval period as cemeteries were closed (post AD 1536) at a time when the Danish population was on the rise (Christensen 1988). Approximately 800 square meters of this cemetery complex were excavated beginning in 1972 until 1997; however, burials were only recovered from 1978, 1979, and 1981, identified as SBT78, SBT79, and SBT81, respectively (Nielsen 1982a; 1982b; Becher 1999). Estimates for the number of individuals recovered from Black Friars cemetery vary from 520 (based on skeletal inventories) to 979 (based on the ADBOU collection record). These discrepancies are likely due to the archaeological recording on site; for example, the 1981 burial inventory contains 680 grave entries, but only 661 of those entries were true burials (Nielsen 1982b). Considering this then, the skeletal assemblage from the Black Friars cemetery complex is made up of approximately 661 to 710 individuals with well over half belonging to the post-medieval period (Mollerup and Boldsen 2010).

2.4 DANISH HISTORICAL TIMELINE

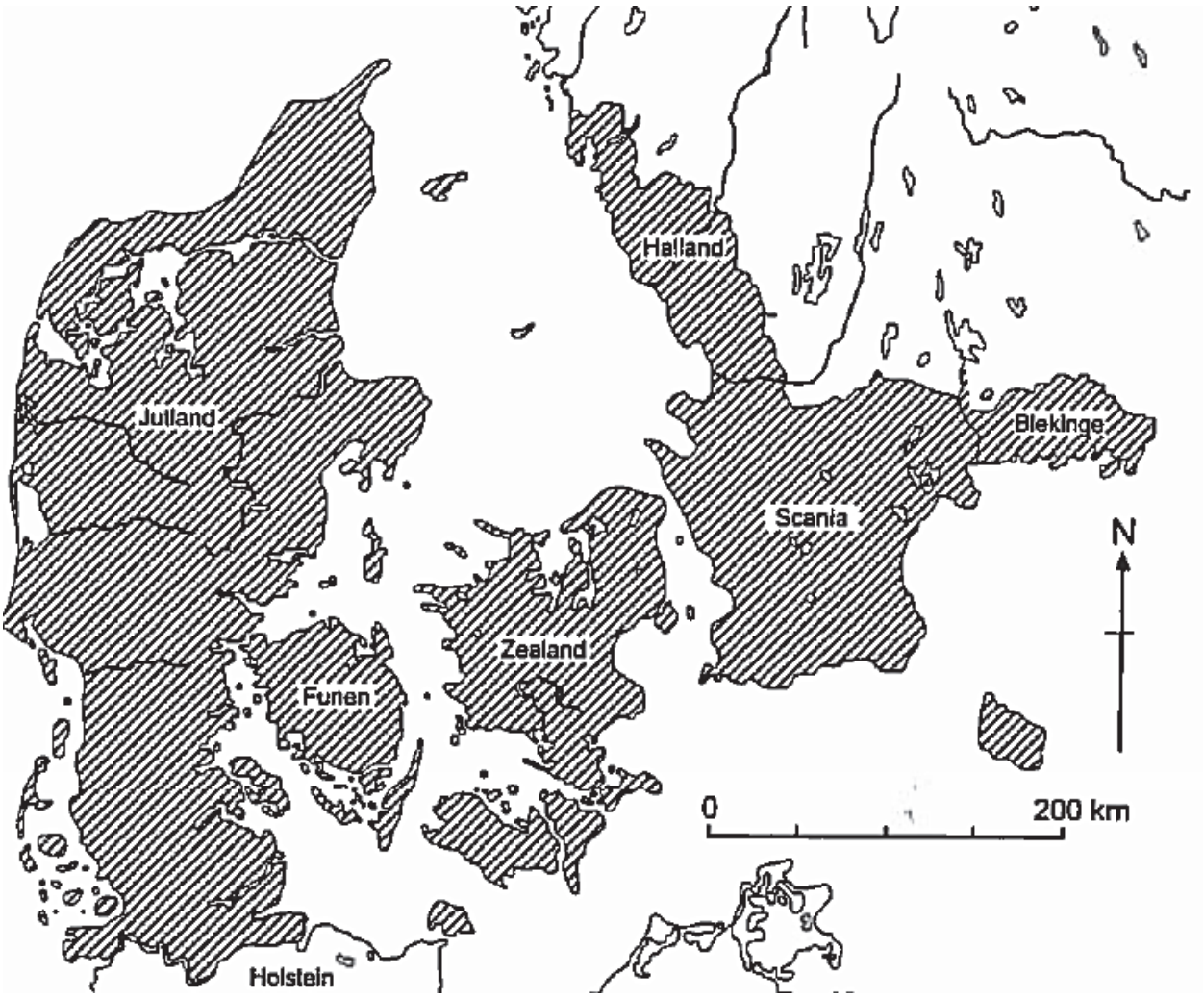
The focus of this study will be on the medieval and post-medieval periods as shown in Table 2.1 below. While the dates of these periods may shift depending on geographic location, the dates selected and discussed here relate to cultural, political and environmental events clearly

demarcated in Denmark's history. The early medieval period begins with the adoption of Christianity at the conclusion of the Viking Age to the beginning of the Great Famine in the early 14th century. The high Middle Ages refers to the period from the Great Famine and the Black Death up until the Protestant Reformation in the early 16th century. This division in the medieval period is historically relevant and warranted specific discussion; however, it would be noted that the medieval skeletal remains cannot be defined within these specific time periods and will be classified as medieval only. The post-medieval period is defined as the short period between the Reformation and when Denmark lost large holdings of land to Sweden in the mid-17th century (Oakley 1972; Hybel and Poulsen 2007; Randsborg 2009; Yoder 2010). Figure 2.1 shows the geographic expanse of Denmark during the medieval period, whereas the post-medieval period saw the loss of Halland, Scania and Blekinge to Sweden.

Table 2.1 Historical Time Periods in Medieval and Post-medieval Denmark

Time Period	**Dates**
Early Medieval Period	AD 1050-1300
High Medieval Period	AD 1300-1536
Post-Medieval Period	AD 1536-1660

Figure 2.1 Map of Medieval Denmark



(Hybel and Poulsen 2007:xxiii; with permission from N. Hybel and B. Poulsen)

2.5 THE EARLY AND HIGH MEDIEVAL PERIOD (AD 1050-1536)

2.5.1 The Emerging Danish Nation during the Middle Ages

At the conclusion of the Viking Age the emerging Danish nation developed under the rule of Svend Estridsen and his five sons who ruled successively from AD 1074 to 1134. It was during the reign of these brothers that the early medieval period experienced the death of King Knud in Odense who had imposed heavy taxes on his people. Following his death, Knud was succeeded by his brother Oluf who was blamed by the people for widespread famine until after

his death in AD 1095. The youngest brother, King Niels finally introduced stability into the early medieval period as Denmark began to flourish from increased shipping and trade (Lauring 1976; Sawyer and Sawyer 1993). Following this was the period of the Valdemars beginning with Valdemar the Great from AD 1157 to 1182. During the rule of Valdemar the Great, order was re-established in Denmark with the help of Absalon, a nobleman's son who eventually gained control over the Danish Church in the later 12th century (Lauring 1976). In AD 1202 when Valdemar the Victorious (2nd) ascended to the throne after his brother's death, there was growing concern with the southern Danish and German border. Concerned with keeping this trading route open, Valdemar made many land conquests to the south. This was eventually retaliated by Heinrich von Schwerin, the Black Count, when he captured Valdemar and his son and held them hostage for three and a half years until Valdemar agreed to a large cash settlement and not to re-invade the northern German provinces (Lauring 1976). Once released, Valdemar rescinded his promise and marched into Germany, but lost and returned to Denmark. While his German expansion was unsuccessful, Valdemar did conquer Estonia during his reign in AD 1219 and also established the Jyllandic Laws, which were the first wide-spread laws in Denmark and remained in place until AD 1683 (Lauring 1976). Similar to the early succession of Sweyn Estridon's sons, Valdemar also had three sons, Eric, Abel and Christopher, who ascended the throne between AD 1241 and 1259. Eric, the eldest was crowned king but his brother Abel rebelled almost immediately, an act which culminated in the burning of Odense and the eventual murder of Eric. Abel ruled for only two years after Eric's murder until the youngest brother, Christopher took the throne. During his reign, Christopher dealt with trouble from northern Germany and the Slesvig region in addition to trouble with the Church and nobles who collectively wanted to curtail the power of the king. In AD 1282 Christopher signed a Royal

Charter putting more power in the hands of the nobility in Denmark; he was eventually murdered in AD 1286 (Lauring 1976; North 2012).

At the end of the 13th century and into the beginning of the 14th century Denmark experienced a financial crisis and severe land disruption at the hands of Eric Menued and Christopher the 2nd. The rule of Eric Menued from AD 1286-1319 created many financial hardships for Denmark with his overspending made worse by Christopher the 2nd who the Danish people distrusted (North 2012). On the brink of financial collapse, the Danish nobles acted without the king and asked for help from Count Gerhard of Rendsberg, a Duke of Holstein to rescue Denmark from financial crisis. Gerhard acted immediately and had the Danish nobility elect his nephew, Count Valdemar, to rule Denmark, effectively having Christopher the 2nd de-throned. Christopher however, did not concede and re-acquired his crown by dividing the control of Danish territory between Count Gerhard, who ruled for his nephew, and Count John of Kiel, an associate of Gerhard's. Once divided, Gerhard controlled Fyn and most of Jylland and John controlled Skåne and Sjælland, Christopher the 2nd at this time had no property as the Danish king (Lauring 1976; North 2012). Eventually, the heavy-handed ruling imposed by Gerhard and John were opposed by the Danish masses and Gerhard was murdered and the German forces were driven out of Denmark. At that time, the son of Christopher the 2nd, Valdemar, was recognized as the rightful heir of Denmark. Valdemar Alterdag ruled Denmark from AD 1340 to 1375, and during his reign he managed to reunite and liberate Denmark, a task that was continued after his death by his grandson Oluf who during his reign was advised by his mother Margaret (Lauring 1976; Sawyer and Sawyer 1993; North 2012).

Known as the 'uncrowned queen', Margaret continued to strengthen the re-united Denmark and for a period also had control over Norway when her Norwegian-born husband

died. With the death of her son Oluf in AD 1387, Margaret continued her political pursuits by adopting her grandnephew who became king under the name Eric of Pomerania. During the rule of Margaret and Eric of Pomerania, Denmark became the center of the Nordic Union (Union of Kalmar) and reclaimed much of its financial strength through the introduction of Øresund dues in AD 1429, creating extensive revenue for the Danish monarchy until its abolishment in AD 1857 (Lauring 1976; North 2012). It was during this period after the rule of Eric of Pomeranian that Denmark again found itself without a proper king and saw the shift to an elected royalty with Christian the 1st who ruled from AD 1448-1481 (Derry 1979; Sawyer and Sawyer 1993). As a young king Christian had both control over Denmark and the Holstein and Slesvig provinces and signed a treaty to keep the provinces forever united. While successfully establishing Danish control over the contentious southern Jylland region, Christian was unable to keep control over the Shetland and Orkney islands as they were given to Scotland as a dowry for his daughter's marriage to King James the 3rd. This lack of money and poor economic forethought characterized the rule of Christian the 1st (Lauring 1976; Derry 1979; Sawyer and Sawyer 1993). In AD 1481, Christian's son Hans ascended the Danish throne and re-established the Nordic Union where he was king of all three countries. During his reign unrest stirred again in the Slesvig and Holstein provinces under the rule of his brother Frederick who attempted to occupy the Ditmarshes region which during Scandinavian history had been continuously occupied by the Frisians who belonged neither to Germany or Denmark (Lauring 1976). During this battle both Denmark and Germany suffered losses; however, the weakness of Hans' army was noted by the Swedish peasants who rose up and successfully removed Hans as their king and broke the Nordic Union once more. At the end of his reign, King Hans passed the crown to his son Christian the 2nd (AD 1513-1523) who, under the guidance of his mother, used Holland as a model of perfection in

Europe and put into place various laws and sanctions to help the Danish peasants and middle class (Derry 1979). Christian the 2nd also successfully re-acquired Sweden and strengthened the Nordic Union. At the close of the high medieval period, Frederick the 1st (AD 1523-1533) and Christian the 3rd (AD 1535-1559) ruled Denmark over a 36 year period which was fraught with religious upheaval and general dissatisfaction within the Holstein and Slesvig provinces. It was this civil unrest that would eventually lead to the Count's War in Denmark prompting the Protestant Reformation in Scandinavia (Lauring 1976; Sawyer and Sawyer 1993).

2.5.2 The Spread of Christianity and Population Growth

The medieval period in Denmark and most of Europe was one of economic, political, demographic, and perhaps most imposing climatic fluctuation that influenced the course of history. Following the decline of the Viking Period after AD 1047, Christianity successfully replaced Pagan belief systems throughout Denmark (Oakley 1972), marking the beginning of the medieval period. This religious conversion, in addition to the growing power of Danish royalty throughout the 10th and 11th centuries contributed to the growth and appearance of new settlements throughout the country as towns and cities became important interconnected strongholds (Andren 1989; Hybel and Poulsen 2007). With the spread of Christianity, churches were built across the landscape with estimates suggesting that at least 2700 parish churches were present in the early part of the medieval period before AD 1300 (Wienberg 1993). Based on the proliferation of these local parish churches, estimates suggest that during the early medieval period, the population of Denmark was roughly one million people (Christensen 1938; Benedictow 1993), with much of this population clustered around the arable land pockets of the country, namely the Skåne plain (now Sweden), most of Sjælland and Fyn, and eastern Jylland (Hybel and Poulsen 2007). While cities and town were growing rapidly during the early

medieval period, particularly between AD 1200-1350 (Hybel and Poulsen 2007), it is estimated that only ten percent of the population occupied these urban environments. Denmark was predominantly agrarian with the majority of the population (approximately 90 percent) remaining part of the rural class until after the 13th century (Benedictow 2003; Yoder 2006). This population growth and expansion during the early medieval period began as early as AD 700 during the Viking Age and levelled-off by the 14th century (Hybel and Poulsen 2007). It was in the early 11th century that the Scandinavian countries began to form as states under centralized Kingship and Church rule with regular taxes established in the early medieval period (Andren 1989). Whereas population growth during the Viking Period prompted the emigration of young males to other countries, the early medieval population growth led to significant settlement expansion and stability within Denmark (Andren 1989; Orrman 2003a).

2.5.3 Emerging Agriculture and Diet during the Medieval Period

Agriculture in Northern Europe was developed around 4000 BC (Hybel and Poulsen 2007) with the first farmers of Denmark immigrating into the region sometime during the New Stone Age which began in 2700 BC (Oakley 1972). During the Middle Ages, subsistence in Denmark was based on both cultivated crops and supplemented by various forms of hunting, fishing and gathering from waste and meadow lands throughout the country (Orrman 2003a; Hybel and Poulsen 2007). Inconsistencies in soil composition across Denmark however, effectively pushed cultivated food production to the clay soils of the east and left the sandy soils of western Jylland for animal husbandry (Hybel and Poulsen 2007). Grain was the most vital crop produced in Denmark throughout the early and high Middle Ages with estimates suggesting that up to 75 percent of the daily diet for adults consisted of grain and grain products (Hybel and Poulsen 2007).

During the early and high medieval periods the primary foodstuffs were agricultural produce such as rye and barley with the majority of the household income being spent on food products (Adamson 2004). First introduced into Denmark at the beginning of the 12th century, rye was grown and used primarily for bread and barley which was most popular for bread and malt and originated from north eastern Germany (Orrman 2003a). It was because of this ability to grow barley and oats in a colder, moist environment that throughout the medieval period Denmark was able to continually export grain and enjoyed similar success to that seen in Western Europe (Orrman 2003a). This success of the grain industry in Denmark was partly dependent on the introduction of specific agricultural technologies including the development of the plough between the 11th and 12th centuries and the introduction of water, wind and horse mills in the 12th and 13th centuries (Hybel and Poulsen 2007). Bread was considered the most cost-effective food where the calories to expense ratio was three to four times higher than for meat (Adamson 2004). While meat was available during the medieval period it was up to four times more expensive, as agricultural land took precedence over livestock habitat, and was mostly enjoyed by the upper tiers of society (Adamson 2004). Most diets during the medieval period were supplemented with garden and wild fruits, nuts and some grasses, likely collected from plots of land outside the urban centres (Hybel and Poulsen 2007). Fish was perhaps the most important dietary supplement as coastal settlements had free access to the water and inland settlements were not far from trading points to obtain salted cod or herring (Sawyer and Sawyer 1993). Additionally, religious sanctions against terrestrial mammal consumption on approximately 135 days each year created more demand for fish, as the Church allowed this dietary substitution on holy days beginning in the 7th century (Hoffman 2001).

The majority of the medieval population survived on two meals a day, a midday dinner and an evening supper. Exceptions to this were the elderly, the sick and children who were afforded small snacks or an early morning meal, as well as some craftspeople who needed additional sustenance to complete their long workday hours (Adamson 2004). For the lowest echelons of medieval society, which was approximately 20-30 percent of the entire population, alms from the rich provided them with basic essentials including various foodstuffs and ale (Orrman 2003b). During years of poor crop yields (i.e. beginning of the 14th century), fluctuating grain prices benefitted townspeople who were able to purchase grain at a reduced price and supplement their diet with secondary goods such as meat and fish (Adamson 2004). Those who were not as fortunate were forced to stretch their grain supply by adding beans or sawdust to their bread flour, made worse by times of extreme famine where grass and dead animals were consumed as a last resort (Adamson 2004). Even in times of sufficient grain supply, contaminants such as fungus were a concern for grains that remained damp before harvest or during storage (Adamson 2004). After the initial onslaught of the Black Death in the high medieval period, those that survived were afforded more meat and animal products such as milk and butter as fallow fields were left for grazing land and prompted an increased interest in animal husbandry (Adamson 2004; Hybel and Poulsen 2007). Overall, the entire medieval period was characterized by the introduction of sophisticated agricultural tools such as the wheel-plough and three-course crop rotation (Orrman 2003b). However, there was a continuous disruption of the food supply for all tiers of society, made worse through the onset of disease and unpredictable weather patterns.

In addition to these natural regulators of the food supply during the early and high medieval periods, religious fasts also regulated much of the quality and quantity of food

consumed by the Danish population (Adamson 2004). As Adamson (2004) discusses, with the onset of Christianity in the western world, fasting was not as wide-spread or as strict as the regulations during the early and high medieval periods. It was in beginning of the 5th century that this focus on food became prominent, as overindulgence was linked with sin, specifically carnal desire. Therefore, fasting represented a way in which to cleanse one's body and soul and to control sexuality and desire (Adamson 2004; Albala 2011); fasting was essentially "a conscience ritual of intentional undernourishment" (Albala 2011:43). Fasting was also used as a tool to unite the religious community where fasting contributed to a sense of belonging (Adamson 2004). While the guidelines of fasting changed over the centuries, by the Middle Ages it was generally agreed upon that no meat from warm-blooded animals could be consumed in addition to dairy products or eggs on designated holy days (Adamson 2004). With religion and religious sanctions playing a pivotal role during the early and high medieval periods, fasting was strictly adhered to with food consumption restricted for almost one third of all calendar days each year (Adamson 2004). Exceptions to this rule were the sick, elderly, and children (Adamson 2004).

2.5.4 Climatic Shift between the Early and High Medieval Periods

Before the 14th century, the early medieval period was characterized by stable weather patterns and higher average summer temperatures that contributed to good harvests and grew the Danish population (Fagan 2000). Known as the "Medieval Warm Period," between the 11th and 14th centuries there was a moderate temperature increase of 0.2 degrees in Europe (Hybel and Poulsen 2007). Scandinavia experienced the beginning of this warmer period in the 11th and 12th centuries, while the remainder of Europe was not until the 13th century (Hybel and Poulsen 2007). Described as the "climatic golden age" (Fagan 2000:21), this period of agricultural proliferation saw one of the warmest periods in Denmark (Fagan 2000). Following this prolific

period of agriculture success and adequate foodstuffs for the steadily increasing Danish population, a climate shift that brought substantial rains in the first decades of the 14th century led to wide-spread famine (Jordan 1996; Fagan 2000; Hybel and Poulsen 2007). Known as the "Little Ice Age," the 14th century ushered in a cooler climate and increased precipitation that lasted until AD 1800 (Fagan 2000). Beginning with poor crop yields in AD 1315, food shortages spread as summer crops were both flattened by torrential rains and failed to ripen properly leading to an insufficient quantity of food and inadequate nutrition (Jordan 1996; Fagan 2000). This first year significantly contributed to widening the gap between agricultural production and demand, a trend that would continue for another six seasons (Jordan 1996). The following year AD 1316 was considered the worst year for cereal crops in all of the medieval period (Fagan 2000). Cereal crops failed as the spring rains kept farmers from planting enough seeds to sustain the population. Estimates suggest that shifting weather trends responsible for the Great Famine devastated over 400,000 square miles of Northern Europe and affected as many as 30 million people, as its continuous presence was felt for a full seven years between AD 1315 and 1322 (Jordan 1996). In addition to poor agricultural yields, livestock also suffered from inadequate food with many left to starve or forage on their own. As a result of these diminishing livestock herds less manure was available to fertilize the fields, contributing to further agricultural hardship (Fagan 2000). While the famine was felt across the Danish landscape and elsewhere in Europe, city inhabitants were perhaps most at risk, as food shortages and the introduction of diarrheal diseases from poor and experimental diets led to disease outbreaks (Fagan 2000). While the beginning of the 14th century marks the beginning of the Little Ice Age in Europe, this period was marked not by constantly colder temperatures, but rather climatic fluctuations that caused warm summers and moderate winters, but also torrential rains and devastatingly cold periods

leading to an inconsistent and unreliable food supply (Fagan 2000; Yoder 2006). By AD 1322 these climatic shifts subsided enough to allow for economic, agricultural and population rebound in Denmark, but this calm was followed closely by the Black Death in AD 1349. This "climatic seesaw" (Fagan 2000:47), and disease devastation contributed to what has been referred to as the Late Medieval Agrarian Crisis (LMAC) (Gissel 1981; Jäger 1981). First discussed by Abel (1976), the LMAC was the result of multiple culminating factors including, large scale land desertion, a decreased workforce, a reduction in grain demand, civil unrest and religious upheaval. During this period there were no new towns established anywhere in Scandinavia (Andrén 1989) as the masses experienced a wide-spread and obvious reduction in the standard of living.

2.5.5 The Black Death

Derived from the Latin phrase, "atra mors," the Black Death is considered the worst "demographic disaster" (Benedictow 2004:3) in human history. With a lethality rate of 80 to 100 percent, the Black Death could kill within a few hours of infection (Benedictow 2004). The colloquial term "plague", derived from the Latin "plaga", originated in northern Europe and was used to describe the symptoms of a stroke (Benedictow 2004). It was in an AD 1349 Norwegian correspondence from King Erik Magnus Eriksson that the first written record of "plaga" appeared in Scandinavia (Benedictow 2004). Contrasting theories have emerged regarding the etiology of the Black Death ranging from the bacteria *Yersinia pestis* to haemorrhagic fever filoviruses (Yersin 1894; Scott and Duncan 2001). While Benedictow (2004) argues that fleas found on rats were the main transmitter of the Black Death, the epidemiology of this transmission system is based on the contemporary strain of the *Yersinia pestis* bacteria and too slow for what was experienced in Europe in the Middle Ages. As Scott and Duncan (2001)

argue, a direct person-to-person contact system would have been necessary to spread the Black Death as fast as it did and over great distances in a relatively short period of time; the long incubation period in the flea-rat-human system would not have sustained the disease. This debate is also present at a molecular level where Drancourt et al. (1998) and Raoult et al. (2000) were able to extract *Yersinia pestis* DNA from the pulp cavity of teeth, but Thomas et al. (2004) showed no evidence of the bacteria in any confirmed archaeological plague victims. Considering this etiological debate, it is likely that the Black Death was carried into Denmark by ship from Southern Norway into the coastal province of Halland in the summer of AD 1349 (Sawyer and Sawyer 1993; Benedictow 2004). However, arguments have been made that the disease could have also been introduced into Halland from Oslo in Norway, Idd in south eastern Norway or even from eastern England (Benedictow 2004). Once in Denmark, the Black Death immediately invaded the southern province of Skåne, and by AD 1350 was present in Jylland to the far west, evidenced through a sharp increase in mortality rates in church records (Benedictow 2004). Similar to the arguments made by Scott and Duncan (2001) and Cohn (2002), the Black Death spread quickly throughout Denmark and likely would have needed human-to-human contact to maintain its virulence, strengthening the virus argument for this disease. After the initial devastation of the Black Death in the middle of the 14th century, there were smaller resurgences of the plague in the 1360s and late 1370s in Lund, Roskilde, and Ribe (Hybel and Poulsen 2007). These later outbreaks of the disease, while less fatal than the initial strain of the Black Death, still contributed to the population deficit which did not fully recover until the beginning of the 19th century (Hybel and Poulsen 2007).

2.5.6 Early and High Medieval Population Demography

During the early medieval period life expectancy was low as poverty "was a permanent feature of the Middle Ages" (Mollat 1986:1) and contributed to poor housing, inadequate clothing, disease outbreak, substandard hygiene practices and malnutrition (Mollat 1986; Livi-Bacci 1991; Hybel and Poulsen 2007). From the early 11th century onwards there was an increase in population size in Denmark which introduced economic strain and resultant poverty for the lowest income families (Mollat 1986; Benedictow 2003; Youngs 2006). This urban expansion also contributed to an increase in epidemics that created "severe demographic stress" (Petersen et al. 2006:114). Regular intervals of disease outbreak would have been commonplace in these medieval urban communities resulting in a population decrease and an inability to recover as these diseases would have affected adolescents and young adults in their reproductive years (Petersen et al. 2006). As Paine (2000) discusses, in normal contemporary populations the risk of dying is highest during the initial phase of life with a steady decline into adolescence; however, this pattern is not always reflected in archaeological populations, where a large proportion of older children, adolescents and young adults may enter the skeletal record. For example, in the medieval St. Mikkel Viborg sample in Denmark survivorship was reduced in the older children and adolescent groups suggesting these urban individuals were exposed to specific conditions driving this unexpected mortality patterns, like periods of disease outbreak (Petersen et al. 2006).

This is not to say that these medieval populations were not experiencing a similar mortality pattern as that seen within contemporary populations, but these expected patterns may have been masked by larger trends driven by long term catastrophic episodes (Paine 2000). For example, infants would have been particularly vulnerable due to obstetrical complications,

disease and infection, and malnutrition living in these urban environments (Palubeckaitė et al. 2002; Youngs 2006); however, the demographic profile of Danish populations at this time (Paine and Boldsen 2002) do not adhere strictly to this expectation suggesting that factors influencing mortality were multi-dimensional and could affect any age group within a medieval population.

For adult men and women there was some disparity in life expectancy with women experiencing higher mortality rates between 20 to 35 years and men between 35 to 50 years (Sellevold 1989a, 1989b; Sawyer and Sawyer 1993). This elevated risk of dying for females in this younger age category was likely the result of childbearing complications or post-partum infection (Sawyer and Sawyer 1993; Petersen et al. 2006; Boldsen 2007). However, for both men and women adult mortality was highly dependent on morbidity patterns seen earlier in life. Boldsen (2007) and DeWitte and Wood (2008) both discuss how individuals that experienced early life stress events were more likely to have higher mortality rates in adulthood as the effects of early life stress may predispose an individual to increase frailty (Palubeckaitė et al. 2002; DeWitte and Wood 2008) or may activate differential frailty already biologically present (Boldsen 2007). Further, sexual buffering may have also impacted these frailty and visible mortality patterns. Sexual buffering is the concept that females are less biologically vulnerable to stress insults than males producing a health gradient between the sexes (DeWitte 2010). However, cultural influences and the historical preference for males or females may skew this biological vulnerability and observed mortality rates. For example, when looking at victims of the Black Death, DeWitte (2010) illustrates that males show higher vulnerability than females even when both sexes are predisposed to the plague based on early life stress events. Looking specifically at the medieval Danish Tirup skeletal sample, Boldsen (2007) found that mean life expectancy rates were 20 years for females and 25 years for males, with less than 50 percent of

the population reaching these ages. However, of the individuals who did reach early adulthood, up to 20 percent survived until the 8th decade of life (Boldsen 2007). This low life expectancy was fairly constant throughout the medieval period with a slow improvement during the post-plague period likely the result of decreased population density and better access to resources (Benedictow 2003), improved hygiene and better living conditions (Youngs 2006), and a shift in socioeconomics with an increased reliance on animal husbandry and meat products (Benedictow 2003; Hybel and Poulsen 2007). Despite these improvements however, it was not until the mid-18th century that life expectancy had risen into the fourth decade of life in Denmark (Benedictow 2003).

2.5.7 Cattle Farming

The importance of cattle farming in Denmark was not full recognized until after the mid-14th century when the decimation of widespread famine and the Black Death had passed (Hybel and Poulsen 2007). In the high Middle Ages Denmark experienced land desertion on a large scale in part due to poor weather conditions in the early 1300s and further exacerbated by the population decline after the Black Death period in the mid-14th century (Hybel and Poulsen 2007). Animal husbandry has always been a part of the Danish landscape since the Neolithic period (Randsborg 2009), particularly in rural environments, and while some larger-scale cattle rearing had begun in the 12th and 13th centuries on deserted land, it wasn't until after AD 1350 there was a wide-spread economic interest in animal husbandry, specifically cattle breeding. While cattle had been used in the early medieval period for dairy, the high Middle Ages were characterized by cattle breeding for meat (Hybel and Poulsen 2007), an industry that flourished as cattle had access to large plots of grazing pasture on deserted land (North 2012). Despite the population decline experienced after the Black Death, animal husbandry thrived as less labour

was required than agricultural pursuits and more land was available (Randsborg 2009). These lands desertions were not equally distributed across Denmark with the western province of Jylland experiencing the most vacancies beginning in the late 1300s and culminating in the late 1400s, making western Denmark the seat of cattle production (North 2012). Where an average farmer may have had 3-4 cows during the early medieval period, at the end of the 15th century a peasant farmer could have had as many as 15 cattle in their herd (Hybel and Poulsen 2007). One benefit of this cattle expansion was a possible shift in dietary patterns where the consumption of meat and dairy products in the early part of the 15th century may have risen (Hybel and Poulsen 2007). As Denmark's reliance on cattle breeding expanded, trade of meat, dairy products and cattle was also increasing, particularly in Germany (Dahlbäck 2003). By the early 15th century salted meat and cattle hides were being heavily traded, with markets becoming saturated with these products around AD 1450 (Hybel and Poulsen 2007). At the height of production, Scandinavia was considered the third major supplier of cattle and cattle products in Europe (Hoffman 2001). This steady increase in cattle production intensified across all of Denmark, replacing the previously successful horse breeding industry between AD 1100 and 1300 (North 2012). The pinnacle of this cattle trade and production was in the early to mid-1500s, where approximately 25,000 heads of cattle were driven south annually into northern Germany a trend that continued well into the post-medieval period (Dahlbäck 2003; Hybel and Poulsen 2007).

2.6 THE POST-MEDIEVAL PERIOD (AD 1536-1660)

2.6.1 Political Unrest and Shifting Borders

During the post-medieval period Denmark experienced substantial agricultural change, a new investment in trading opportunities, and a renewed interest in urban towns; this period was also marked by political struggles and changing geographic boundaries (Randsborg 2009). This

shifting of Danish land rites was politically charged as the post-medieval period was continually plagued with conflict between Denmark and Sweden that had begun with the establishment of the Nordic Union in AD 1397 (Derry 1979). After the Nordic Union was established in an attempt to centralize power and strength equally between all three countries (Norway, Sweden and Denmark), Denmark became increasingly identified as the central authority and claimed land rights in Norway, Finland and even Sweden for a period, creating a tumultuous relationship resulting in constant conflict through the late and early post-medieval periods (Derry 1979). This animosity reached its peak in the 17th century when Denmark almost lost its independence at the hands of Sweden as land was being constantly traded between various ruling monarchs (Randsborg 2009). While first established under the rule of Margaret, the Nordic Union from the Middle Ages into the post-Reformation period was a constant struggle for the Danish monarchy and rebellious peasants (Derry 1979). In addition to these external threats, Denmark also experienced civil war at the dawn of the Reformation that arguably ushered in this new religious reform (Oakley 1972). Known as the Count's War, this Danish civil war was the result of feuding Catholic and Protestant beliefs which rallied the Catholic masses against the largely Protestant nobility. During these two years of civil war, proponents of Christian the 2nd with the support of Count Christoffer of Oldenburg tried to restore the crown to the rightful heir, while the nobility of Denmark, worried by the rebellious peasant masses, elected Christian the 3rd a known Lutheran to fight against this Catholic rebellion (Lockhart 2004). As the war raged across Denmark with the Protestant side gaining ground, this civil unrest eventually reached the island of Fyn in June AD 1535 (Lockhart 2004). Despite Christian the 2nd supporters resisting in Copenhagen and Malmö, eventually they surrendered and Christian the 3rd was appointed as king of Denmark (Lockhart 2004). With Christian the 3rd as the new leader of Denmark the nobility

who had helped his cause in the Count's War were granted social advantages and new land claims, while the Catholic peasant groups of Denmark experienced increased economic and social sanctions (Lockhart 2004). Because of the close relationship between Denmark and Northern Germany, Protestantism was inevitable in Scandinavia as the demand for religious reform continually grew after Martin Luther posted his 95 theses in Wittenberg, Germany (Oakley 1972). It was the Count's War that led to this widespread change, as the rule of Christian the 3rd marked the beginning of Denmark's Golden Age receptive to cultural maturation, social engagement and religious reform (Randsborg 2009). Following the rule of Christian the 3rd in AD 1559, Frederick the 2nd enjoyed a relatively stable rule of Denmark with his political gain over the island of Øsel and a fruitless seven year war against Sweden from AD 1563-1570. The purpose of this war was to re-establish the Nordic Union, but in reality created greater animosity between these warring nations (Randsborg 2009). At the conclusion of the reign of Frederick the 2nd in AD 1588, Christian the 4th became the king of Denmark. As the king of both Denmark and Norway (Dano-Norwegian Kingdom), Christian the 4th enjoyed the benefits of sole control over the Baltic via the Øresund which separated the Sjælland and Skåne provinces. With increased trade and traffic through the Øresund, the second half of the 16th century was prosperous for Denmark (Oakley 1972; Randsborg 2009). It was during this period that urban control switched to Copenhagen and Malmö as the Øresund was the "commercial link" between the West and East of Europe (Andren 1989:602). As a strictly Protestant entity, the Dano-Norwegian Kingdom was called to fight during the 30 Years' War when the Catholic enemy began defeating Protestant forces. As one of the first countries to engage in the 30 Years' War, Denmark attempted to quell the Catholic forces of northern Germany in AD 1625 but failed (Anderson 2011). After this defeat in the 30 Years' War, unrest was still present in this early

post-medieval period as the Torstenson War began in AD 1643 when Sweden with the help of the Dutch simultaneously invaded Jylland in the west and the Skåne province in the east (Randsborg 2009). This defeat at the hands of the Swedes cost Denmark the islands of Gotland and Øsel, as well as Norwegian territory. Denmark was also forced to cede the Skåne and Halland provinces for 30 years (Randsborg 2009), marking the end of the early post-medieval period in Denmark.

2.6.2 Post-medieval Population Demography

Within European society, the post-medieval period was dominated by the peasant class, making up approximately 85 percent of the population with the remaining 15 percent of society being represented by the middle class and nobility (Anderson 2011). While the population in Scandinavia slowly recovered from the high medieval decline to approximately 1.4 million people in the late 1500s, mortality rates were still high among children reaching as much as 50 percent in rural regions (Anderson 2011). Despite relatively stable weather patterns and a return to agricultural success with wheat, rye and barley crops, the cattle production success of the early 16th century was challenged by outbreaks of the cattle plague in AD 1518 and 1559. These plague outbreaks diminished livestock herds and consequently led to a rise in unemployment and an increase in the urban poor population (Anderson 2011); this urban influx lead to overcrowding conditions similar to that experienced in the early medieval period (Anderson 2011). There was an overall decline in the Danish population in the post-medieval period likely a hangover from the events of the high Middle Ages leaving the Danish population at an estimated 825,000 by AD 1645 at which point settlement and growth began to rebound (Randsborg 2009; North 2012).

2.6.3 Diet during the Post-medieval Period

While many of the staple food items remained the same from the medieval into the post-medieval period, two important aspects changed: 1) meat consumption and cattle products were more readily available, and 2) new foodstuffs were being introduced into Europe from the new world after AD 1520 (Anderson 2011). Bread, similar to the medieval period, was the staple food item in all of Europe during the post-Reformation period. The majority of breads for the peasant classes were made from rye and barely grains with the upper classes and nobility enjoying more refined and less coarse grains (Anderson 2011). New items being introduced into Europe included tomatoes and potatoes. Because potatoes were a hardy vegetable and could be grown in various climatic conditions, they became widely used in the late 16th and 17th centuries (Anderson 2011). The expansion of European markets to include various foreign foodstuffs also contributed to shifting dietary patterns where new spices such as saffron, cinnamon, ginger and cloves could be added to recipes changing the way food was prepared and consumed (Anderson 2011). During the 16th century the prominence of wine and ale was also marked as only children enjoyed non-alcoholic beverages and the remainder of the population consumed homemade ale or imported wine, as water was deemed unfit to consume (Anderson 2011). Fish was also an important dietary item during the post-medieval period as herring from the North Sea established Aalborg as an important commercial trade city distributing herring along the Danish coast into the Baltic region (North 2012). Despite the introduction of new food items and larger commercial pursuits, the post-medieval period was not immune to famine or disruptions of the food supply. Between AD 1594 and 1597 another widespread famine swept through much of Europe due to shifting weather patterns (Fagan 2000; Hybel and Poulsen 2007). In addition to

the shifting availability of food and the introduction of foreign items, new religious considerations about food were also ushered in during the post-Reformation period.

During the century leading up to the Reformation in Denmark, there was marked increase in public interest in the Church with rebellion against the ridged sanctions of Catholicism (Randsborg 2009). Not surprisingly, as King Christian the 3rd demanded Protestantism become the religious doctrine for all of Danish society, there were changes in the public's opinion of certain religious practices, namely fasting (Benedictow 2003). Considered a devotional act, fasting during the medieval period was well-entrenched; however, with the crumbling of Catholicism in the mid-16th century in Scandinavia, individuals began to question the necessity of fasting to gain salvation (Albala 2011). Fasting was generally opposed by the newly reformed Protestants due to the poor management of this religious sanction during the medieval period; specifically the lack of consistency and enforcement of food restrictions, the ability to obtain a dispensation from fasting and, the different rules in place from region to region (Albala 2011). It was because of this increasing discontent within the general population that fasting was reassessed by Pope Alexander the 6th who lowered restrictions on foodstuffs such as butter and increased the number of exceptions that were granted throughout the year (Albala 2011). Once these new rules were ushered in by Pope Alexander in AD 1491, the growing Protestant population questioned why other similar foodstuffs could not also be reintroduced and what the benefit of fasting was if the rules could be changed so easily (Albala 2011). As Protestantism grew throughout Europe, fasting became more of a private, voluntary matter where individuals could decide which foodstuffs they would or would not consume during specific calendar days (Albala 2011).

2.7 SUMMARY OF THE MEDIEVAL TO POST-MEDIEVAL PERIOD IN DENMARK

The early medieval period in Denmark saw the establishment of new towns and the growth and evolution of agriculture (Hybel and Poulsen 2007). Characterized by relatively stable weather the early medieval Danish population mainly subsisted off of a grain diet and local foodstuffs (Fagan 2000; Adamson 2004). Despite these stable climatic trends and adequate food for the majority of the population, this period also experienced low life expectancy, especially for infants and children (Youngs 2006). In contrast, the high medieval period in Denmark was marked by the beginning of the Little Ice Age which brought unpredictable weather patterns leading to wide-spread famine in the first decades of the 14th century (Jordan 1996; Fagan 2000). This climatic devastation was promptly followed by the Black Death which decimated nearly 50 percent of the Scandinavian population (Benedictow 2004). The sharp population drop resulting from these high medieval disasters led to a socioeconomic shift in Denmark where cattle production became important for trade in larger European markets (Hybel and Poulsen 2007). Towards the end of the high medieval period Danish diets were likely influenced by the expanding meat and dairy trade that was flourishing at this time; however, the primary grain staple of the diet remained the same throughout the medieval and post-medieval periods (Anderson 2011). Despite the devastation of the Black Death and the early 14th century famine period, those who survived in late medieval period likely had better over health as resources were more abundant due to the sharp decrease in the Danish population.

The post-medieval period was mainly characterized by an increase in urban expansion due to diminished agricultural work which introduced new concerns such as pathogen exposure from over-crowding and food contamination (Anderson 2011). This urban expansion would have also created specific strains on the food supply chain as more and more foodstuffs would have

needed to be brought in from the countryside to feed the expanding urban population. Changing religious beliefs also contributed to the post-medieval diet as fasting rules became more lenient and the population was allowed to take control of their own food consumption habits during holy days (Albala 2011). Despite this economic prosperity however, the post-medieval period was also marked by continual conflict with Sweden resulting in the loss of agricultural land needed to feed the expanding population (Hybel and Poulsen 2007). While the medieval period would have been host to many fluctuations in health, the post-medieval period experienced mainly conditions of extreme hardship that ushered in the Reformation and continued steadily through the 16th and early 17th centuries.

CHAPTER 3: LITERATURE REVIEW – OSTEOLOGICAL CONTEXT

3.1 INTRODUCTION

This chapter will focus on an in-depth discussion of skeletal structure, bone metabolism, bone cell dynamics and the influence of stress on these processes. Specific attention will be paid to the osteological indicators of stress in the skeleton and their use to assess health and well-being in past populations.

3.2 SKELETAL STRUCTURE AND GROWTH

Bone is the foundational tissue of the human body, providing the necessary structure and support to all overlying and interconnected biological systems (Rosenfield 1996). Human bone is comprised of both organic and inorganic materials and when fully mineralized has a composition of 20 percent water, 35 percent organic materials and 45 percent inorganic materials (by weight) (Carter and Beaupré 2001; White and Folkens 2005). The structural protein collagen makes up nearly 90 percent of the organic component of bone, with hydroxyapatite (bone mineral comprised of calcium carbonate and calcium phosphate) making up the majority of the inorganic component of bone (Carter and Beaupré 2001). While bone development begins in utero, this dynamic tissue is continually modelled and remodelled in size and shape throughout the lifetime of an individual, with primary bone mineralization occurring during the growth and development period (Leonard and Zemel 2004). This initial mineralization of bone is referred to as modelling, where bone cells lay down the primary scaffolding of the skeletal system to maintain bone shape and mass during the growth and development period. After this primary modelling of bone has occurred, bone remodelling is required to maintain calcium homeostasis within the skeleton and to repair any bone that may be damaged (Buckwalter et al. 1995; Giustina et al. 2008). This

modelling and remodelling process continues throughout life with 10 percent of the entire skeleton being turned over every year (Parfitt 1977).

3.2.1 Osteoblast Cells and the Creation of Bone

All skeletal elements are comprised of three types of bone cells that form the foundation of skeletal integrity, osteoblasts, osteoclasts, and osteocytes, which form, remodel and maintain bone, respectively (Vaughan 1975). Osteoblasts are the primary bone cells that begin to differentiate in utero and are responsible for bone formation and deposition (Ducy et al. 2000; Carter and Beaupré 2001). Osteoblasts contribute to the formation of both the organic and inorganic components of bone through the production of type 1 collagen and through the regulation of calcium and phosphate (Manolagas 2000). It is through this type 1 collagen synthesis that the osteoblast cells secrete osteoid and other proteins creating the primary bone structure that will eventually become mineralized (Kronenberg 2003). The average lifespan of an osteoblast cell is approximately 3 months (Parfitt 1994), and within this lifespan all osteoblast cells will be a part of a basic multicellular unit (BMU) (Manolagas 2000). A BMU is made up of both osteoblasts and osteoclasts and maintains skeletal equilibrium as bone is equally deposited and resorbed by these units (Manolagas 2000). Not only do osteoblasts play an imperative role in bone formation, mineralization, and remodelling, but are also responsible for the synthesis and secretion of molecules required to stimulate the production of osteoclast cells (Ducy et al. 2000). Despite the similar structure of osteoblasts and osteoclasts and their working relationship within BMUs, these cells stem from different precursors that dictate their unique metabolic function (Manolagas 2000).

3.2.2 Osteoclast Cells and Bone Remodelling

Osteoclasts are primarily identified as bone resorbing cells, but also play an important role in bone formation and the regulation of overall skeletal mass (Rifken and Gay 1992). Despite the resorptive function of these cells, osteoclasts are imperative for the successful growth and development of an individual, as their resorbing function ensures that all skeletal elements maintain an optimal calcium composition, and that damaged bone is removed and replaced by new bone (Giustina et al. 2008). It is well understood that osteoclasts participate in a multistep process during their functional lifespan. This process begins with the establishment of osteoclast cells within the embryonic skeleton, followed by a maturation sequence into adult cells that begin their resorptive duties within BMUs throughout the skeleton (Teitelbaum 2000). Similar to osteoblasts and their production of osteoid, osteoclast cells also manipulate their extracellular environment during bone resorption. This extracellular environment is created by the unique membrane feature only visible in osteoclast cells during the bone destruction process (Teitelbaum 2000). Upon recognizing degraded bone, an osteoclast cell will situate this unique membrane between itself and the degraded bone. Once this extracellular environment becomes sealed, the osteoclast cell begins the process of acidification that demineralizes both the organic and inorganic components of the bone (Blair et al. 1986). How the osteoclast recognizes when to halt this destructive process is not as well understood, but may be controlled by receptors that indicate calcium levels and essentially cause the osteoclast to switch-off the demineralization process when the older, calcium-deprived bone has been successfully removed (Väänänen and Horton 1995).

3.2.3 Osteocyte Cells and Bone Maintenance

The metabolic function of osteocyte cells is far less understood; however, there is general agreement regarding their basic maintenance and communication function in bone (Vaughan 1975). Osteocytes are the most abundant bone cells within the skeleton (Parfitt 1977), and have a considerable lifespan of approximately 20 years (Weinstein et al. 1998). Osteocytes, sometimes referred to as "sensor cells," are considered maintenance cells because of their ability to communicate and dictate the function of osteoblast and osteoclast cells (Aarden et al. 1994). While osteoblasts and osteoclasts are created from precursor cells, osteocytes are created directly from osteoblast cells that become encapsulated within the boney matrix of the forming skeleton (Carter and Beaupré 2001). Within each BMU osteoblasts are organized into rows and create new bone in a linear pattern; however, during the bone formation process, some osteoblasts become trapped within the boney matrix being created by surrounding cells. Once these osteoblasts become encapsulated, they begin to differentiate into osteocytes with distinct cellular bodies housed in the lacunae and cytoplasmic processes housed in the canaliculi (Noble et al. 1997). This cellular organization of osteocytes effectively connects all cells to one another through the canaliculi, including osteoblasts and osteoclasts, creating a communication network where the osteocyte maintains homeostasis within the skeleton (Aarden et al. 1994; Manolagas 2000). In order for the skeleton to maintain homeostasis, there must be cohesion between all three cell types; therefore, once one osteoblast cell has begun to differentiate into an osteocyte cell, an immature osteoblast cell will differentiate into a mature cell and will replace the old osteoblast in its former position within the BMU (Palumbo et al. 1990).

3.3 BONE MINERALIZATION

All skeletal elements are built upon a framework established by the embryonic mesenchymal cells during the initial stages of skeletal formation (Wagner and Karsenty 2001). In utero, these mesenchymal cells cluster together to form the structural precursors of the entire skeleton (Wagner and Karsenty 2001). The mesenchymal cells that precede all skeletal elements migrate from three distinct cell lineages where they will differentiate into either chondrocytes (cartilage cells) or osteoblasts (Hall and Miyake 2000; Olsen et al. 2000). The craniofacial skeleton is created through cranial neural crest cells that migrate from the neural tube; the axial skeleton is created through paraxial (somatic) mesoderm cells, and the appendicular skeleton is created by cells from the lateral plate mesoderm (Bronner-Fraser 1994; Tam and Trainor 1994; Cohen and Tickle 1996; Cooper 2004). Once established, these skeletal precursors will either contribute to intramembranous or endochondral ossification (Scheuer and Black 2000).

3.3.1 Intramembranous Ossification

As the primary mode of skeletal mineralization, intramembranous ossification begins in utero and continues throughout life during the bone remodelling process (Scheuer and Black 2000). During intramembranous ossification, mesenchymal cells differentiate directly into osteoblast cells in a one-step process where ossification begins immediately after cellular differentiation. While intramembranous ossification continues throughout life as bone is continually remodelled without a cartilage precursor, the primary bones that form through this type of ossification are the bones of the cranial vault, the face, and many elements of the thorax (Scheuer and Black 2000). It has been suggested that primary skeletal elements derived from this type of mineralization are imperative for protection and survival immediately after birth,

particularly the bones of the cranial vault to protect the brain, and the bones of the face and thorax to facilitate feeding and respiration (Holden 1882).

3.3.2 Endochondral Ossification

Endochondral ossification occurs in the majority of skeletal elements; particularly the long bones with all elements being preceded by a cartilage matrix (Vaughan 1975). In utero, mesenchymal cells that cluster to form the endochondral skeleton differentiate into chondrocytes creating a cartilage matrix that will eventually mineralize. Once created, these matrices will be mineralized by the osteoblast cells, those mineralized first in utero are referred to as the primary ossification centers, where matrices mineralized after birth are known as secondary ossification centers (Scheuer and Black 2000). The primary ossification centers make up the majority of the skeleton, with secondary centers being represented by the hands and feet, all long bone epiphyses and elements of the thorax. While the primary and secondary ossification centers create the skeletal template, it is the growth plates located between these templates that allow for the linear growth of the skeleton (van der Eerden et al. 2003; Giustina et al. 2008).

3.4 BONE GROWTH

3.4.1 Growth Plates and Linear Growth

The growth plate is comprised of three zones of well-organized specialized chondrocyte cells: the resting zone, the proliferative zone and the hypertrophic zone (Giustina et al. 2008). The resting zone is the furthest zone from the diaphysis and where chondrocytes replicate slowly before moving into the proliferative zone (Hunziker 1994). The proliferative zone is characterized by an increase in cell replication followed by the hypertrophic zone located directly along the bone diaphysis (Nilsson et al. 2005). Once cells have entered into this hypertrophic zone they begin to increase in size and move into the extracellular matrix along the

diaphyseal border, where these hypertrophic chondrocytes are able to attract blood vessels, bone cells, and then begin to calcify the boney matrix. With the attraction of osteoblasts and blood supply, hypertrophic chondrocytes begin their natural cell death as bone mineralization continues (Nilsson et al. 2005). This process of laying down new layers of bone along the proximal and distal diaphyseal borders is what contributes to linear growth.

3.4.2 Appositional Growth

While bone length is obtained through the delicate balance between chondrocyte proliferation and osteoblast mineralization in the growth plates, bone mass is increased through the interaction of osteoblast and osteoclast cells during appositional growth (Vaughan 1975). Appositional growth is described as the process in which bone modelling and remodelling creates an increase in bone width or girth (White and Folkens 2005). Shortly after the appearance of primary ossification centers, skeletal elements are surrounded by a periosteum which is a protective sheath that covers the cortical bone (Buckwalter et al. 1995). Within this protective layer the osteoblast cells line up along the outer surface of the bone and begin to secrete osteoid to be mineralized, effectively increasing bone width (Brainerd Arey 1965). During this bone formation process on the outer surface of the bone, a similar destructive process occurs on the endosteal surface as osteoclasts resorb bone. Essentially as the osteoblasts are laying down new skeletal tissue on the outer surface, bone is being equally removed from the inner surface allowing for bone width expansion without increased cortical bone thickness (Buckwalter et al. 1995).

3.4.3 Hormonal Influence on Skeletal Growth

In addition to the cellular component of bone growth, there are also specific hormones that contribute to skeletal maturation, particularly growth hormone (GH) and insulin-like growth

factor 1 (IGF-1). GH and IGF-1 are closely related metabolic hormones that work together to maintain skeletal homeostasis (Giustina et al. 2008). GH is synthesized through a variety of central and peripheral signals within the body as well as through input from sex hormones and thyroid hormones (Giustina et al. 2008). GH is secreted for the majority of the lifecycle and is usually complete after 60 years of age (Giustina et al. 2008). GH stimulates the growth of cartilage during longitudinal bone growth and can either act directly on the chondrocytes or in a more systemic capacity through interaction with IGF-1 (Ohlsson et al. 1998). Up until the 1980s it was believed that GH worked in a systemic capacity only, rather than directly at the site of the growth plate; however, this theory was abandoned when research showed that injecting GH directly into the growth plate will stimulate chondrocyte proliferation and increase growth (Isaksson 1982). GH has a direct effect on the proliferation of osteoblast cells in developing bone, but has also been shown to affect the mesenchymal precursor cells by promoting osteoblastogenesis and chondrogenesis (Giustina et al. 2008). In addition to the function of GH on skeletal growth, GH also directly impacts IGF-1 as GH stimulates the synthesis and secretion of IGF-1 via the liver. Once synthesized, IGF-1 functions at both a local level and a systemic level contributing to bone growth (Giustina et al. 2008). Similar to GH, IGF-1 directly impacts bone formation and osteoblast cells by increasing collagen synthesis and decreasing collagen degradation (Ohlsson et al. 1998). It is through this intricate hormonal system and a detailed understanding of the skeletal maturation process that the impact of stress can be identified and linked to changes in skeletal structure.

3.5 THE STRESS RESPONSE

First introduced into biological research in the 1930s, “stress” is defined as any perceived or actual disruption of biological homeostasis caused by either physiological or psychological

factors (Selye 1936; Selye 1973; Charmandari et al. 2005; Chrousos 2009). In reaction to this perceived or actual disruption, the body undergoes what is referred to as the "stress response," also known as the "fight or flight" response, which signals multiple physiological and behavioural responses via the central nervous system (Cannon 1914). The "stress response" is a time limited response that promotes survival by increasing the function of primary systems (i.e. heart rate, respiration, and energy release) and halts secondary systems (i.e. reproduction, digestive function, and growth) (Chrousos and Gold 1992; Habib et al. 2001); this regulation is accomplished through the release of glucocorticoids (Tsigos and Chrousos 2002).

Glucocorticoids are endogenous steroids released by the hypothalamo-pituitary-adrenocortical (HPA) axis and are present in nearly all cellular and physiological systems (Herman and Cullinan 1997; Charmandari et al. 2004). When the stress system is activated the body releases these steroids to fulfil two functions: 1) to alert the individual to the stress, and 2) to preserve homeostasis (Herman and Cullinan 1997). Glucocorticoids are an essential component of normal bone metabolism and growth; however excessive levels of these steroids, produced during times of prolonged or chronic stress, can have a detrimental effect on skeletal tissues (Charmandari et al. 2004; Miller et al. 2007; Henneicke et al. 2011)

3.5.1 Skeletal Stress and Glucocorticoids

Glucocorticoids have been studied extensively in a clinical context for their adverse effects on bone (Manelli and Giustina 2000; Weinstein 2001; Rehman and Lane 2003), with these negative effects first recognized 80 years ago by Harvey Cushing (1932). While many of these studies focus on the effects of pharmaceutical glucocorticoids, the natural endogenous form of glucocorticoids has also been shown to have a negative effect on skeletal growth and development (Charmandari et al. 2005). The relationship between endogenous and exogenous

glucocorticoids has, however, shown some disparity with the endogenous form seemingly less potent than the manufactured form (Henneicke et al. 2011). Despite this disparity, endogenous glucocorticoids circulating at an elevated level for a prolonged period can still produce skeletal changes (Dennison et al. 1999). Additionally, bone type has also been shown to react differently to glucocorticoid saturation with differing chemical signatures reflecting the heterogeneous nature of cortical and trabecular bone (Ninomiya et al. 1990; Weinstein et al. 1998). Within this heterogeneous framework, interconnected skeletal segments have also demonstrated differing sensitivity to the effects of glucocorticoids with the axial skeleton considered more vulnerable to growth disturbances than then appendicular skeleton (Weinstein et al. 1998). Essentially, these tissue specific vulnerabilities to glucocorticoids can be related to receptor prevalence and location, differing osteoblast frailty (Henneicke et al. 2011) and unique governing mechanisms of the metabolic process in different skeletal tissues (Ninomiya et al. 1990). The negative effects of glucocorticoids on bone metabolism are primarily through their ability to disrupt the differentiation and proliferation of osteoblast cells (Cooper et al. 1999). This disruption includes i) a reduction in osteoblastogenesis and the creation of new bone cells, ii) an increase in premature cell death, and iii) an increase in osteoclast lifespan and their functionality (Manelli and Giustina 2000: O'Brian et al. 2004). This negative effect of glucocorticoids on osteoblast cells can occur during any phase of the cell cycle, affecting precursor cells in utero and mature cells throughout the life course (Cooper et al. 1999). Glucocorticoids have also been shown to alter the function of bone cells, specifically inhibiting the production of type 1 collagen in osteoblast cells (Canalis and Delany 2002) and disrupting the differentiation of mesenchymal cells into mature osteoblast cells during skeletal maturation (Cooper et al. 1999). An excessive release of glucocorticoids has also been shown to interfere with the bone demineralization

process as osteoid remains unmineralized and, therefore, stimulates the removal process carried out by the osteoclast cells (Chambers and Fuller 1985; Cooper et al. 1999).

3.5.2 Osteocalcin

During normal bone metabolism in adult and subadult years, the modelling and remodelling of bone is accompanied by the production of biochemical by-products (Christenson 1997; Rauch 2008). Osteocalcin is a non-collagenous protein made up of 49 amino acids and is secreted by the bone forming osteoblast cells. Osteocalcin regulates skeletal mineralization and influences bone cell activity (Delmas 1995; Calvo et al. 1996; Neve et al. 2013). While a small amount of osteocalcin is deposited into the blood stream after synthesis, the majority of this protein is found in the inorganic hydroxyapatite of bone making it less susceptible to degradation over time (Calvo et al. 1996; Collins et al. 2000; Smith et al. 2005; Neve and Corrado 2011). Considered the most sensitive chemical indicator of bone mineralization, a decrease in osteocalcin levels has been clinically shown to correlate with an increase in circulating glucocorticoid levels (Delmas 1995; Neve et al. 2013). This disruption of osteocalcin production is associated with the overlap between the glucocorticoid receptors found in skeletal tissues, and the osteocalcin promoter where the production of osteocalcin is repressed by an over-saturation of this steroid (Stromstedt et al. 1991; Morrison and Eisman 1993). While present in all bone types, osteocalcin is most prevalent in lamellar bone (Ingram et al. 1994; Calvo et al. 1996). Indicative of osteoblast new bone creation, osteocalcin is more closely associated with the bone mineralization process than bone synthesis (Calvo et al. 1996). Because of this close link with the mineralization process, quantifying the amount of osteocalcin can be difficult. Studies have shown that if bone mineralization is stimulated through dietary malnourishment, the amount of osteocalcin can fluctuate (Calvo et al. 1996). Osteocalcin levels can also vary based on age with

the peak value occurring around puberty, reflecting rapid linear growth (Calvo et al. 1996; Szulc et al. 2000). Elevated levels of osteocalcin also occur during the infancy and childhood stage of maturation (Hauschka et al. 1989; Szulc et al. 2000). At birth, the high level of osteocalcin continues to increase until the end of the first month and remains high until the end of the third year (Magnusson et al. 1995; Mora et al. 1997) where it remains stable until puberty for males and females (Szulc et al. 2000). Reflecting growth velocity over chronological age, these elevated levels of osteocalcin correspond with rapid bone growth and mineralization periods (Robins 1994; Szulc 2000). Once skeletal maturation is complete, osteocalcin levels remain relatively stable with a slow decline reflecting a reduction in bone remodelling with age. Women however, experience a slight increase in osteocalcin during menopause (Vanderschueren et al. 1990; Ingram et al. 1994; Calvo et al. 1996). Additionally, women can experience fluctuating osteocalcin levels during pregnancy where osteocalcin levels are up to 50 percent lower than normal during the first and second trimesters (Martinez et al. 1985; Cole et al. 1987). Osteocalcin may also be elevated during menstruation, particularly during the luteal phase (Calvo et al. 1996). Diet and activity have also been shown to affect osteocalcin levels; where underweight individuals have lower osteocalcin levels, overweight individuals can have elevated levels of osteocalcin up to 25 percent higher than an age-matched average-weight individual (Bell et al. 1985; Fonseca et al. 1987). Similarly, in individuals with higher activity levels and increased skeletal strain, osteocalcin levels can be up to 50 percent higher than age-matched sedentary individuals (Bell et al. 1988). Interestingly, while many internal and external factors can affect osteocalcin levels, diseases that do not directly involve bone seem to have no effect on normal osteocalcin production (Slovik et al. 1984).

3.5.3 Archaeological Studies of Osteocalcin

From an archaeological perspective, the study of osteocalcin and other similar non-collagenous proteins (NCPs) was developed with the methodological hope that these NCPs could provide another way in which to date fossilized material or to contribute to paleodietary reconstructions (Ajie et al. 1991). Because the preservation of collagen can be greatly hampered by diagenetic changes post-mortem, the extraction and quantification of NCPs was developed to further explore which proteins best preserve in ancient bone and how they may be used to explore archaeological research. While there are a variety of these NCPs that have been explored in archaeological research, osteocalcin is arguably the most prevalent and favoured due to its excellent preservation (Huq et al. 1990; Collins et al. 1998). The use of NCPs to explore paleopathological changes in skeletal materials has also been initiated in skeletal studies by Tuross (1991) and Cattaneo et al. (1994). These studies, focusing on the immunological detection of extracted of NCPs, demonstrated the viability of using biochemical signatures to explore changing health trends in archaeological populations. Similar to clinical studies on NCPs, fluctuating levels of these biochemical markers, can be used to approach paleopathological research previously unattainable through skeletal remains alone. In addition to the interpretive value of studying NCPs in an archaeological context, many of these studies have also focused on extraction protocols and diagenetic changes to these biochemical markers. Discussed by Smith et al. (2005) and Collins et al. (2002), the survival of organic remains in bone is highly dependent on the conditions of burial after death specifically, soil pH, microbial activity and burial environment (Elster 1991; Grupe and Turban-Just 1996). In addition to these post-depositional changes, Collins et al. (2002) also discussed the impact of perimortem changes such as funerary practices which can alter the biological environment of the body and the

underlying skeletal tissue affecting the long term preservation of these NCPs. After death bone becomes unstable outside of its natural organic environment and begins to crystalize immediately (Trueman et al. 2004). As crystallinity increases through the process of Ostwal ripening (small crystals dissolve and reform as larger crystals), the level of protein preservation in bone decreases (Roberts et al. 2002; Trueman et al. 2004). However, the rate of this crystallinity is dependent on the speed of organic decomposition and surrounding soil type, evidenced in the results of Hedges and colleges (1995). While the preservation of NCPs in archaeological remains is not infinite, the complete or partial extraction of these proteins from archaeological sites over multiple millennia (e.g. Grupe and Turban-Just 1996; Smith et al. 2005) demonstrates the long-term viability of these biochemical markers and the need to expand their analytical potential in archaeological research. From a simplistic perspective, the better preserved the overall bone is, the better preserved the NCPs (Grupe and Turban-Just 1996; Collins et al. 2000; Collins et al. 2002; Smith et al. 2005). There has also been an additional focus on extraction protocols for NCPs with growing interest in the demineralization process and whether or not similar techniques can be used between traditional collagen extraction and NCPs (Cleland et al. 2012). Depending on the type of protein to be extracted, Cleland and colleagues argue that there are both benefits and drawbacks to traditional hydrochloric acid (HCl) extractions over ethylenediaminetetraacetic acid (EDTA) methods; however, with osteocalcin EDTA provides a higher yield at the cost of an additional purification step.

3.6 BIOARCHAEOLOGICAL EVIDENCE OF SKELETAL STRESS

It is based on clinical knowledge of glucocorticoids and their effect on skeletal growth and development, that osteologists are able to assess the impact of stress on skeletal maturation. Because there is individual variation in how stress is internalized (Goodman and Armelagos

1989; Wood et al. 1992) and how stress manifests in different skeletal elements (Weinstein et al. 1998; Cooper 2004), there are a variety of ways in which osteologists can assess stress and the specific nature of these disturbances.

Defined by a limited data set, osteological questions regarding biological processes must be identified from skeletal remains alone (Steckel 2005). As Armelagos and VanGerven argue, "human skeletons represent answers and the goal of osteology is to frame the questions" (2003:53) and it is the responsibility of osteologists to define what types of data can be obtained from studies of skeletal remains. It is during the growth and development period (birth to approximately 20 years of age) when variation is introduced into the skeleton, as biological systems are rapidly maturing into their adult form (Bogin 2001); as such, subadult growth is considered to be the most sensitive indicator of biological or cultural fluctuations (Lewis 2000). While the basis of osteological research is in the study of skeletal variation, the primary objective is to explore the variety of influencing factors, known as stressors, which can lead to these developmental deviations (Hoppa and FitzGerald 1999; Humphrey 2003). Because human growth and development is well-established as one of the best indicators of overall health (Eveleth and Tanner 1990), these patterns of maturation are consistently used as a gauge of stress and the impact of that stress on overall skeletal health. While these stressors may be brought upon by genetic, socioeconomic, environmental, and psychosocial factors (Johnston and Zimmer 1989), their resultant effect on skeletal growth is considered "deceptively simple because bone is limited in its potential responses" (Goodman and Armelagos 1989:228), but not without specific limitations.

In general, osteological studies are able to identify that a stress event has occurred based on the presence of lesions, or the alteration of linear growth; however, the challenge is in

defining the cause of that stress. While many factors are identified as potential catalysts of stress, these factors are generally considered non-specific (Goodman et al. 1988; Larsen 1997; Armelagos and VanGerven 2003). Additionally, it must be recognized that examples of stress in archaeological populations are not necessarily defined by one single factor, but are rather the result of multiple, confounding factors that affect the skeleton in a collaborative manner (Goodman et al. 1988; Klaus and Tam 2009). Important to consider here is how the impact of one type of stress may affect the manifestation of another, or if adaptation to one stressor can hinder or help the skeleton in its response to any subsequent exposure to stress (Goodman et al. 1988). Further to these complicating factors, is that stress will not always manifest within the biological systems of the body, as evidence shows some physiological stress does not cause an increased release in glucocorticoids (Mason 1968a, 1968b; 1971; Mason et al. 1976). Conversely, the effect of perceived stress is also a challenging factor for anthropological analyses, as perceived threat has been identified as the most consistent activator of the "stress response" system (Mason 1968a, 1968b; 1971; Mason et al. 1976; Goodman et al. 1988). Because osteological analyses are limited to the skeleton only, it becomes a matter of questioning whether it is the perceived stress, or the experienced stress that is being biologically recorded. While perceived threat has been shown to be the most consistent activator of the "stress response," there has been no correlation of this activation to the deeper tissues of the skeleton. This manifestation of perceived stress may perhaps be more apparent in the soft tissues of the body, as it has been shown that the soft tissues are more vulnerable to endocrine fluctuations than the skeleton (McCance 1960; McCance et al. 1961, 1962).

When trying to accurately assess stress in the skeleton, the hierarchical nature of the body's biological systems must be considered. Discussed in detail from an osteological

(Goodman et al. 1988) and clinical perspective (Weinstein et al. 1998; Cooper 2004), stress affects the skeleton in a graded manner. At the individual level, the impact of stress can be affected by an individual's age, sex, genetic susceptibility, or socioeconomic status. Depending on the type and duration of stress, individuals of diverse constitutions will inevitably react differently, producing a variety of physiological changes (Goodman et al. 1988). Additionally, at the cellular level there is a hierarchy of vulnerability, with teeth considered more resilient and buffered against stress than bones (Garn et al. 1965). Because of the variety of responses to stress within the skeleton, based on intrinsic and extrinsic factors and their interaction, osteologists are only able to obtain a certain level of specificity in stress analysis based on the current methods employed and their limitations.

Lesion-based methods of assessing stress are well-defined in the osteological literature and are arguably the main source of information regarding the health of archaeological populations (Lewis and Roberts 1997). Porotic hyperostosis (PH) and cribra orbitalia (CO) are frequently used as indicators of stress due to their prevalence in many archaeological populations. While these lesions manifest in different regions of the cranium, PH on the posterior parietal bones and CO along the orbital plate, both stress indicators occur as the result of similar metabolic processes (Angel 1966; Stuart-Macadam 1989). For both PH and CO there is a characteristic expansion of the diploe with resulting macroscopic porosity of the cortical bone surface (Stuart-Macadam 1985). While in a North American context these indicators are defined under the umbrella term of 'porotic hyperostosis,' in Europe CO is much more common than PH leading some researchers to argue that they are not necessarily linked by the same type of stress or perhaps indicate a variation in severity (Lewis 2000). This recognition is significant as it demonstrates the underlying variability of the skeleton and how the concurrent expression of

stress indicators in one population does mean it is a universal pattern, as the manifestation of stress is not that simple. CO and PH can be associated with a variety of causes, but most commonly with iron deficiency anemia, resulting from poor nutrition or genetic factors (Huss-Ashmore et al. 1982), with more recent research suggesting that the iron deficiency anemia causing these lesions may be the result of pathogens rather than nutritional deficits (Stuart-Macadam 1992). Current research further explores the etiology of PH and CO by demonstrating that severe iron deficiency anemia is perhaps not the best explanation for these lesions, as this type of anemia causes a restriction in red blood cell production, not an increase, which is necessary for the characteristic expansion of the diploe seen in these stress lesions (Walker et al. 2009). However, this argument has been further countered by Oxenham and Cavil (2010) who argue that CO and PH can be tied to anemias and the arguments by Walker et al. (2009) are faltered as they do not consider the clinical knowledge surrounding the increased erythropoietic (formation of red blood cells) activity that occurs in the bone marrow with the onset of iron-deficiency anemia. Despite these ongoing debates, CO and PH are arguably the best indicator of stress in regards to the specific cause of these lesions, but to date there is no method to assess the time period or duration of these stress events.

Enamel hypoplastic lesions (EHL) are considered the most versatile indicator of stress as the time period in which the stress occurred, its duration, and its potential severity can be assessed (Goodman et al. 1980). Similar to stress disruption in bone, EHL form when there is a systematic disturbance of biological homeostasis and appear as lines or pits within tooth enamel (Pindborg 1970). As the tooth enamel forms, it does so in a predictable pattern as ameloblasts (enamel forming cells) lay down new enamel during the growth and development period; therefore, the period in which these lesions form can effectively be measured and translated into

a chronological age estimate (Goodman and Song 1999). In addition to providing a time period when stress was experienced, the pattern of EHL may indicate systemic versus localized trauma or infection, if multiple teeth are affected (King et al. 2005). EHL have been associated with a variety of causes, but are most often linked with nutritional deficiencies, including calcium deficiency, gastrointestinal disorders, and weaning stress (Sweeney et al. 1969; El-Najjar et al. 1978; Nikiforuk and Fraser 1981; Goodman and Armelagos 1988). Arguably, the primary benefit of employing EHL in studies of stress is the fact that these lesions will not remodel over time, and remain a permanent marker of early childhood stress (Goodman and Song 1999); however, this permanent record can only provide a limited timeline of stress specificity before approximately 12 years when enamel formation is complete (Lewis 2000).

Harris lines, visible only through radiographic analysis, are another type of growth arrest lesion used to assess stress in the subadult years of life. Harris lines are formed when there is a disruption of the cartilage growth plate causing an interruption in the bone formation process (Park and Richter 1953; Park 1964; Garn et al. 1968; Larsen 1997). As Park (1964) explained, Harris lines first form in the organic matrix of the bone, but become incorporated into the hard tissue, as bone mineralization occurs almost immediately after the stress event has passed creating a radiopaque line of disruption. It is only after a stress episode has subsided and growth has resumed that these lines will appear; however, this does not necessarily indicate that the stress has completely dissipated, but rather has subsided enough to allow growth to resume (Mays 1995). Therefore, this method of analysis has specific limitations in defining the timeline of a stress event, or if indeed the event has passed completely. As with many of these methods, it cannot be assumed that stress was only present if there is an indicator to denote a fluctuation in homeostasis, as many researchers have identified the need for "sufficient stress" to illicit a

skeletal response (Steckel 2005:325). This is also addressed in the clinical literature of stress disruption and the assumption that stress manifests only once it has passed a threshold of specific severity and duration (Tsigos and Chrousos 2002; Charmandari et al. 2005). Unfortunately in an osteological context this threshold of severity and duration is still poorly understood and not easily accessed, as studies of stress more often than not focus solely on the immediate environment and do not necessarily consider the long-term stress processes that lead to skeletal changes (Goodman et al. 1988). Studies show that Harris lines appear most commonly after the initial six months of life and usually plateau around five to six years of age (Clarke and Gindhart 1981); however, these lines can disappear over time as a result of normal bone remodelling. Because the skeleton undergoes a continual process of skeletal remodelling (Parfitt 1977; 1994), the analysis of Harris lines in adult remains is limited (Dreizen et al. 1964). In addition to the complications surrounding the formation of Harris lines, there is also a lack of consensus regarding their cause with potential correlations to childhood illness and disease (Garn et al. 1968; Marshall 1968), nutritional deficiencies (Blanco et al. 1974), and psychological stress (Sontag and Comstock 1938).

In addition to lesion-based methods of stress analysis in archaeological populations, studies of linear growth and development have also been used to assess stress, specifically body size. As discussed, during times of stress the body will redirect energy to vital operating systems, effectively disrupting normal growth which can alter the overall skeletal maturation of an individual if this stress occurs at a critical time of maturation (Sommer 1996). What is unique about studying stress from a linear growth perspective is that endocrine disturbance is scored based on subtle, gradient changes in relation to the remainder of the skeleton, while studies of lesions focus purely on presence or absence. While lesions can be scored based on their

frequency, it is still primarily based on a presence or absence binary classification, whereas growth and development methods approach stress by assessing element variation within the larger skeletal system. A limiting factor of studying stress from this linear growth perspective however, is the effect of mechanical loading and how factors such as handedness (Steele 2000) may produce skeletal responses that give the impression of stress, but cannot be verified or denied as such by skeletal remains alone.

It is well established that certain elements within the skeleton are correlated to overall body stature and body mass (Anderson et al. 1976; McHenry 1992; Aiello and Wood 1994; Porter 1999; Ruff 2002; Spocter and Manger 2007). Using these indicators of body size as proxies for what is considered normal growth and development, it has been shown that during times of physiological and psychological stress, these indicators may be reduced in size as a result of growth disruption during critical periods of maturation (Scott 2009). This method of assessing linear growth allows for a better understanding of the timeline of stress and its potential severity by looking at the number of indicators affected over a certain period of time. Evidence suggests that the more body size indicators being affected, the more severe the stress at that critical period of maturation. Despite the benefit of this method in identifying stress along a timeline continuum, there is no way to associate a reduction in body size indicators with any specific type of stress, unless associating the calculated time period of stress with known cultural or environmental events.

Stature has also been used as a means by which to examine the impact of stress on skeletal maturation. While the calculation of stature is fraught with difficulties in osteological research, the measurement of long bone lengths has been used successfully to assess individual growth in clinical settings (Maresh 1943, 1955). Based on long bone length measurements within

and between populations, osteological research has been able to use these clinical data to assess the expected growth trajectories of a population and identify individuals that fall outside of those parameters. These outliers may be the result of prolonged stress during critical periods of growth and maturation and can be associated with a variety of factors, predominantly environmental factors including: nutritional deficiencies (Huss-Ashmore et al. 1982), socioeconomic status (Cardoso 2007), climatic variation (Frisancho 1979), or psychosocial stress (Eveleth and Tanner 1990). One of the primary difficulties with studies of stature, aside from methodological challenges, is that measures of stature that deviate from the norm are more often than not presumed to represent stress without enough focus on genetic variability or secular changes in populations over time, especially when archaeological populations are compared indiscriminately to contemporary populations. Even if these genetic considerations are accounted for, the calculation of adult stature only provides a static assessment of stress, attained at maturation and does not consider the possibility of growth fluctuations that may have occurred earlier in life, but left no skeletal evidence due to biological recovery.

While all of these methods have potential to demonstrate the impact of stress on the skeletal system they lack certain specificity. Many researchers argue that knowing the age at which stress occurred influences osteological conclusions regarding health and survival (Lewis 2000); or that knowing the cause of the stress event may indicate changing cultural practices or environmental trends (Goodman et al. 1988); therefore, access to this knowledge is important, but can be difficult to ascertain based on the current methods of stress assessment. Lesion-based methods are perhaps the best indicators to specify the potential cause and duration of stress; however, it is the subtleties of the growth-based approaches that can better address the ever-imperative question of stress threshold (the point at which homeostasis is disrupted enough to

cause physiological change) and severity (the amount of stress experienced by an individual to cause any one particular type of disruption or delay). At this stage, osteological researchers can be fairly specific in the identification of a stress event whether through lesion-based analyses or changes in linear growth and development. The downfall, however, is the difficulty in moving beyond the simple recognition that stress has occurred. Goodman et al. (1988) argued that because there is such a variety in how stress can manifest within the skeleton, it is imperative that researchers begin to address the intra- and inter-variability for each type of stress indicator and the impact of that stress on "functional competence" (195).

3.7 BRINGING THE STRESS DATA TOGETHER

One of the main challenges of osteological research is working with cross-sectional data, and the inability to observe skeletal fluctuations at the individual level over an extended period of time (Lewis 2007). Particularly for studies of stress, being able to observe how an individual reacts and adapts over time to negative influencing factors provides insight into the biology of the "stress response" and the resultant health outcome. While there has been clinical research conducted regarding the visible impact of stress in living populations (Eveleth and Tanner 1990; Bogin 1999), subtle changes within the skeleton are generally unobservable. However, despite the limitation of a cross-sectional data set, osteological research focusing on skeletal maturation sequencing has provided a means by which to move beyond static assessments of stress at the time of death to incorporate periods of disturbance throughout the lifespan (Goodman and Armelagos 1988; Humphrey 1998). Stress in past osteological research has been approached from both adult and subadult perspectives, with each age cohort suited to different research objectives. However, subadult examples arguably make up the majority of the literature as these individuals provide "the most sensitive indicator of change" (Goodman and Martin 2002:19).

More recently however, there has been a shift towards looking at stress on a continuum through the entire span of growth and development and the impact of stress on later life events. As Armelagos and Van Gerven (2003) argued, osteologists have become impeded by their inability to move beyond simple descriptions of stress to explore the intricate analytical possibilities of this type of skeletal research. While there has been intensive studies of skeletal stress incorporating multiple methods of analysis (see Larsen 1997; Hoppa and FitzGerald 1999; Steckel and Rose 2002; Lewis 2007), it is only recently that this multifaceted research has moved beyond a static point in time to focus on the implications of these stress events on health. Discussed by Goodman et al. (1988), the only attempt by osteologists to truly look at the continuum of stress though life via lesions has been in the study of mortality, first explored in an anthropological context by Meindl and Swedlund (1977). Meindl and Swedlund argued that by considering stress events from both the subadult and adult years of life a better understanding could be gained regarding the impact of stress on long term health outcomes. As they described, this was a method in which to isolate and quantify the adult and subadult relationship and the impact of stress beyond a single period in time (Meindl and Swedlund 1977). This timeline approach to stress was also tested in early osteological studies by White (1978), Cook and Buikstra (1979), and Rose et al. (1978). In all three examples, the authors determined that early childhood stress events, evidenced by EHL, could be correlated with an increase in adult mortality rates. Because EHL do not remodel over time, this method of associating early life stress events to later adult health helped to better explore the underlying mechanism of the stress process.

Building on these earlier studies and the relationship between early life stress events and adult mortality, there has been an increasing focus on studying the relationship between multiple

stress indicators. While the study of multiple stress indicators is not a new approach in osteological research, the comparison of these indicators and their contribution to later adult mortality provides a new perspective on stress severity and if some indicators produce greater vulnerability later in life. In translating indicator frequencies into patterns of adult mortality, researchers have been able to show how these indicators compare to one another in the assessment of stress (Hummert and VanGerven 1983; Miles and Bulman 1994; Ribot and Roberts 1996; Steckel 2005; Klaus and Tam 2009). While Goodman et al. (1988) argued that stress occurs within a hierarchical framework, and that an individual experiencing stress would not necessarily show evidence in the skeleton, it was not until the work of Miles and Bulman (1994) and Ribot and Roberts (1996) that this was clearly demonstrated in archaeological remains. Miles and Bulman in their study found that linear growth disruption could occur in skeletal elements with no evidence of stress lesions present. Conversely, Ribot and Roberts found that despite high frequencies of stress lesions in their sample, linear growth was virtually unaffected and showed no evidence of being impeded by stress. The results of these two studies challenged how stress was assessed at this time, by demonstrating there is not necessarily a straightforward relationship between stress and the skeletal reaction. Based on these studies and earlier theoretical assumptions, it was illustrated that when an individual experiences stress they do not necessarily demonstrate the entire gamut of stress responses. Importantly, this suggests that the hierarchical underpinning of the skeletal response to stress can perhaps be accessed in osteological research.

Another important consideration that came out of this mortality based research was the possibility of identifying the severity of stress episodes in different populations. Steckel (2005) found that some indicators of stress differently affected adult mortality, with linear growth

disruption as the variable least likely to affect mortality and EHL being the most likely to increase adult mortality. Based on the literature, it is known that teeth are more resistant than bone to disruption during growth (Garn et al. 1965); therefore, this would suggest that EHL are produced during periods of more severe stress than other indicators affecting the reminder of the skeleton. This type of analysis also has applicability for the comparison between populations and how the severity of stress indicators may change over time or reflect geographic differences. By expanding osteological analyses to look at which stress indicators are more predictive of adult mortality, it should be possible to 'rank' these indicators in terms of severity which may help to better elucidate their causes.

The concept of catch-up growth is also an important consideration for osteological studies of stress. First introduced by Prader et al. (1963), catch-up growth is based on the foundation that every individual has a growth trajectory that they will follow under ideal conditions. If however, an individual deviates from this growth curve due to stress, there is the capacity for them to regain their trajectory if the stress event passes before the cessation of growth (Prader et al. 1963; Tanner 1986). As Tanner (1986) outlined, growth is "self-stabilizing or target-seeking" (167), and will push the organism to regain their normal growth trajectory. Because of this self-stabilizing mechanism, individuals that experience growth disruption early in childhood may not show signs of stress as the result of catch-up growth. Considered an invisible mechanism in osteological research due to cross-sectional data sets, catch-up growth is an important consideration for studies of stress, particularly in respect to severity and duration. To date, catch-up growth has not been extensively studied in osteology; however, its potential use for studies of stress has been recognized. Clark et al. (1986) focused on how early life stress events could affect adult lifespan and overall health, similar to the discussed studies of adult

mortality patterns. To explore this relationship between early life events in adult skeletal remains, Clark and colleagues focused on vertebral growth, as vertebral growth shows fast velocity in early childhood and is likely susceptible to stress insults. The authors argued that using different indicators of growth, such as vertebral measurements, provided a more reliable way to explore the impact of stress on later life health, as later developing skeletal elements always had the capacity to realign with their growth trajectories, while earlier maturing elements did not and could provide a record of early life stress events (Clark et al. 1986). From this pioneering study and a later study conducted by Clark (1988), these researchers demonstrated the plausibility of assessing a static skeleton from a timeline perspective and the use of catch-up growth to demonstrate whether stress was prolonged (affecting all aspects of the vertebra) or acute (affecting only the earliest maturing vertebral elements).

Porter and Pavitt (1987) also explored the use of vertebral canal measurements but combined this linear growth data with lesion-based methods of analysis. In combing these two types of stress assessment methods, Porter and Pavitt successfully demonstrated the variability in the timeline of when certain indicators actually manifest within the skeleton, suggesting that vertebral growth is first affected by stress followed by the production of lesions in the terminal stages of growth. The benefit of this comprehensive analysis of linear growth indicators and lesions is the potential to identify how the visibility of stress can change over time. When looking at linear growth indicators alone, catch-up growth has the potential to mask stress events, but when coupled with a lesion-based analysis, the masking effect of catch-up growth can be averted. Despite the difficulties associated with catch-up growth, its identification in skeletal remains provides a unique opportunity to look at the timing of skeletal recovery and any possible impact this may have on the production of other indicators of stress.

The acknowledgement of skeletal sequencing and the timing of specific growth events has also become an innovative way to explore stress in archaeological populations (Humphrey 1998; Scott 2009). Because certain skeletal elements will reach adult maturity before others, it has been argued that some elements will be more vulnerable to the effects of stress during their critical maturation period. While skeletal sequencing was defined in detail by Humphrey (1998), the application of this sequencing pattern to the timing of stress was further explored by Scott (2009). By focusing on the overall size of certain skeletal elements compared to a population standard, Scott was able to demonstrate the variability in size discrepancies between different skeletal elements. When plotted against each other in a sequential order, it was possible to identify periods of stress when multiple skeletal elements known to mature at the same chronological age all demonstrated a reduction in growth. Further to this, Scott was also able to identify accelerated growth following these periods of stress in later maturing elements, possibly representing catch-up growth. While Clark et al. (1986) and Clark (1988) also focused on stress sequencing, this study was the first to incorporate multiple skeletal elements in a timeline approach to stress. A significant outcome of this research was the ability to identify invisible stress episodes that from a traditional lesion-based perspective would be overlooked.

This move towards identifying invisible stress has also been explored in microstructure analyses, first discussed by Martin and Armelagos (1979; 1985) and expanded more recently by Simpson (1999); FitzGerald et al. (2006); Schilling et al. (2008); and Temple et al. (2012). Martin and Armelagos (1985) argued for the need to combine microscopic and macroscopic data and highlighted the important fact that stress does not necessarily lead to gross macroscopic skeletal changes. This new innovation of using microstructure analysis has focused predominantly on enamel defects, but has also been used to assess the shape and morphology of

osteons within the remainder of the skeleton. The benefit of microstructure analysis is the ability to compare what is visible at a microscopic level to that at the macroscopic level, establishing a better understanding of the threshold in which stress must reach before leaving a visible insult in the skeleton.

Similar to these studies of microstructure there has also been exploration into bone mineral density (BMD) and its applicability to stress analysis (Van Gerven et al. 1985; McEwan et al. 2005). Most recently, McEwan and colleagues (2005) used radiographic analysis to look at BMD in juvenile radii to see if there was a correlation between BMD and visible stress lesions. Because BMD is known to increase in a linear fashion with age, the authors questioned whether deviations from this linear pattern were indicative of stress. It was found that BMD is preserved in the skeleton at the cost of other architectural structures, again highlighting the hierarchical nature of stress manifestation. The authors argued that only severe stress would be capable of altering BMD in the skeleton, as bone mineralization is a vital function which seems to be better buffered against disruption. This innovative technique to assess stress provides another important vantage point to look at stress impact on the skeleton and the need to explore the possibilities of a hierarchical ranking system for these skeletal changes.

The study of stress in osteological research is predominantly focused on skeletal remains; however, innovative techniques employing soft tissues can perhaps expand and contribute to these strictly skeletal analyses. Using archaeological hair, recent research has demonstrated the ability to extract cortisol which can then be used to identify hormonal levels associated with stress shortly before death (Webb et al. 2010; 2015). This type of collaborative data is significant for stress research, as direct hormonal evidence can perhaps be correlated with skeletal evidence of stress depending on the type of soft tissue used and its preservation. While the examples

discussed by Webb et al. (2010) focused on adult remains, there is a possibility that organic material from subadult remains could be employed to assess the actual levels of stress hormone in the body at the time of death and if there is associated skeletal evidence of this stress. Because the subadult skeleton is extremely susceptible to perturbations, this method could perhaps be an important step forward in identifying how quickly stress translates from the biological soft tissues into the hard tissues of the skeleton.

3.8 THEORETICAL LIMITATIONS IN THE STUDY OF SKELETAL STRESS

3.8.1 The Osteological Paradox

One of the biggest theoretical challenges to come out of osteological research in recent decades has been the "Osteological Paradox" (Wood et al. 1992) and the implications for the way in which skeletal stress is assessed in archaeological populations. As discussed, skeletal disruption controlled by chemical reactions, is continually used as a gauge for health and well-being in past populations; however, with the introduction of the Osteological Paradox, this seemingly straightforward relationship between skeletal changes and overall health was challenged. Wood and colleagues focused on three main conceptual issues that had, as they argued, been overlooked in the osteological literature at that time: demographic non-stationarity, selective mortality, and hidden heterogeneity in risks (Wood et al. 1992). All three issues tied into how researchers at the time were making direct conclusions about once living populations from deceased individuals; particularly pertinent was their concern of selective mortality.

Selective mortality focuses on the argument that skeletal samples will never fully represent the entire population at risk of dying at any particular age, but only those individuals who did in fact die, as these were the non-survivors. Wood and colleagues argued that all skeletal samples were therefore, under-representative of the once living population who were at risk of

dying, inevitably creating a selectivity bias. While some authors argue that a skeletal sample will always be under-representative of the once living population due to preservation issues and a lack of full skeletal recovery (Waldron 1994; Saunders 2008), Wood and colleagues insisted that, even if it were possible to obtain a perfectly random sample it would still be under-representative of the living population from which it was derived, as these individuals are only the non-survivors, not all who were at risk of dying (Wood et al. 1992).

Selective mortality also addresses skeletal disruptions (i.e. lesions and linear growth) and how these disruptions are used to infer health. Wood and colleagues argued that the interpretation of lesions is difficult in skeletal remains, as lesion frequencies may be under-represented due to a delay in skeletal manifestation, or that the frequency of lesions may be over-represented because the non-survivors who died were more likely to have lesions. The authors reasoned that because lesions are associated with illness, mortality would be selective for individuals in a population that had lesions. Wood and colleagues also discussed selective mortality in regards to growth and development patterns, considering linear growth a more subtle indicator of stress. The authors argued that studies of stature in particular, consistently associate short stature with increased stress, as this relationship has been continually observed in contemporary populations (Eveleth and Tanner 1990; Bogin 2001). While recognizing this consistent trend in contemporary research, the authors still challenged this assumption, and argued that variations in growth may indicate variations in mortality, as mortality is likely selective for stature, similar to lesions. Assuming that stature variation within a population is normally distributed, the authors pointed out that in times of high mortality, not only will short individuals enter the skeletal record, but individuals of average or tall stature as well. However, in times of low mortality, only the weakest or most frail will die, therefore the shortest. Based on

this reasoning, Wood and colleagues concluded that like lesions, stature and growth patterns cannot be approached in a straight forward manner, and as more lesions may equate with better health, shorter skeletons may also indicate better overall health as the result of low mortality.

Complicating the concept of selective mortality is the third caveat of the Paradox and that is hidden heterogeneity in risks. Hidden heterogeneity refers to the fact that any skeletal population is made up of various individuals that will have varying frailty and susceptibility to disease and the risk of dying. It cannot be assumed that all individuals will react similarly to stress, as individuals are exposed to unique internal and external forces that contribute to individual heterogeneity in terms of frailty (Eisenberg 1992; Palubeckaitė et al. 2002; Boldsen 2007). Boldsen (2007) argued, that stress events can manifest in the skeleton in one of two circumstances: 1) these lesions form due to heterogeneity in an individual's immune system or 2) these lesions can occur by activating other pre-existing variables leading to frailty. Based on this understanding of individual heterogeneity then, it must be recognized that frailty is not fixed and can change, impacting how stress manifests within the skeleton. While this understanding of heterogeneity complicates the ability to define whether frailty is inborn or acquired from early life stress events (Boldsen 2007), it is assumed that individuals exposed to chronic stress will eventually manifest lesions or display growth disturbance reflective of differential frailty. Therefore, this understanding of frailty contributes to the discussion of stress severity rather than the manifestation of stress over time which is important as it separates these key elements of the stress response system and how it ultimately affects skeletal tissues.

Based on the arguments made by Wood and colleagues, the seemingly straightforward relationship between outward skeletal manifestations of health and the actual health status of archaeological populations was called into question. The authors demonstrated the countless

intricacies that must be considered in osteological research and how the study of health, whether through an assessment of lesions or growth and development patterns, must consider the influence of extrinsic factors, intrinsic vulnerability, and of course how health actually expresses itself within the skeleton.

3.8.2 Considerations of the Paradox

Not surprisingly, there was a considerable amount of comment on the Osteological Paradox immediately after publication (Cohen, Eisenberg, Hutchinson, Jankauskas and Česnys, Katzenberg, Lukacs, McGrath, Abella Roth, Ubelaker, Wilkinson 1992; Goodman 1993; Cohen 1994) and more recently (Wright and Yoder 2003) with discussions of whether the concerns raised by Wood and colleagues were valid for researchers studying skeletal remains. While many of the initial commenters did agree that osteologists must be stringent in their conclusions drawn from skeletal research, many also argued that Wood and colleagues overlooked some of the basic foundational aspects of osteological research and how researchers conduct their analyses. At the forefront of this rebuttal was the work of Goodman (1993), who argued that the Paradox is not paradoxical at all, but rather an interpretation without all of the facts. One of the main arguments made by Goodman against Wood and colleagues was that many of the interpretations made from the provided examples were based on single indicators of health, and overlooked the analytical possibilities when evaluating multiple indicators. Discussing the relationship between lesion analyses and studies of linear growth, Goodman made the excellent point that any type of health indicator provides different information contributing to what is known of skeletal health. For example, growth data provides cumulative measures of health as the skeleton matures on a continuum until adulthood, while lesions provide very time specific information regarding health; therefore, “the bones and teeth of the dead reflect conditions at death *and* conditions

during life" (Goodman 1993:282, emphasis added). Goodman also pointed out the very significant oversight by Wood and colleagues in their hypothetical reconstruction of a population, and that was their omission of age at death information. In the authors' hypothetical situation, there is Group 1 that has no exposure to stress and as such displays no skeletal lesions; Group 2 however, is exposed to moderate stress that is widespread and lasts a prolonged period without many deaths, therefore skeletal lesions are present; and finally Group 3 is exposed to severe stress with many casualties at the onset of the illness, and as such no lesions manifest in the skeleton. Wood and colleagues argued that Groups 1 and 3 would look identical in the skeletal record and that a lack of lesions cannot automatically be associated with better health, as no lesions may also indicate acute severe stress that did not have time to manifest in the skeleton before death. Goodman (1993) was quick to dissolve this possibility that "better health makes for worse skeletons" (Wood et al. 1992:356) by demonstrating that despite lesions frequencies being the same, the individuals from Group 1 would have lived, whereas the individuals from Group 3 would have died and would have shown an earlier mean age at death. Goodman argued that calculating age-at-death mortality profiles within these hypothetical groups is a way in which to avoid the supposed paradoxical nature of the argument made by Wood and colleagues. Further to this, Goodman also challenged the assumption that shorter individuals are inherently more vulnerable and stated that the model used by Wood and colleagues was too simplistic. Even though it has been shown that shorter stature is tied to higher mortality, it is also based on a prerequisite that stress has occurred earlier during skeletal maturation and has compromised an individual's overall health and constitution. Therefore, stature can be associated with a risk of dying, but other factors can also contribute to this increased mortality, which was overlooked by the authors in their analysis.

In addition to these arguments made by Goodman (1993), Cohen (1994) also responded to the Osteological Paradox, particularly focusing on Wood and colleagues assumption that those who die are always the most vulnerable. As Cohen argued, "the sample of deaths in a population will always include both a selected and random component" (631) as there are always unforeseeable factors that select individuals to be included into skeletal samples, even when they are not considered at risk or vulnerable (e.g. accidental death). Cohen applied this reasoning to stature fluctuations visible between the Paleolithic and Neolithic period. Where Wood and colleagues would argue the decrease in stature indicates better health, Cohen demonstrated that because the sample is made up of the most vulnerable and a random component, the general decline in stature was likely due to cultural shifts and a decline in nutrition, not the result of only the weakest dying in times of low mortality.

It was not until the work of Saunders and Hoppa (1993), that the mortality bias discussed by Wood and colleagues was fully explored and considered from a methodological perspective, creating a standard of accountability when using non-survivors to make inferences about survivors in a population. Saunders and Hoppa addressed whether or not there was a significant difference in morphology and physiology between the survivors and non-survivors of childhood, and whether or not there would be "significantly different subadult stature distributions than their corresponding living populations" (128). While considering various factors that may affect mechanisms of growth and development, the authors determined that there was significant variation in growth between survivors and non-survivors, with the survivors generally showing enhanced growth; however, these individual differences were almost negligible when assessed from an aggregate level as the majority of studies using skeletal remains are (Saunders and Hoppa 1993). Therefore, Saunders and Hoppa concluded that using a non-survivor sample to

draw conclusions about the health of the survivor group in a skeletal sample was methodologically viable.

3.9 METHODOLOGICAL LIMITATIONS IN THE STUDY OF SKELETAL STRESS

3.9.1 Lesion-based Assessments of Stress

One of the biggest challenges for osteologists is the variability in skeletal preservation, affected by numerous factors such as soil composition (Behrensmeyer 1978), predator disturbance (Marshall 1989), freeze thaw climatic cycles (Micozzi 1996), chemical breakdown (Gill-King 1996), skeletal age at death (Stojanowski et al. 2002), etc. As a result of these numerous factors, the archaeological record is inherently biased in what remains are initially discovered and of those how many are preserved well enough for osteological analysis (Waldron 1994). Particularly in the study of stress lesions, preservation can be a large limiting factor as some of these indicators are located on skeletal elements inherently delicate and prone to post-mortem loss or excavation damage (e.g. orbital plate fragility; the loss of teeth during excavation). While preservation issues can negatively affect both stress lesions and growth analyses of stress, the interaction between lesions associated with stress and taphonomic damage is arguably more difficult to discern in archaeological remains (Aufderheide 2011).

Another challenge with the study of stress lesions is the lack of standardization between the various methods of assessment. While multiple osteological studies have focused on identical stress lesions, the methods used and the theoretical foundation in which they are approached can differ, producing variable results and inconsistent interpretations (Lewis and Roberts 1997; Jacobi and Danforth 2002). As Jacobi and Danforth (2002) discussed, inter-observer error when assessing cribra orbitalia and porotic hyperostosis can be minimal between observers; however, more discrepancies and error are introduced when assessing more complex aspects of these

lesions such as the degree of healing. While there has been some attempt to improve this lack of standardization through new technologies and refined methodology (e.g. Suter et al. 2008; Naveed et al. 2012; Hassett 2014; Scott and Hoppa 2015), differing opinions in how these lesions can be approached and studied still produces variability in how stress is interpreted in archaeological remains. While this variability does not necessarily produce problems within a particular study, it can make cross-population and temporal studies difficult to compare as results produced using one method of assessment do not necessarily produce the same results when compared using a secondary method. In addition to this lack of consistent methodological approaches, there is also a growing disparity between comparing different stress indicators and their timeline of manifestation in the skeleton (McHenry and Schulz 1976; Maat 1984; Mays 1995; Ribot and Roberts 1996; Nowak and Piontek 2002; McEwan et al. 2005; Papageorgopoulou et al. 2011). While the causes of these stress lesions can range from malnutrition to parasitic infections (e.g. Sontag and Comstock 1938; Garn et al. 1968; Marshall 1968; Sweeney et al. 1969; Blanco et al. 1974; El-Najjar et al. 1978; Nikiforuk and Fraser 1981; Huss-Ashmore et al. 1982; Goodman and Armelagos 1988; Stuart-Macadam 1992; Walker et al. 2009) there is no definitive connection between certain stressors and resultant lesions, making this comparison challenging when researchers are trying to compare different lesion types to better explore the timeline of manifestation (Ribot and Roberts 1996). As discussed, the stress response is a heterogeneous process where biological tissues and skeletal elements are affected differently; therefore, there should not be an assumption that all skeletal stress lesions will be comparable.

Within this comparison of multiple stress-lesions there is also the challenge of intra-observer error and the consistency of researchers and their ability to accurately and precisely

identify these lesions. Because stress studies now focus on development stages when assessing lesions, observation has moved beyond a simple determination of presence or absence increasing intra-observer error. Additionally, intra-observer error is not consistent across these different lesion types, within intra- and interobserver error varying widely depending on the methods used and experience of the researcher (e.g. Danforth et al. 1993; Macchiarelli 1994). These intra- and interobserver error challenges may also be affected by age differences and employing similar methods of observation across all age groups. Whereas adult skeletal structure is dominated by lamellar or mature bone, subadult remains have more woven or immature bone which may be mistakenly identified as pathological when in reality it is healthy bone. Similarly, size and shape differences encountered when observing skeletal lesions across various age categories can distort the visualization of these stress indicators (e.g. Scott and Hoppa 2015).

There has also been an increased discussion of how these lesions form and if they are always associated with stress or poor health. For example, Papageorgopolou et al. (2011) argued that because Harris lines cannot be consistently associated with other indicators of stress, they may in fact represent normal growth events rather than stress events. Similarly, Alfonzo et al. (2005) and Alfonso-Durruty (2011) argued that hormonal shifts during maturation that speed up and slow down the growth process may lead to the formation of Harris lines. These studies are an important consideration in the osteological study of stress lesions as they address the assumption that all skeletal changes that deviate from expected patterns are unequivocally associated with stress or poor health. These theoretical considerations of stress lesions are warranted to ensure that the interpretations being made based on skeletal changes have biological validity.

3.9.2 Linear Growth Assessments of Stress

Archaeological speaking, osteologists are limited in how growth data is collected and measured. From a clinical perspective, growth and development studies usually focus on longitudinal measurements that allow the researcher to collect multiple data points from one individual over an extend period of time, effectively creating an individual growth curve (Eveleth and Tanner 1990; e.g. Maresh 1955, 1970). Archaeological studies of growth and development, however, must rely on cross-sectional data to create population growth curves. While cross-sectional studies have the advantage of displaying population trends of growth and maturation, the drawback of these datasets is a lack of information regarding individual growth velocity, as measurements are taken from multiple individuals at only one period of time, the time of death (Hoppa and FitzGerald 1999; Lewis 2007). Based on these cross-sectional population growth curves, archaeological populations are regularly compared to other archaeological samples or are compared to modern growth studies to assess changing patterns of maturation (Johnston and Zimmer 1989).

Some of the challenges associated with cross-sectional growth studies are difficulties in accurately comparing measurements between populations when overall growth patterns can differ based on genetics. It has been well-established that the growth and development sequence remains consistent across all human populations (Humphrey 1998); however, the timing at which certain growth landmarks are attained may differ as a result of genetic factors, geographical differences or temporal changes in growth patterns (King and Ulijaszek 1999). Because not all populations will have the same genetically predetermined adult body size and stature, comparing cross-sectional measurements of growth has been a consistent challenge for osteologists (Saunders 2008).

Methodological issues of aging and sexing techniques are also an important consideration for osteologists. Growth curves are the basis of growth studies with skeletal measurements usually plotted against chronological age to track maturation trends through the subadult years of life. Accurate determination of chronological age is of utmost importance, as poor assignment of age can greatly skew the growth curve of a population (Merchant and Ubelaker 1977). It has been well-established that dental formation is far less affected by stress than the remainder of the skeleton (El-Nofely and İşcan 1989; Smith 1991) and as such, subadult dentition is a key source of chronological age information over other skeletal data (Lewis and Garn 1960). While early studies used a combination of both skeletal and dental eruption criteria for aging subadult remains, later studies began to focus solely on dental formation patterns (Saunders 2008). For many of these later growth studies the Moorrees et al. (1963a,b), or the Schour and Massler (1944) aging techniques were used, but not consistently, as aging techniques are usually modified within each study to suit specific research questions.

The determination of sex can also hinder growth and development studies of subadults, particularly due to a lack of consistent and accurate subadult sexing techniques. While some techniques have been established within the literature (Gleiser and Hunt 1955; Hunt and Gleiser 1955; Boucher 1957; Weaver 1980), the majority of researchers do not attempt to assign sex to subadult remains. The limitation of this methodological challenge for studies of growth and development is the differing rates of maturation between males and females which has been well-established within the clinical literature (Eveleth and Tanner 1990; Bogin 2001). When creating a growth curve from subadult remains, the intermixing of males and females, especially during the adolescent period of maturation, may cause skewing of the data as females generally mature two years earlier than males (Bogin 2001). The majority of growth and development

studies focus on younger subadults before the onset of puberty; therefore, these sexual differences are minimized between the sexes but are still an important consideration for osteologists and their observations of growth pattern variation.

CHAPTER 4: MATERIALS AND METHODS

4.1 BLACK FRIARS SKELETAL MATERIAL

4.1.1 Selection Criteria

For this study, all skeletal data were collected from the Black Friars cemetery sample curated at the ADBOU facility, University of Southern Denmark. A total of 203 individuals (out of a maximum of 710) were examined with 152 adults and 51 subadults. For the adult individuals (over 20 years) the primary criterion for selection was preservation. This included preservation of the cranial vault and face in addition to the long bones and vertebral column. Adult individuals selected for this study were also those who had recorded archaeological information including a grave number and registry in the archaeological site report. For the subadults (birth – 20 years), all identified individuals with archaeological information were assessed; poorly preserved individuals were not excluded due to a reduced sample size.

4.2 ARCHAEOLOGICAL DATING OF THE BLACK FRIARS POPULATION

4.2.1 Arm Position

Using the original Black Friars site reports from 1979 and 1981, temporal classification of each individual was assigned on arm position, cemetery location and for some individuals relative dating techniques used at the time of excavation. The majority of dating that has been completed on the Black Friars cemetery sample has been based primarily on arm positioning and the distinct pattern that emerged during the Middles Ages (Kieffer-Olsen 1993; Jantzen et al. 1994). Four distinct arm positions have been identified: A) arms at the sides, B) hands on the pelvis, C) hands over the abdomen, and D) hands crossed over the chest (Kieffer-Olsen 1993; Jantzen et al. 1994). Position A is the earliest seen in the medieval period and was dominant up until the beginning of the 14th century. The transition from position A to B occurred during the

late part of the 13th century up until AD 1350 with the introduction of position C. It was not until the middle of the 15th century that position D was introduced, but was most prevalent in the later part of the 16th century (Mollerup and Boldsen 2010). As shown in Table 4.1 below, the Black Friars sample demonstrates a similar distribution of this arm position patterning spanning the medieval and post-medieval periods. For the Black Friars sample, only three positions were recorded at the time of excavation: 1) at sides; 2) over pelvis; and 3) over chest. It was therefore, assumed that individuals in the Black Friars population with arm position 2 likely represented both those defined by positions B and C described by Kieffer-Olsen (1993) and Jantzen et al. (1994), spanning the period between the late 13th century up until the middle of the 15th century. The subtle differences between positions B and C as discussed by Kieffer-Olsen (1993) and Jantzen et al. (1994), were likely not distinguished at the time of the Black Friars excavation as the four-position classification was published after excavation was complete.

Table 4.1 Distribution of Arm Positions between Time Periods in the Black Friars Cemetery Sample

Time Period	**Arm Position***			
	1 (beginning of 14th cen.)	**2** (late 13th to middle 15th cen.)	**3** (late 16th cen.)	**Unknown**
Medieval	21	29	0	11
Post-medieval	7	80	37	18
Total	28	109	37	29

* Arm position designations based on Kieffer-Olsen (1993) and Jantzen et al. (1994) classifications. Position 1 = A; Position 2 = B and C; Position 3 = D

4.2.2 Burial Location

Burial location within the cemetery was also used to aid in temporal classification as those buried within the church and to the east were most predominantly from the medieval period and those recovered in the central section of the cemetery complex and the church

courtyard were largely post-medieval (Boldsen and Mollerup 2006; Mollerup and Boldsen 2010). Using arm position (when available) and geographic location, burials were assigned a temporal classification. Of the 152 adult individuals studied, 147 were classified as either medieval or post-medieval using site report data. Similarly, of the 51 subadult individuals, 48 were classified as medieval or post-medieval, as shown in Table 4.2 below.

The remaining five adults and three subadults were without arm position data as they were excavated in the 1978 field season and no site report was available. However, site maps did show that the 1978 excavation season only removed burials from the east side of the church complex that fell within the medieval burial region outlined by Mollerup and Boldsen (2010). Therefore, these individuals were confidently classified as medieval.

Table 4.2 Distribution of Adults and Subadults between Time Periods in the Black Friars Cemetery Sample

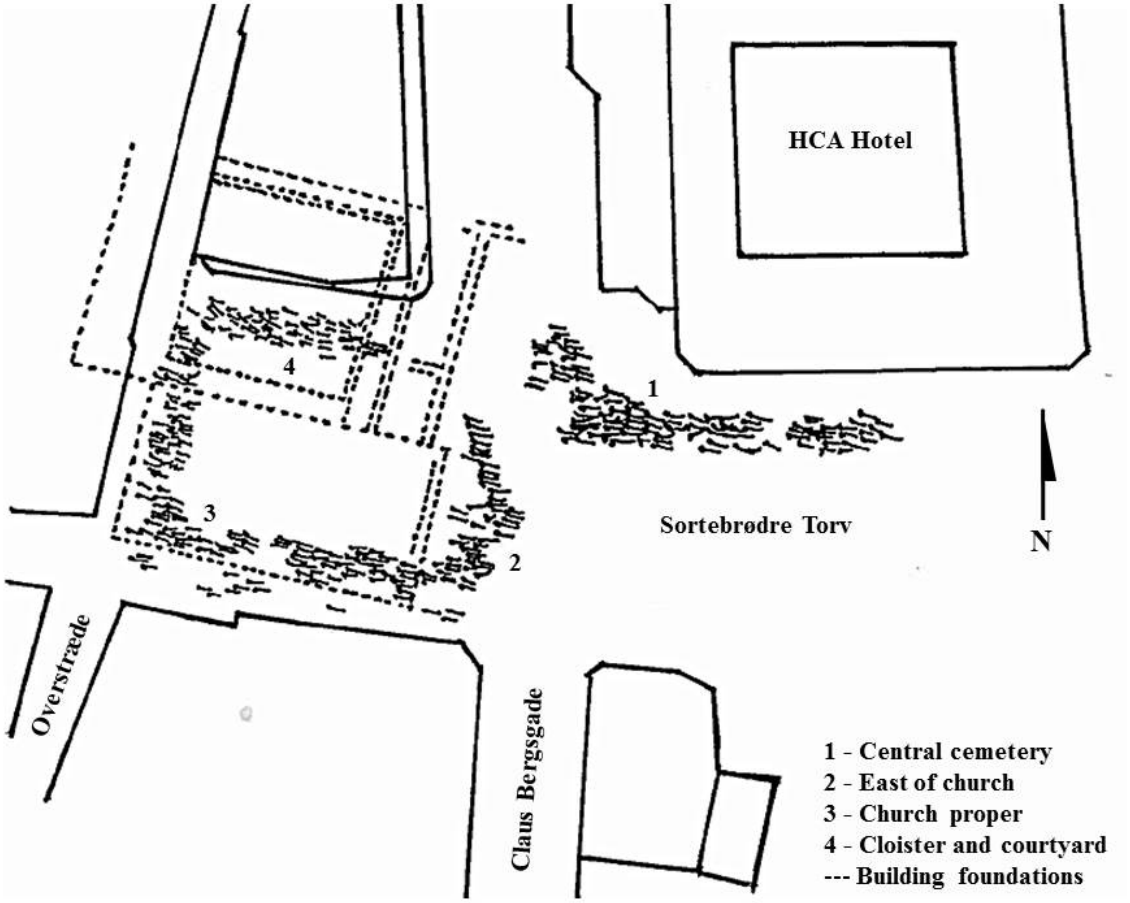
Time Period	Adults	Subadults	Total
Medieval	52	9	61
Post-medieval	100	42	142
Total	152	51	203

4.2.3 Relative Dating Techniques

For all 1979 and 1981 burials, in addition to arm positioning and burial location, relative dating methods used at the time of excavation and recorded in the site reports were also used. Relative dating was based on grave position in relation to the surrounding church structures that could be accurately dated and compared with other surrounding graves dated at the time of excavation. In circumstances where relative dating designation in the site reports disagreed with the burial location and arm positioning designations, the relative dating method was used for classification. This disagreement occurred for 13 burials, where temporal classification was based on relative dating; ten burials were moved from medieval to post-medieval classification

and three burials were moved from post-medieval to medieval classification. In switching these individuals based on the site report data, it is important to note that the arm positioning still agreed with these shifts as there is great overlap between these distinct phases. Further, many of these individuals were located along the perimeter of geographically ambiguous regions where medieval and post-medieval burials may have overlapped; therefore, their shift from one temporal period to another is not inconsistent with what might be expected in a cemetery with such a long burial history as Black Friars (Figure 4.1).

Figure 4.1 Black Friars Site Map



(Modified from Nielsen 1982, with permission from Odense Bys Museer).

Further, the distribution of medieval and post-medieval individuals in this Black Friars sample is similar to the temporal distribution reported by Mollerup and Boldsen (2010), with the

highest number of medieval burials located inside the church proper and directly east of the church complex, and the greatest number of post-medieval burials located in the central cemetery region and the church courtyard and cloister regions. Whereas the central cemetery and church proper designations of this research match closely with Mollerup and Boldsen (2010) there is some discrepancy between the medieval and post-medieval burials located inside the church courtyard and cloister walk regions. Because the designation between these two regions was difficult to discern using the archaeological excavation reports these two regions needed to be combined; possibly skewing the number of medieval and post-medieval burials that have been previously reported in this area. The burials located east of the church also show some discrepancy with higher post-medieval burials in the Mollerup and Boldsen study. Again, this is likely the result of some individuals being buried on or near the threshold of the church complex. For this study, only 19 individuals east of the church were used as compared to the 128 selected in the Mollerup and Boldsen study; therefore, it is not surprising that there is a difference between the patterns emerging. Significant to note, however, is that despite the differences between the number of medieval and post-medieval graves present in this region, both studies do show a similar pattern with more medieval burials located east of the church compared to post-medieval burials, as shown in Table 4.3 below. Additionally, it was not possible to confirm the burials used in the Mollerup and Boldsen analysis and it can be assumed that not all of the same individuals were selected; therefore, slight differences in temporal patterns based on burial geography would be expected.

Table 4.3 Comparison of Burial Location by Time Period in the Black Friars Cemetery Sample

Present Study		
Burial Location	**Medieval**	**Post-medieval**
Central cemetery	2%	98%
East of church	74%	26%
Church proper	76%	24%
Cloister and courtyard	5%	95%

Mollerup and Boldsen (2010:42)		
Burial Location	**Medieval**	**Post-medieval**
Central cemetery	9%	91%
East of church	55%	45%
Church proper	78%	22%
Cloister and courtyard	17%	83%

4.3 SEX DETERMINATION AND AGE ESTIMATION

For each individual a standard osteological inventory sheet was completed in addition to photographs taken in the anatomically correct position. Skeletal elements were recorded as present or absent with the level of fragmentation also recorded. For all adult individuals sex was assigned using both pelvic and cranial scoring methods. Pelvic traits included the ventral arc, subpubic concavity, ischiopubic ramus ridge (Phenice 1969), the greater sciatic notch (Buikstra and Ubelaker 1994; Walker 2005), preauricular sulcus (Milner 1992), arc composé or composite arch (Genovés 1959; Bruzek 2002), subpubic angle (White and Folkens 2005), and sacrum curvature (St. Hoyme and İşcan 1989). Cranial traits included the nuchal crest, mastoid process, supra-orbital margin, glabella, mental eminence (Buikstra and Ubelaker 1994). After amalgamating the pelvic and cranial scores, if there was discrepancy between the two in terms of sex determination, favour was granted to the pelvic assignment of sex. Overall skeletal structure

(i.e. robusticity) was also assessed when assigning sex, but was always considered with regard to the pelvic or cranial scores. Table 4.4 shows the sex distribution of the Black Friars sample based on temporal period, for all adult individuals it was possible to determine sex. Sex was not determined for any of the subadult individuals due to inconsistent results in the established methods of analysis (see Cox and Mays 2000).

Table 4.4 Distribution of Sex between Time Periods in the Black Friars Cemetery Sample

Time Period	Female	Male	Total
Medieval	23	29	52
Post-medieval	55	45	100
Total	78	74	152

Adult age was determined for each individual using the pubic symphyses (Todd 1921; Brooks and Suchey 1990) and the auricular surface (Lovejoy et al. 1985; Meindl and Lovejoy 1989). Because these methods have limitations, particularly in accurately aging older adults with arbitrary age divisions, these standard osteological methods were further supplemented with aspects of the Transition Analysis method (Boldsen et al. 2002; Milner and Boldsen 2012). Transition Analysis was not used as the primary method for age estimation as it is not as accurate as experience-based assessments of individual skeletons (Milner and Boldsen 2012). Therefore, attempts were made to integrate supplementary skeletal elements aside from the pelvis when estimating age, as discussed by Milner and Boldsen (2012). In particular, femoral aging techniques were used to secondarily assess the age estimates given to the oldest adult individuals, as pelvis traits have been consistently shown to under-estimate the oldest individuals in a population (Hens et al. 2008; Jackes 2011). Fovea capitis lipping and spicule formation along the greater trochanter were used (Milner and Boldsen 2012). In the few instances where

femoral age was greater than the estimate made from the pelvic characteristics, the femoral age took precedent. For all adult individuals age estimation was possible.

For all subadult individuals age estimation was based on dental formation and eruption as well as epiphyseal closure. A standard dental inventory was completed for all subadult individuals identifying all teeth present, teeth lost ante-mortem, and root development in loose teeth that could be removed from the alveolar bone. Tooth formation and root development were assessed using the Moorrees and colleagues (1963) standards. These standards were chosen for their ease of use and the continued evolution and refinement in various studies (i.e. Demirjian et al. 1973, Demirjian and Goldstein 1976, Anderson et al. 1976). Tooth eruption was based on the Ubelaker standard (Ubelaker 1989). While this Ubelaker standard was constructed based on Native American populations, its use in this research was only to determine a wide age range that was further refined using root development standards, as well as supplementary data from epiphyseal development and closure throughout the skeleton. Because dental formation is considered the least susceptible to episodes of stress (Garn et al. 1965) and therefore a fairly reliable indicator of subadult age, formation was considered the primary source for age estimation with eruption used as a secondary mode of assessment. In instances of excellent preservation the majority of epiphyses throughout the skeleton could be assessed and recorded including the long bones, the vertebral column, and bones of the cranial vault, especially in young infants. Epiphyseal closure timing was estimated from the Schaefer et al. (2009) summary standards and only fusion ages were recorded, not appearance ages. Because taphonomy (e.g. soil pH) and varying bone densities in subadult remains contribute to the absence of epiphyseal bones in the archaeological record (Gordon and Buikstra 1981), it was difficult to determine if the epiphysis had not yet appeared or was simply not recovered and therefore omitted. Age

estimation was possible for all subadult individuals. Using the same age classifications as Chamberlain (2000), the adult and subadult age distribution in the Black Friars sample is shown below (Tables 4.5 and 4.6), with a further division of the subadult data into developmental categories outlined by Bogin (2001).

Table 4.5 Age Distribution between Time Periods in the Black Friars Cemetery Sample

Age (years)	Medieval	Post-medieval	Total
0-14	8	37	45
15-29	16	36	52
30-44	33	63	96
45-59	4	5	9
60+	0	1	1
Total	61	142	203

Table 4.6 Age Distribution in Subadults between Time Periods in the Black Friars Cemetery Sample

Age (years)	Medieval	Post-medieval	Total
0-1	0	8	8
2-4	2	3	5
5-7	3	4	7
8-13	3	21	24
14-18	1	6	7
Total	9	42	51

4.4 PATHOLOGICAL ANALYSIS

For both adults and subadults, a visual pathological assessment was completed with a specific focus on diseases known to affect medieval Danish populations, including tuberculosis, treponemal disease, and leprosy (e.g. Møller-Christensen 1953, 1958, 1961, 1967, 1978; Bennike 1985; Lefort and Bennike 2007; Boldsen 2005, 2008; Boldsen and Mollerup 2006). The criteria outlined in these studies and Ortner (2003) were used to assess each individual with additional

guidance from members of the ADBOU research team (Dr. Jesper Boldsen, Ulla Høg Freund, and Peter Tarp). Additionally, visual assessments to identify trauma (e.g. fractures), degenerative disease (e.g. osteoarthritis), dental disease (e.g. caries), and other skeletal anomalies were also made for all adult and subadult individuals. Once this data was collected in a qualitative format it was translated into quantitative scoring for ease of comparison. Pathology categories included: periostitis, tuberculosis, leprosy, treponema, fractures, degenerative spine changes, Schmorl's nodes, degenerative joint changes (excluding the spine), and dental abscesses. Using the qualitative descriptions of these pathological conditions all adult and subadults were given a score for each of the outlined pathologies: 0 (none) – no pathology visible, or 1 (possible) – either confirmed or possible pathology present. Because the focus of this dissertation was on indicators of skeletal stress, scoring these pathological conditions in a simple classification was considered sufficient to assess the interaction between these conditions and skeletal indicators of stress. A detailed description of pathological changes was recorded at the time of initial observation along with photographs; therefore, this data may be revisited for further analysis in the future.

4.5 STRESS LESION DATA

For all adult and subadult individuals stress indicators, including enamel hypoplastic lesions, cribra orbitalia, porotic hyperostosis, and Harris lines were analyzed and recorded. These particular lesions were chosen for this study as their use indicate periods of stress has been well-established in the osteology literature (e.g. Angel 1966; Park 1964; Garn et al. 1968; Sweeney et al. 1969; Pindborg 1970; Steinbock 1976; El-Najjar et al. 1978; Nikiforuk and Fraser 1981; Huss-Ashmore et al. 1982; Goodman and Armelagos 1985; Goodman and Armelagos 1988;

Stuart-Macadam 1989; Goodman and Rose 1990; Mays 1995; Lewis and Roberts 1997; Walker et al. 2009).

4.5.1 Cribra Orbitalia (CO) and Porotic Hyperostosis (PH)

Cribra orbitalia (CO) and porotic hyperostosis (PH) were similarly scored for adult and subadult individuals as it has been argued that both lesions have a similar etiology characterized by cortical destruction (Angel 1966; Stuart-Macadam 1989). Cribra orbitalia and porotic hyperostosis were scored only if the lesions were visible to the naked eye and could be confirmed through the use of a hand lens if necessary. Similar to the recording conditions of EHL, both CO and PH were observed in bright natural light supplemented by the use of a standard desk lamp. CO and PH scoring was based on the classification scheme of Stuart-Macadam (1982) and supplemented by the photographs and descriptions by Ribot and Roberts (1996). This classification scheme was selected for this research for its ease of use and repeatability and its standardization in osteological studies. Each individual was scored in one of 5 categories: absent (0) – no evidence of lesions; mild (1) – slight pitting, no expansion of bone, scattered foramina; moderate (2) – scattered large and small foramina, some coalescence of foramina, no expansion of bone; advanced (3) – foramina have linked to trabecular structure, substantial coalescence, some expansion of bone; and severe (4) – severe pitting, gross expansion of bone, trabecular exposure, substantial coalescence of foramina, deformation of normal contour (after Stuart-Macadam 1982, and Ribot and Roberts 1996) (Appendix A.1 and A.2). All 152 adults and 21 subadults were scored for both CO and PH. Seventeen subadult individuals had either CO or PH scores and 13 individuals had no CO or PH data due to absent skeletal elements. Repeated observations of CO and PH were also made to test intra-observer

error selecting the same 21 adult individuals used for the EHL rechecks and five of the 17 subadults individuals.

4.5.2 Enamel Hypoplastic Lesions (EHL)

For the analysis of enamel hypoplastic lesions (EHL), all present teeth, both deciduous and permanent, were scored. An EHL defect was only counted if it was visible with the naked eye, but could be confirmed through the use of a hand lens if necessary. All lesions were observed in bright natural light and supplemented with a standard desk lamp. For each adult and subadult individual a standard inventory of all teeth was completed including data on teeth lost ante-mortem and those absent for study. All visible lesions were then recorded onto the dental inventory chart with each tooth classified into a specific grouping category. All teeth were scored at the time of initial data collection with the knowledge that only specific teeth would eventually be used for this study, this was done in the event that further analysis required the assessment of different teeth or in the event of using a different method of analysis. Due to the difficulty of observing these small enamel defects, grouping raw counts into categories was done to decrease the potential of intra-observer error. The classification categories were as follows: absent (0) - no evidence of defects; mild (1) - maximum of one defect present; moderate (2) – two to three defects present; severe (3) – more than three defects present (Appendix A.3). While all teeth can show evidence of EHL it was decided that the best tooth for identification was the mandibular canine, left, which was preferentially chosen to remain consistent throughout the sample. When absent, the right canine was used. This decision was based on the fact that the anterior teeth show the most susceptibility to these defects (Cutress and Suckling 1982; Goodman and Rose 1990) and have the longest period of enamel formation in medieval Danish populations (Reid et al. 2002; Reid and Dean 2006; Reid and Ferrell 2006). This long period of enamel formation allows

the mandibular canine to capture the most data during the developmental period. When the mandibular canines were not present or damaged, the left central incisor was selected as it also shows a long maturation period (Reid and Dean 2006). If the central incisor was missing the maxillary canine was used followed by the maxillary premolar. As shown in Tables 4.7 and 4.8 below, the majority of adult and subadult individuals had EHL data for the mandibular canine, central incisor, or maxillary canine which cover the longest developmental period 1.5 - 6.2 years, 1.1 - 5.0 years and 1.7 - 5.3 years, respectively (Reid and Dean 2006). Boldsen (2007) argues that the use of multiple teeth introduces bias as one stress event may manifest on multiple teeth inflating the EHL count. Therefore, this study only used one tooth for each individual, eliminating the possibility of double counting the same stress event. Further, all teeth selected had a similar developmental time period arguably providing similar data with minimal bias. In three individuals (two adult, one subadult) the maxillary premolar was used for EHL analysis as there were no other teeth present. There were six adult and 15 subadult individuals who had no dental data.

Table 4.7 Distribution of Adult Teeth used in EHL Assessment

Tooth	Mandibular canine	Maxillary central incisor	Maxillary canine	Maxillary premolar 1
Adults n=152	105	27	12	2

No data n=6

Table 4.8 Distribution of Subadult Teeth used in EHL Assessment

Tooth	Mandibular canine	Maxillary central incisor	Maxillary canine	Maxillary premolar 1
Subadults n=51	31	3	1	1

No data n=15

Repeated observations of EHL were also made to assess intra-observer error. Twenty-one adults and 17 subadults that covered the entire duration of data collection were selected. In testing a

non-random sample, the goal was to assess any identifiable changes between observations taken at the beginning of the project to observations taken after the learning curve period. A third round of rechecks was also conducted on the 21 adult individuals.

4.5.3 Harris Lines (HL)

Harris lines (HL) were scored for all adult and subadult individuals using radiographic analysis of the left and right radii and tibiae. Initially 34 adults and 23 subadults were radiographed in both an anterior-posterior (AP) and medial-lateral (ML) orientation. Because of time constraints it was assumed that taking radiographs in only one orientation (standard AP) would be sufficient. However, early analysis of these adult and subadult subsamples demonstrated that the ML view provided optimum visibility to identify all HL present and therefore, better accuracy for analysis (Scott and Hoppa 2015). During a subsequent visit to Denmark, the remainder of the adult individuals were then radiographed in the ML view; however, time limitations and inconsistent results from the preliminary study did not warrant further radiographs for the remaining subadult individuals. In total, 141 adults and 23 subadults were radiographed in both the AP and ML orientations. All radiographs were taken at the Odense University Hospital by radiograph technicians on a Siemens Ysio machine. The standard source to bone distance was 115 cm with adult KvP and mAs values ranging between 49-52 and 3.0-3.2, respectively and subadult kVp and mAs values ranging between 46-52 and 1.3-3.1, respectively. These ranges allowed for better image capture and were adjusted by the radiographic technicians based on bone preservation, weight/density, and overall size, particularly in the subadults. Once captured, the radiographic images were exported from their DICOM format into the Materialize MIMICS software program. Each individual image was then observed and scored. HL were only counted if they met two standard criteria: 1) the line crossed at least half of the diaphyseal shaft,

and 2) the line was predominately transverse in orientation (Mays 1985; Clark 1981; Mays 1995). All adult and subadult individuals were first scored in the AP orientation followed by the ML orientation with data kept blind between observations; this was done to ensure no biases between assessments. All radiographs were magnified to 200 percent in a standard position when viewed in MIMICS to ensure consistency during analysis. The thresholding function, used to demarcate areas of increased density in the image, was also used when identifying HL. While this function was used to help identify faint lines within each image, it was not used exclusively to determine when HL were present. Based on the previous study by Scott and Hoppa (2015), Harris lines were grouped into categories reflecting differing levels of severity: absent (0) - zero lines present; low (1) – one to three lines present; moderate (2)- four to eight lines present; and high (3) - nine or more lines present (Appendix A.4). While both the tibiae and radii were radiographed, the distal tibia was selected for the assessment of HL due to its higher prevalence for the formation of these lines and its continued use in osteological studies (e.g. Follis and Park 1952; Park and Richter 1953; Park 1964; Garn et al. 1968; Hughes et al. 1996). For the adults, 135 individuals were scored in the ML view using the left distal tibia and replaced with right when absent, and nine scored in the AP view. Eight adult individuals had no data available.

For the subadult individuals there was no consistent pattern regarding orientation that emerged in the preliminary study (Scott and Hoppa 2015); therefore, the AP orientation was used to establish consistency as the majority of subadult individuals only had data in this orientation. Using the left distal tibia and replacing with the right when absent, 40 subadults were analyzed in the AP view, and 11 individuals had no radiographic data.

To assess intra-observer error 25 adults and 10 subadults with radiographs in both orientations were selected for a second round of observations. Similar to the original scoring

protocol, all individuals were first observed in the AP orientation followed by the ML orientation with all scoring data kept blind between the rounds of observation. The 35 individuals chosen for HL intra-observer error were not the same individuals used for the EHL, CO, and PH rechecks. The individuals for HL were chosen at random as all observations were made within a similar time period and did not exhibit the same time depth between observations as the other indicators of stress.

4.6 GROWTH AND DEVELOPMENT DATA

4.6.1 Body Size Indicators (BSI)

In addition to the standard stress indicators, body size indicator (BSI) and cortical thickness data were also collected for all adult individuals. Introduced by Scott (2009), the BSI method uses a suite of 56 skeletal measurements that correlate with overall body size (16 measurements from the cranium and 40 measurements from the infra-cranial skeleton). The standard osteological measurements established by Moore-Jansen et al. (1994) were primarily used for the cranial and long bone measurements, with more specific body size measurements taken from descriptions found in Trotter and Gleser (1958); Anderson et al. (1977); McHenry (1992); Aiello and Wood (1994); Holland (1995); Porter (1999); Ruff (2002); Raxter et al. (2006); and Spocter and Manger (2007). Measurements were only collected if bone preservation was adequate and specific landmarks could be consistently determined. Both digital sliding calipers and spreading calipers were used for all cranial measurements, vertebral measurements and some long bone measurements. A standard osteometric board was used for all long bone lengths, including the talus and calcaneus with a flexible measuring tape used for midshaft circumferences.

Once collected, all left side measurements were used to determine if a linear correlation was present between each BSI. To assess these correlations, individuals were divided into sex groupings as size and shape variations between males and females may affect the correlation between these measurements. Only BSIs with an R-value of 0.60 (60%) or higher with at least two other measurements were kept to ensure the highest correlation between the BSIs selected. From this initial correlation analysis using all original 56 BSI measurements (Scott 2009), 31 infra-cranial and four cranial BSIs met the R-value criterion in the Black Friars female group and 29 infra-cranial and one cranial BSI in the Black Friars male group. These correlated BSIs were then further reduced by eliminating measurements with an overlapping maturation timeline or measurements that were redundant (e.g. femur midshaft width and femur midshaft circumference). The final BSI list consisted of 20 measurements for females and 19 measurements for males. Once established, the male and female BSI measurements were then ordered chronologically based on established maturation timing (Schaefer et al. 2009) (Tables 4.9 and 4.10). While the timeline of maturation may shift in different populations based on a variety of factors, the *sequence* of maturation remains fixed (Humphrey 1998); therefore, using standard maturation timing to determine the chronological ordering of each BSI measurement was considered sufficient for this analysis.

Table 4.9 Female BSI Measurements in Chronological Maturation Sequence

Female BSI Measurements	Maturation Age Average (yrs) (Schaefer et al.2009)
BSI1 - maximum length of talus	9.0
BSI2 - transverse diameter of tibial facet of talus	9.0
BSI3 - distal humerus epiphysis breadth	11.0
BSI4 - maximum humerus length	11.5
BSI5 - femur midshaft circumference	12.0
BSI6 - tibia midshaft circumference	12.0
BSI7 - transverse diameter of radius head	12.3
BSI8 - maximum radius length	14.0
BSI9 - maximum superior/inferior diameter of femoral head	14.0
BSI10 - maximum femur length	14.0
BSI11 - second metacarpal length	14.3
BSI12 - anteroposterior diameter of humeral head	15.0
BSI13 - maximum tibia length	15.0
BSI14 - proximal tibia breadth (plateau)	15.0
BSI15 - maximum length of calcaneus	15.5
BSI16 - maximum ulna length	16.0
BSI17 - biepicondylar diameter of distal femur	16.0
BSI18 - T12 anteroposterior diameter of superior surface	20.0
BSI19 - L1 anteroposterior diameter of superior surface	20.0
BSI20 - L5 anteroposterior diameter of superior surface	20.0

Table 4.10 Male BSI Measurements in Chronological Maturation Sequence

Male BSI Measurements	Maturation Age Average (yrs) (Schaefer et al.2009)
BSI1 - femur midshaft circumference	12.0
BSI2 - tibia midshaft circumference	12.0
BSI3 - maximum humerus length	13.5
BSI4 - distal humerus epiphysis breadth	13.5
BSI5 - second metacarpal length	16.3
BSI6 - transverse diameter of radius head	16.5
BSI7 - maximum fibula length	17.0
BSI8 - maximum radius length	17.5
BSI9 - femur head breadth	17.5
BSI10 - biepicondylar diameter of distal femur	17.5
BSI11 - maximum femur length	17.5
BSI12 - maximum tibia length	18.4
BSI13 - proximal tibia breadth (plateau)	18.4
BSI14 - anteroposterior diameter of humeral head	18.5
BSI15 - maximum ulna length	18.5
BSI16 - max. length of calcaneus	19.0
BSI17 - T12 transverse diameter of superior surface	20.0
BSI18 - L1 transverse diameter of superior surface	20.0
BSI19 - L5 transverse diameter of superior surface	20.0

Once the BSI maturation sequence was complete, all measurements were chronologically paired and analysed using regression analysis with the earlier maturing variable on the x-axis and the later maturing variable on the y-axis. Because each BSI measurement only needed to be correlated with two other measurements to be included in the final BSI list, not all chronological pairs were correlated; therefore, only BSI pairs that met the correlation criterion (R-value >0.60) were analyzed. For females, 113 regression pairs were analyzed and for males 54 pairs. Using a

95% confidence interval for each chronological pairing it was possible to visually identify if an individual fell within, above, or below the confidence interval for each of the BSI pairs. Analyzing all BSI pairings in this chronological order then, allowed for an assessment of growth fluctuations between specific maturation periods. These regression data were then recorded to visually illustrate the periods of individual growth fluctuation. Because each BSI variable was compared to multiple other variables with overlapping periods of maturation, a summary designation was assigned to each BSI based on how it fluctuated when compared to other BSI measurements. Below in Table 4.11 is a regression analysis excerpt (SBT81 GR212) demonstrating the results of the analysis and the determined summary designation for each BSI variable. The first column represents the multiple variable pairings determined from the correlation analysis results; the second column represents the focus variable (i.e. the later maturing variable driving the regression analysis results); the third column represents the physical position of the focus variable from the regression analysis (i.e. within the 95% confidence interval (0), above the confidence interval (+) or below the confidence interval (-)); and the fourth column represents the summary designation for each variable.

Table 4.11 An example of a BSI Pattern Map with Summary Designation for Focus Variables (SBT81 GR212)

Variable Pairs (Female)	Focus Variable	Regression Analysis Results	Summary Designation
BSI1 compared to BSI2	2	+	+
BSI1 compared to BSI3	3	0	0
BSI2 compared toBSI3		-	
BSI1 compared toBSI4	4	-	-
BSI2 compared to BSI4		-	
BSI3compared to BSI4		-	
BSI1 compared to BSI5	5	-	-
BSI3 compared to BSI5		-	
BSI4 compared to BSI5		-	
BSI1 compared to BSI6	6		
BSI4 compared to BSI6			
BSI5 compared to BSI6			
BSI1 compared to BSI7	7	+	+
BSI2 compared to BSI7		0	
BSI3 compared to BSI7		+	
BSI4 compared to BSI7		+	
BSI5 compared to BSI7		+	
BSI3 compared to BSI8	8	-	-
BSI4 compared to BSI8		-	
BSI1 compared to BSI9	9	0	0
BSI2 compared to BSI9		-	
BSI3 compared to BSI9		0	
BSI4 compared to BSI9		+	
BSI5 compared to BSI9		+	
BSI6 compared to BSI9			
BSI7 compared to BSI9		-	
BSI8 compared to BSI9		+	

Table 4.12 BSI Summary Designation Code

Early Maturing BSI	Later Maturing BSI	Summary Designation
0	+	+
0	-	-
0	0	0
-	+	0
-	-	-
-	0	-
+	+	+
+	-	0
+	0	+

To determine the summary designation for each BSI, a simple code was created to ensure consistency and repeatability in the method. As shown in Table 4.12, the code was based on the relationship between the earlier and later maturing BSIs and how the overall size of the earlier maturing BSI affected where the later maturing BSI fell within the regression analysis. With multiple BSI pairs displaying different regression results, the final summary designation was based on the most consistent classification assigned to each BSI using the code in Table 4.12. This designation summary was completed for all individuals using this method and a final calculation was completed to determine the proportion of variables that fell above and below the confidence interval within each time period and sex group.

Intra-observer error was not assessed for the BSIs as consistency and precision was already established during previous research (Scott 2009). However, to ensure consistency between individuals, measurements that were difficult to capture (e.g. maximum width of proximal tibia across plateau) were reviewed and practiced in advance of data collection.

4.6.2 Cortical Bone Thickness

Using the HL radiographs, cortical bone thickness was also recorded for each adult individual in both AP and ML orientations when possible. Cortical thickness was captured at the midshaft of the tibia and radius (both sides) using the MIMICS software measurement tool at a 200 percent magnification to remain consistent across individuals. The AP value was calculated as the average of the anterior and posterior measurements and the ML value was calculated as the average of the medial and lateral measurements. The left tibia was used to calculate the average AP and ML thickness and replaced with the right side when absent. For the adults, 134 individuals had data for both AP and ML cortices, nine individuals had ML data only and nine individuals were unobservable. Cortical thickness measurements were not captured for the subadults as inconsistencies related to age, sex and growth introduce extensive bias into the analysis. Intra-observer error was not conducted for cortical thickness as these measurements were captured along the clearly demarcated edges of the outside cortical bone and the inside medullary cavity.

4.7 BIOCHEMICAL DATA

In addition to using growth- and lesion-based data to assess stress, osteocalcin was also identified as a potential indicator of poor health because of its relationship with bone formation and glucocorticoid release as discussed in Chapter 3 Section 3.5.1 and 3.5.2. This study of osteocalcin was a pilot project to test extraction protocol and analytical potential by comparing any results with the observed macroscopic skeletal disruptions associated with stress. Twenty adult individuals were chosen for this preliminary biochemical exploration (Appendix B.1). Selection was based on preservation, as poor preservation has been shown to negatively affect protein extraction in archaeological samples (Brown and Brown 2011). Only adult remains were

used for this preliminary analysis as fluctuating growth patterns during the subadult years have been shown to interfere with osteocalcin production (Gundberg et al. 2002), making it difficult to identify if osteocalcin fluctuations are reflecting stress events or normal growth cycles. For each of the 20 adults, two samples were collected, one from the femur and one from the clavicle. Two samples were taken to test the argument that glucocorticoids affect regions of the skeleton differently based on receptor density in the surrounding soft tissues (Weinstein et al. 1998). Therefore, the femur was used as a proxy for the appendicular skeleton and the clavicle as a proxy for the axial skeleton. While more centrally located skeletal elements would have been preferred for the axial comparison, the thin cortical bone found on the ribs, vertebrae, sternum and pelvic bones were not suitable for sample collection. All femoral samples were taken on the posterior portion of the shaft inferior to the lesser trochanter. All clavicle samples were taken on the inferior portion of the shaft medial to the conoid tubercle. All samples were collected at the ADBOU laboratory under the supervision of Dr. Lillian Skytte, Institute of Physics, Chemistry and Pharmacy at the University of Southern Denmark. Approximately 50mg of bone powder was harvested from the cortical bone of the femur and clavicle in each individual using a standard electrical Dremel (model 225). The bone surface was first cleaned by removing the outer layer of cortical bone with the Dremel approximately 1cm by 3cm, followed by a sterilization of the Dremel tip before the sample was collected. The tip was first cleaned in distilled water and sterilized in an ethanol flame. The Dremel was allowed to cool before the bone sample was collected. All samples were collected onto aluminum foil and then directly funneled into a glass vial. All attempts were made to keep the bone powder free of contaminants from the surrounding boney tissue at the sample site, especially loose dirt fragments.

In addition to these 20 individuals, bone powder and a raw bone sample was also procured from the surface collection remains of the Aarhus Vestergade Site to use as a control. Contemporaneous to the Black Friars cemetery, the femoral sample taken from the Aarhus Vestergade site was used to establish and test the osteocalcin extraction protocol in advance of the Black Friars analysis. Approximately 100mg of bone powder was collected from the Aarhus femur using the Dremel method discussed above, and an additional 100mg was harvested by hand at the University of Manitoba, BDIAL Laboratory. To test the impact of heat (generated from the Dremel) on protein preservation, a preliminary test study was done comparing the amount of protein extracted from heat-harvested bone powder, versus hand crushed bone powder. The hand crushed bone samples were collected by using a hacksaw to remove a small portion of the cortical bone from the femur. Once removed this small bone fragment was put into an ultrasonicator bath of distilled water for 45 minutes until the water ran clear and was left for 24 hours to dry completely. This bone sample was then crushed by hand using a marble mortar and pestle and crushed until the powder was a similar consistency to the Dremel collected samples.

4.7.1 Protein extraction

All biochemical analysis was conducted at the Manitoba Centre for Proteomics & Systems Biology at the John Buhler Research Centre at the University of Manitoba. Dr. Neeloffer Mookherjee and Grace Choi (Department of Immunology) were responsible for establishing the protocol for the biochemical analysis and training. In advance of using the Black Friars sample for biochemical extraction, the Aarhus Vestergade femoral sample was used to test the extraction protocol, discussed below. Following this successful protocol development stage, all 40 Black Friar samples were then analyzed. The protein extraction protocol developed was

based on the methods outlined by Cleland et al. (2012) with ethylenediaminetetraacetic acid (EDTA) selected as the extraction agent over hydrochloric acid (HCL), as the stable pH of EDTA retains the structure of the proteins better than HCL in bone material. For this preliminary step of extraction all 40 samples were treated with an EDTA and protease inhibitor cocktail (PIC) mixture; 9000µl of EDTA was added to 90µl of PIC. The EDTA contributes to the demineralization of the boney tissue encasing the proteins, while the PIC renders the protease in the bone non-functional which halts the protein degradation process. 200µl of this EDTA PIC mixture was then added to each of the 40 vials and then vortexed to ensure the EDTA PIC mixture saturated as much of the bone powder as possible. All samples were then continually oscillated at 4 degrees Celsius for 24 hours. Following this 24-hour oscillation period, the samples were centrifuged at 4 degrees Celsius for 10 minutes at 10,000xg relative centrifugal force (RCF). Following this step, the supernatant was collected off of the top of each vial with 5µl being added to 40 small vials and the reminder to another 40 larger vials. The supernatant was divided like this so that the small vials could be used for the microBCA process and the remaining larger vials for the ELISA analysis. After dividing the supernatant samples each day, all vials were kept at -80 degrees Celsius and kept on ice once removed from the freezer this was to minimize the freeze-thaw process. This supernatant collection process was repeated for 4 days.

Once the supernatant was collected, all samples were filtered to remove any EDTA still present. Using Amicon Ultra-0.5 centrifugal Filter Devices, each filter membrane was cleaned to remove the glycerine used to keep the filters moist before laboratory use. Using 400µl of phosphate-buffered saline solution (PBS), each filter tube was centrifuged for 15 minutes at 13,000xg RCF. After this initial cleaning, 500µl of each supernatant sample was added to the

cleaned filters and centrifuged for 15 minutes at 13,000xg RCF. After this initial filter of the supernatant samples, 350µl of PBS was added to the filter tubes to rinse the membranes and centrifuged for 15 minutes at 13,000xg RCF. After this secondary cleaning the clarity of the samples was visually assessed. Because many samples still had a yellowish colour, the samples were again filtered for a total of three cleaning cycles. While not all samples were consistently yellowish, all were filtered the same so as to not introduce variability in the established protocol between certain samples. Once the filtering was complete, approximately 50µl of each sample was removed from the filter tubes and transferred into vials; additionally, 150µl of PBS was added to these vials as well, to ensure all samples were at approximately 200µl. Because the samples were further diluted by PBS during the microBCA and ELISA processes, this addition at the end of the filtering stage did not negatively dilute the supernatant. All samples were stored at -80 degrees Celsius.

4.7.2 MicroBCA Protein Analysis

For the microBCA analysis a Thermo Scientific 23235 kit was used to measure the quantity of protein present in the supernatant samples. A standard was mixed using 450µl of buffer 1 (PBS and double distilled water) and 50µl of bovine serum albumin (BSA). 100µl of buffer 1 was then added to columns one and two, rows B through H on the plate, followed by 100µl of the standard mix in rows one and two, rows A and B. From this a serial dilution was performed downwards from column B through G leaving wells H blank with no solution. After these standard columns were established, 95ul of the standard solution and 5µl of each supernatant sample were added to the 40 sample wells. At this stage the microBCA reaction mix was created using regents A, B and C at a ratio of 25:24:1, respectively. Once mixed, 100µl of the reaction solution was added to all 40 sample wells and 16 standard wells. The plate was then

incubated for approximately 45 minutes until the wells developed a purple/blue colour. Based on the colour change of the top standard wells, multiple plate reads were conducted to ensure the standard was not overdeveloped (i.e. too dark). Once optimal colour change was reached, the plate was read at 560nm with protein levels recorded.

4.7.3 Enzyme-linked immunoassay (ELISA)

For the ELISA analysis a R&D Systems Quantikine ELISA – Human Osteocalcin (DSTCN0) kit was used to quantifying the amount of osteocalcin protein present in each sample. After determining the concentration of proteins for each sample from the micoBCA results, differing amounts of each sample (Appendix B.2) were extracted from the individual vials and mixed with PBS to bring all samples to 50µl. 100µl of the Assay Diluent (RDI-117) was then added to each well in addition to 50µl of each sample. Similar to the micoBCA analysis, two columns of standards were also prepped following a similar serial dilution method (Appendix B.3). Once all samples were added during this initial step, the plate was put on an oscillator at room temperature for two hours. Following this, the plate was washed using the buffer solution from the ELISA kit. 300µl was added to each well, dumped and blotted dry on clean paper towels. This washing step was repeated four times to ensure all material not adhered to the primary antibody that was pre-coated on the plate was removed. After washing, 200µl of the secondary antibody was added to all 40 sample wells and 16 standard wells. Because the secondary antibody is light sensitive the plate was covered and left to oscillate for two hours at room temperature. The plate was again washed four times. At this stage Colour Reagent A and Colour Reagent B were mixed together creating the substrate solution. 200µl of the substrate solution was then added to all 56 wells. This substrate solution is also sensitive to light and was covered during incubation. Kept at room temperature, the incubation timing of this substrate

solution was based on the full development of the standard columns, similar to the microBCA analysis. Once the standards were fully developed, 50µl of the stop solution was added to each well and gently mixed by hand until the colour of all wells turned from green to yellow. After this colour change, signalling the full activation of the stop solution, the plate was read at 450nm and 540nm and osteocalcin levels were calculated by creating a standard curve in Excel with the concentration from each dilution multiplied by the dilution factor as per the instructions from the ELISA kit. All osteocalcin levels were recorded as ng/ml from 4µg of starting bone material. Two dilutions were completed for this osteocalcin data. The first dilution was 1:1 with 14 samples developing beyond the top standard. While these osteocalcin levels were still recorded within the standard curve for the data, the samples were rerun at a 1:4 dilution in an attempt to capture those outliers. With the second dilution only three samples fell above the top standard and within the standard curve and deemed sufficient for this analysis.

CHAPTER 5: RESULTS

5.1 INTRODUCTION

This chapter presents the results of this research beginning with an overview of intra-observer error followed by the analysis of the lesion-based observations including assessments of both pathological and stress indicators. Growth and development-based analyses are also discussed with a specific focus on body size indicators and cortical thickness measurements. This chapter concludes with the analysis of the osteocalcin data.

5.2 INTRA-OBSERVER ERROR

Intra-observer error was calculated for the primary macroscopic stress indicators (i.e. CO, PH, EHL and HL), comparing the first and second rounds of scoring, and a third round of scoring for EHL. For CO, PH and EHL a weighted quadratic kappa test (κ_w) was performed. All calculated kappa statistics will fall between -1.00 and 1.00, but most often are between 0.00 and 1.00, with 1.00 representing perfect agreement between scoring rounds and 0 representing the level of agreement that would occur by chance. A negative kappa statistic (<0.00) means that the level of agreement is less than what would be expected by chance (Viera and Garrett 2005). For ordinal, hierarchical data the weighted kappa statistic puts more emphasis on larger score discrepancies than the smaller score discrepancies (Cohen 1960; Cohen 1968; Sim and Wright 2005; Viera and Garrett 2005). A quadratic weighting for the kappa statistic was used to represent the differences between the score categories (i.e. the severity difference between the 0 score category and the 1 score category is not equal to the difference between the 2 score category and the 3 score category) whereas a linear weighting assumes there is no difference between the score categories (i.e. the severity difference between the 0 score category and the 1 score category are equal to the difference between the 2 score category and the 3 score category)

(Fleiss and Cohen 1973; Brenner and Kliebsch 1996; Schuster 2004). While Landis and Koch (1977) established an arbitrary hierarchy for kappa statistic interpretation (Table 5.1), Sim and Wright (2005) argue that the nature of the data being interpreted and inherent observer biases influence the interpretation of the kappa statistic and should be considered.

Table 5.1 Landis and Koch (1977) on the Interpretation of the Kappa Statistic

Kappa Statistic (K_w)	Strength of Agreement
<0.00	Poor
0.00 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.00	Almost Perfect

For CO and PH the kappa statistic between the first and second scoring rounds was 0.860 (standard error = 0.116; confidence interval (95%) =0.632 to 1.088) and 0.909 (standard error =0.094; confidence interval (95%) =0.724 to 1.094), respectively. This included the adult and subadult rechecks. For EHL the kappa statistic was calculated separately for the adult and subadult groups as the adult group had three rounds of scoring and the subadult only two. In this study, EHL left side observations were used for analysis and replaced with the right side when the left was absent. An assessment of the comparability between left and right side observations was not completed for enamel defects as all data was converted into categorical data arguably diminishing any potential bias between the original raw counts. Further, any right side replacements in the primary data were also replaced in the secondary and tertiary observational rounds to ensure consistency. For the adults, the EHL kappa statistic ranged between 0.194 and 0.811 and for the subadults between 0.333 and 0.677 (Table 5.2). For all adult comparisons, except the maxillary canine, the kappa agreement value was highest between the second and third rounds of scoring, demonstrating the predicted learning curve associated with the identification of EHL; however, the calculated kappa statistic between the first and second round

of scoring also demonstrated satisfactory agreement between observations and can be considered a good representation of the population sample. In both the adult and subadult grouping the highest kappa agreement values were for the maxillary first premolar scores. This level of agreement likely represents a bias in the tooth being observed. As discussed by Sim and Wright (2005), the interpretation of the kappa statistic requires an acknowledgement of influencing biases such as an observer's pre-existing knowledge of the subject being scored. For example, EHL are less likely to form on the premolars and as a result stricter criteria may have been inadvertently used in identifying what was and was not considered an EHL. Because this high level of agreement for the first premolar was seen in both the adult and subadult grouping and differs from the generalized trend seen in the remaining comparisons, it is likely that observer bias was in part influencing this kappa statistic result.

Table 5.2 Weighted Quadratic Kappa Statistic Values for Adult and Subadult EHL Comparisons

Adults (n=21)	κ_w	**Std. Error**	**Confidence Interval (95%)**
MNC Score 1 – MNC Score 2	0.679	0.201	0.285 to 1.072
MNC Score 1 – MNC Score 3	0.407	0.141	0.132 to 0.683
MNC Score 2 – MNC Score 3	0.750	0.161	0.434 to 1.066
MXCI Score 1 – MXCI Score 2	0.423	0.236	-0.039 to 0.885
MXCI Score 1 – MXCI Score 3	0.625	0.227	0.181 to 1.069
MXCI Score 2 – MXCI Score 3	0.581	0.195	0.199 to 0.963
MXC Score 1 – MXC Score2	0.480	0.178	0.131 to 0.829
MXC Score 1 – MXC Score 3	0.343	0.204	-0.056 to 0.743
MXC Score 2 – MXC Score 3	0.462	0.176	0.117 to 0.806
MX1PM Score 1 - MX1PM Score 2	0.545	0.206	0.142 to 0.949
MX1PM Score 1 - MX1PM Score 3	0.194	0.362	-0.515 to 0.902
MX1PM Score 2 - MX1PM Score 3	0.811	0.126	0.565 to 1.057

Subadults (n=17)	κ_w	**Std. Error**	**95% Confidence Interval**
MNC Score 1 – MNC Score 2	0.500	0.375	-0.235 to 1.235
MXCI Score 1 – MXCI Score 2	0.333	0.385	-0.421 to 1.088
MXC Score 1 – MXC Score 2	0.400	0.162	0.083 to 0.717
MX1PM Score 1 - MX1PM Score 2	0.667	0.236	0.205 to 1.129

(MNC - mandibular canine; MXCI – maxillary central incisor; MXC – maxillary canine; MXPM1 – maxillary first premolar)

Intra-observer testing for Harris lines was preceded by a comparison of left and right side observations in the distal tibia to ensure comparability when absent left side data was replaced with the right. While this comparison was not completed on the categorical EHL data, for the Harris line raw count data this comparison was necessary; further, the asymmetrical growth of long bones shown to be associated with stress (Albert and Greene 1999; DeLeon 2007) may affect the manifestation and visibility of these lines. For the adult group a paired samples t-test

was used to assess side comparison in the medial-lateral (ML) view with no statistically significant differences between the left and right raw counts (t=1.114; df=14; p=0.284). Similarly, the subadult group also showed no statistically significant differences between the left and right raw counts in the anterior-posterior (AP) orientation (t=1.210; df=9; p=0.257).

For the adult grouping, a paired samples t-test was completed to assess the relationship between the first and second rounds of counts in the ML orientation with no statistically significant differences observed (t=0.295; df=22; p=0.770). For the subadult grouping, there was a statistically significant difference between the first and second round in the AP orientation (t=-2.512; df=9; p=0.033). One subadult individual (SBT81 GR53) demonstrated an exceptional number of Harris lines in the distal tibia making it difficult to clearly observe the true line count; therefore, this individual was omitted from a second paired samples t-test to determine whether or not their noted variability was influencing the differences between observational rounds. Once removed, there were no statistically significant differences between the observational rounds in the subadult subsample (t=-2.121; df=8; p=0.067), suggesting this one individual was likely influencing the original assessment.

Overall, the intra-observer error results suggest satisfactory agreement between preliminary and secondary data collection for all of the stress indicators in both the adult and subadult age groupings. Cribra orbitalia and PH showed the highest level of agreement when using the kappa statistic with variable results for EHL. Variability between the EHL observations was likely influenced by multiple factors including: natural lighting differences at the time of observation, observer eye fatigue, or observer expectation bias when scoring particular teeth. Despite these limitations however, it is assumed that based on these results the data used for further analysis are an accurate reflection of the Black Friars population.

5.3 LESION-BASED ANALYSES

5.3.1 Pathology

Using a chi-square test (χ^2) each of the nine pathological conditions were assessed for any significant differences between males and females and lesion frequency. The results showed that there was a statistically significant difference between males and females in the prevalence of Schmorl's nodes (χ^2=7.028; df=1; p=0.008) and degenerative joints changes (χ^2=6.625; df=1; p=0.010), with males more likely to develop these pathologies over females. No other pathological comparisons between males and females were significant. All adult raw data is presented below in Tables 5.3 to 5.11.

Table 5.3 Cases of Periostitis between Sexes in the Black Friars Cemetery Sample

Sex	Periostitis		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	25	49	74
Female (n=78)	28	50	78
Total (n=152)	53	99	152

Table 5.4 Cases of Tuberculosis (TB) between Sexes in the Black Friars Cemetery Sample

Sex	TB		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	61	13	74
Female (n=78)	56	22	78
Total (n=152)	117	35	152

Table 5.5 Cases of Leprosy between Sexes in the Black Friars Cemetery Sample

Sex	Leprosy		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	73	1	74
Female (n=78)	75	3	78
Total (n=152)	148	4	152

Table 5.6 Cases of Treponema between Sexes in the Black Friars Cemetery Sample

Sex	Treponema		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	51	23	74
Female (n=78)	51	27	78
Total (n=152)	102	50	152

Table 5.7 Cases of Fractures between Sexes in the Black Friars Cemetery Sample

Sex	Fractures		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	64	10	74
Female (n=78)	69	9	78
Total (n=152)	133	19	152

Table 5.8 Cases of Degenerative Spine Changes (DSC) between Sexes in the Black Friars Cemetery Sample

Sex	DSC		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	28	41	69
Female (n=78)	36	42	78
Total (n=152)	64	83	147

Table 5.9 Cases of Schmorl's Nodes between Sexes in the Black Friars Cemetery Sample

Sex	Schmorl's Nodes		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	10	59	69
Female (n=78)	26	52	78
Total (n=152)	36	111	147

Table 5.10 Cases of Degenerative Joint Changes (DJC) between Sexes in the Black Friars Cemetery Sample

Sex	DJC		
	Absent (n)	Possible/Present (n)	Total (n)
Male (n=74)	47	27	74
Female (n=78)	64	14	78
Total (n=152)	111	41	152

Table 5.11 Cases of Dental Abscesses between Sexes in the Black Friars Cemetery Sample

Sex	Dental Abscesses		Total (n)
	Absent (n)	Possible/Present (n)	
Male (n=74)	67	6	73
Female (n=78)	69	9	78
Total (n=152)	136	15	151

Similarly, when assessing the data by time period there were no statistically significant differences between the medieval and post-medieval groupings except in the presence of dental abscesses (χ^2=5.467; df=1; p=0.019) with the post-medieval individuals more likely to have dental abscesses than the medieval individuals. All adult raw data is presented below in Tables 5.12 to 5.20.

Table 5.12 Cases of Periostitis between Time Periods in the Black Friars Cemetery Sample

Time Period	Periostitis		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=52)	30	31	61
Post-medieval (n=100)	63	79	142
Total (n=152)	93	101	203

Table 5.13 Cases of Tuberculosis (TB) between Time Periods in the Black Friars Cemetery Sample

Time Period	TB		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=52)	51	10	61
Post-medieval (n=100)	116	26	142
Total (n=152)	167	36	203

Table 5.14 Cases of Leprosy between Time Periods in the Black Friars Cemetery Sample

Time Period	Leprosy		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=52)	60	1	61
Post-medieval (n=100)	139	3	142
Total (n=152)	199	4	203

Table 5.15 Cases of Treponema between Time Periods in the Black Friars Cemetery Sample

Time Period	Treponema		
	Absent (n)	Possible/Present (n)	Total (n)
Medieval (n=52)	47	14	61
Post-medieval (n=100)	105	37	142
Total (n=152)	152	51	203

Table 5.16 Cases of Fractures between Time Periods in the Black Friars Cemetery Sample

Time Period	Fractures		
	Absent (n)	Possible/Present (n)	Total (n)
Medieval (n=52)	56	5	61
Post-medieval (n=100)	128	14	142
Total (n=152)	184	19	203

Table 5.17 Cases of Degenerative Spine Changes (DSC) between Time Periods in the Black Friars Cemetery Sample

Time Period	DSC		
	Absent (n)	Possible/Present (n)	Total (n)
Medieval (n=52)	32	26	58
Post-medieval (n=100)	71	57	128
Total (n=152)	103	83	186

Table 5.18 Cases of Schmorl's Nodes between Time Periods in the Black Friars Cemetery Sample

Time Period	Schmorl's Nodes		
	Absent (n)	Possible/Present (n)	Total (n)
Medieval (n=52)	23	35	58
Post-medieval (n=100)	44	84	128
Total (n=152)	67	119	186

Table 5.19 Cases of Degenerative Joint Changes between Time Periods in the Black Friars Cemetery Sample

Time Period	DJC		
	Absent (n)	Possible/Present (n)	Total (n)
Medieval (n=52)	47	14	61
Post-medieval (n=100)	109	29	138
Total (n=152)	156	43	199

Table 5.20 Cases of Dental Abscesses between Time Periods in the Black Friars Cemetery Sample

Time Period	Dental Abscesses		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=52)	58	1	59
Post-medieval (n=100)	116	16	132
Total (n=152)	174	17	191

Using a Fisher's exact test and looking at sex specific differences within each time period, there were no significant differences between males and females and any pathological condition in the medieval period. In the post-medieval grouping only the presence of Schmorl's nodes was significantly different between males and females ($p=0.023$) with males more likely to have the pathology over females.

Pathological conditions were also assessed by age group. Because many of these pathologies do not manifest in subadults (e.g. Schmorl's nodes, degenerative changes, to some extent leprosy and treponema), the subadult age groups were only evaluated for periostitis, TB, leprosy, treponema, fractures, and dental abscesses. In the subadult group there were five age categories used, marking known maturation periods (i.e. 0-1 year; 2-4 years; 5-7 years; 8-13 years; 14-18 years) (Bogin 2001). A Fisher's exact test was used for all subadult comparisons due to a reduced sample size. In assessing all subadult individuals there was no statistically significant differences between the presence of pathological conditions by age. Further, there were no cases of leprosy or fractures among any of the subadult age groups. Pathology was then compared by age group within each time period with no significant differences observed. In the medieval grouping there were no cases of leprosy, treponema, fractures or dental abscesses and in the post-medieval group there were no cases of TB, leprosy or fractures. All subadult raw data is presented below in Tables 5.21 to 5.28.

Table 5.21 Cases of Periostitis between Age Categories in the Black Friars Subadult Cemetery Sample

Age Category	Periostitis		Total (n)
	Absent (n)	Possible/Present (n)	
0-1 years (n=8)	4	3	7
2-4 years (n=5)	5	0	5
5-7 years (n=7)	6	1	7
8-13 years (n=24)	17	6	23
14-18 years (n=7)	6	1	8
Total (n=51)	40	11	51

Table 5.22 Cases of Tuberculosis (TB) between Age Categories in the Black Friars Subadult Cemetery Sample

Age Category	TB		Total (n)
	Absent (n)	Possible/Present (n)	
0-1 years (n=8)	7	0	7
2-4 years (n=5)	5	0	5
5-7 years (n=7)	6	1	7
8-13 years (n=24)	23	0	23
14-18 years (n=7)	7	0	7
Total (n=51)	50	1	51

Table 5.23 Cases of Treponema between Age Categories in the Black Friars Subadult Cemetery Sample

Age Category	Treponema		Total (n)
	Absent (n)	Possible/Present (n)	
0-1 years (n=8)	7	0	7
2-4 years (n=5)	6	0	6
5-7 years (n=7)	7	0	7
8-13 years (n=24)	23	0	23
14-18 years (n=7)	6	1	7
Total (n=51)	50	1	51

Table 5.24 Cases of Dental Abscesses between Age Categories in the Black Friars Subadult Cemetery Sample

Age Category	Dental Abscesses		Total (n)
	Absent (n)	Possible/Present (n)	
0-1 years (n=8)	5	0	5
2-4 years (n=5)	5	0	5
5-7 years (n=7)	6	0	6
8-13 years (n=24)	17	0	17
14-18 years (n=7)	5	2	7
Total (n=51)	38	2	40

Table 5.25 Cases of Periostitis between Time Periods in the Black Friars Subadult Cemetery Sample

Time Period	Periostitis		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=9)	7	2	9
Post-medieval (n=42)	33	9	42
Total (n=51)	40	11	51

Table 5.26 Cases of Tuberculosis (TB) between Time Periods in the Black Friars Subadult Cemetery Sample

Time Period	TB		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=9)	8	1	9
Post-medieval (n=42)	42	0	42
Total (n=51)	50	1	51

Table 5.27 Cases of Treponema between Time Periods in the Black Friars Subadult Cemetery Sample

Time Period	Treponema		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=9)	9	0	9
Post-medieval (n=42)	41	1	42
Total (n=51)	50	1	51

Table 5.28 Cases of Dental Abscesses between Time Periods in the Black Friars Subadult Cemetery Sample

Time Period	Dental Abscesses		Total (n)
	Absent (n)	Possible/Present (n)	
Medieval (n=9)	7	0	7
Post-medieval (n=42)	31	2	33
Total (n=51)	38	2	40

For the adult age comparison, individuals were divided into three age categories (i.e. 18-29 years; 30-44 years; 45-60 years) and were tested against all nine pathological conditions. All pathological groups were analyzed using a Fisher's exact test due to the low prevalence of pathology. All pathologies associated with age, degenerative spine changes ($p<0.001$), Schmorl's nodes ($p=0.037$) and degenerative joint changes ($p=0.012$) were all significantly more prevalent in the older age categories, as expected. No other pathology showed a significant difference in prevalence between any of the three adult age groups. All adult raw data is presented below in Tables 5.29 to 5.37.

Table 5.29 Cases of Periostitis between Age Categories in the Black Friars Adult Cemetery Sample

Age Category	Periostitis		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=46)	16	30	46
30-44 years (n=96)	33	63	96
45-60 years (n=10)	4	6	10
Total (n=152)	53	99	152

Table 5.30 Cases of Tuberculosis (TB) between Age Categories in the Black Friars Adult Cemetery Sample

Age Category	TB		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=46)	38	8	46
30-44 years (n=96)	74	22	96
45-60 years (n=10)	5	5	10
Total (n=152)	117	35	152

Table 5.31 Cases of Leprosy between Age Categories in the Black Friars Adult Cemetery Sample

Age Category	Leprosy		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=46)	44	2	46
30-44 years (n=96)	94	2	96
45-60 years (n=10)	10	0	10
Total (n=152)	148	4	152

Table 5.32 Cases of Treponema between Age Categories in the Black Friars Adult Cemetery Sample

Age Category	Treponema		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=46)	30	16	46
30-44 years (n=96)	65	31	96
45-60 years (n=10)	7	3	10
Total (n=152)	102	50	152

Table 5.33 Cases of Fractures between Age Categories in the Black Friars Adult Cemetery Sample

Age Category	Fractures		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=46)	41	5	46
30-44 years (n=96)	85	11	96
45-60 years (n=10)	7	3	10
Total (n=152)	133	19	152

Table 5.34 Cases of Degenerative Spine Changes (DSC) between Age Categories in the Black Friars Adult Cemetery Sample

Age Category	DSC		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=46)	35	10	45
30-44 years (n=96)	25	68	93
45-60 years (n=10)	4	5	9
Total (n=152)	64	83	147

Table 5.35 Cases of Schmorl's Nodes between Age Categories in the Black Friars Adult Cemetery Sample

	Schmorl's Nodes		
Age Category	Absent (n)	Possible/Present (n)	Total (n)
18-29 years (n=46)	17	28	45
30-44 years (n=96)	17	76	93
45-60 years (n=10)	2	7	9
Total (n=152)	36	111	147

Table 5.36 Cases of Degenerative Joint Changes between Age Categories in the Black Friars Adult Cemetery Sample

	DJC		
Age Category	Absent (n)	Possible/Present (n)	Total (n)
18-29 years (n=46)	35	11	46
30-44 years (n=96)	73	23	96
45-60 years (n=10)	3	7	10
Total (n=152)	111	41	152

Table 5.37 Cases of Dental Abscesses between Age Categories in the Black Friars Adult Cemetery Sample

	Dental Abscesses		
Age Category	Absent (n)	Possible/Present (n)	Total (n)
18-29 years (n=46)	41	5	46
30-44 years (n=96)	87	8	95
45-60 years (n=10)	8	2	10
Total (n=152)	136	15	151

This age data was further divided by time period to assess any differences in the presence or absence of these pathologies in the medieval or post-medieval period. Using a Fisher's exact test for the medieval group there was a significant difference in the presence of fractures (χ^2=6.319; p=0.027) (Table 5.38), degenerative spine changes (χ^2=11.814; p=0.001) (Table 5.39) and degenerative joint changes (χ^2=5.716; p=0.041) (Table 5.40). While degenerative changes are expected to increase with age, the number of fractures in the oldest age group was not expected. In the oldest age category (45-60 years) half of the observed individuals had a fracture,

whereas in the youngest age group (18-29 years) of the 15 observable individuals, none showed evidence of a fracture (new or healed) (Table 5.38).

Table 5.38 Cases of Fractures in the Medieval Adult Age Categories in the Black Friars Cemetery Sample

Age Category	Fractures		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=15)	15	0	15
30-44 years (n=33)	30	3	33
45-60 years (n=4)	2	2	4
Total (n=52)	47	5	52

Table 5.39 Cases of Degenerative Spine Changes (DSC) in the Medieval Adult Age Categories in the Black Friars Cemetery Sample

Age Category	DSC		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=15)	12	2	14
30-44 years (n=33)	10	22	32
45-60 years (n=4)	2	2	4
Total (n=52)	24	26	50

Table 5.40 Cases of Degenerative Joint Changes (DJC) in the Medieval Adult Age Categories in the Black Friars Cemetery Sample

Age Category	DJC		Total (n)
	Absent (n)	Possible/Present (n)	
18-29 years (n=15)	10	5	15
30-44 years (n=33)	27	6	33
45-60 years (n=4)	1	3	4
Total (n=52)	38	14	52

Similar to the medieval comparison and using a Fisher's exact test, the post-medieval age groups also showed a significant difference between the prevalence of degenerative spine changes (χ^2=20.913; p=0.000) based on age category with the oldest age group showing the majority of these degenerative changes; however, degenerative joint changes were not significantly different by age (χ^2=5.876; p=0.072) in the post-medieval group. No other pathological comparisons by age in the post-medieval group were significant. Further, when

comparing the three age groups across the medieval and post-medieval time periods using a chi-square test there was a significant difference in the presence of periostitis (χ^2=4.438; df=1; p=0.035) in the middle age group (30-44 years) (Table 5.41). In this comparison, it was more likely for the post-medieval individuals to have periostitis in middle adulthood than the medieval individuals. There was also a significant difference in the presence of dental abscesses using a Fisher's exact test (p=0.047) in the 30-44 year age category with the pathology only present in the post-medieval individuals (Table 5.42). All other comparisons between age groups across the two time periods were not significantly significant.

Table 5.41 Cases of Periostitis within each Adult Age Category Compared between Time Periods in the Black Friars Cemetery Sample

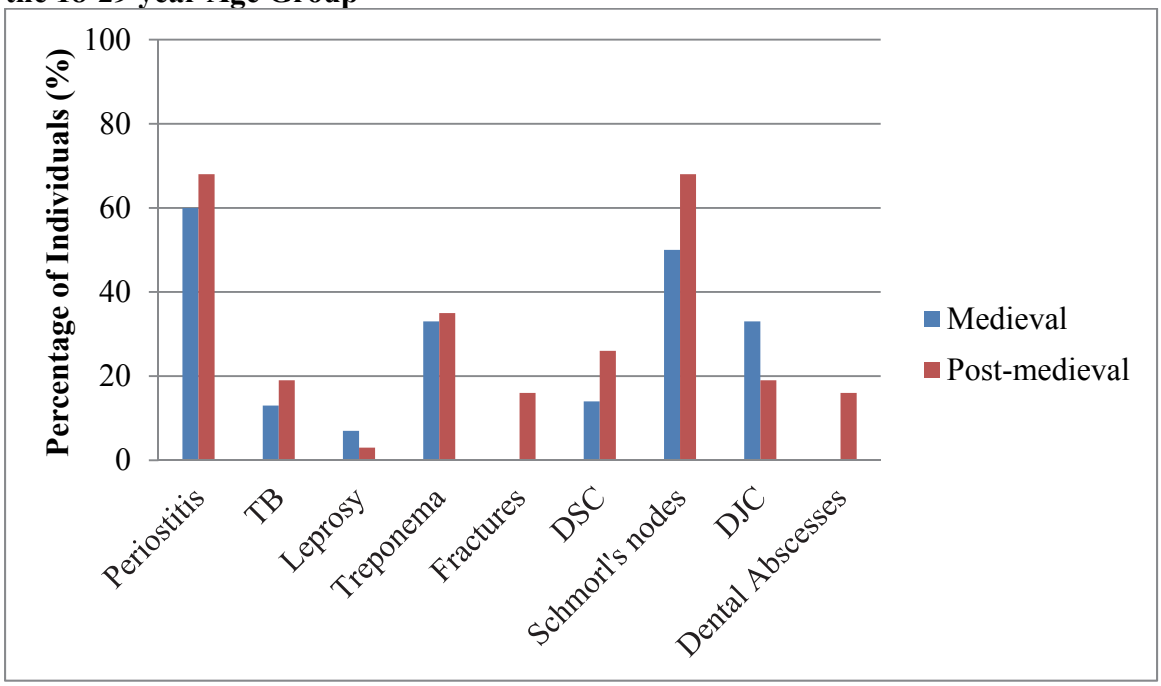
Age Category		**Time Period**		
		Medieval (n)	Post-medieval (n)	Total (n)
18-29 years	Absent	6	10	16
	Possible/Present	9	21	30
	Total	15	31	46
30-44 years	Absent	16	17	33
	Possible/Present	17	46	63
	Total	33	63	96
45-60 years	Absent	1	3	4
	Possible/Present	3	3	6
	Total	4	6	10
Total	Absent	23	30	53
	Possible/Present	29	70	99
	Total	52	100	152

Table 5.42 Cases of Dental Abscesses within each Adult Age Category Compared between Time Periods in the Black Friars Cemetery Sample

Age Category		Time Period		
		Medieval (n)	Post-medieval (n)	Total (n)
18-29 years	Absent	15	26	41
	Possible/Present	0	5	5
	Total	15	31	46
30-44 years	Absent	33	54	87
	Possible/Present	0	8	8
	Total	33	62	95
45-60 years	Absent	3	5	8
	Possible/Present	1	1	2
	Total	4	6	10
Total	Absent	51	85	136
	Possible/Present	1	14	15
	Total	52	99	151

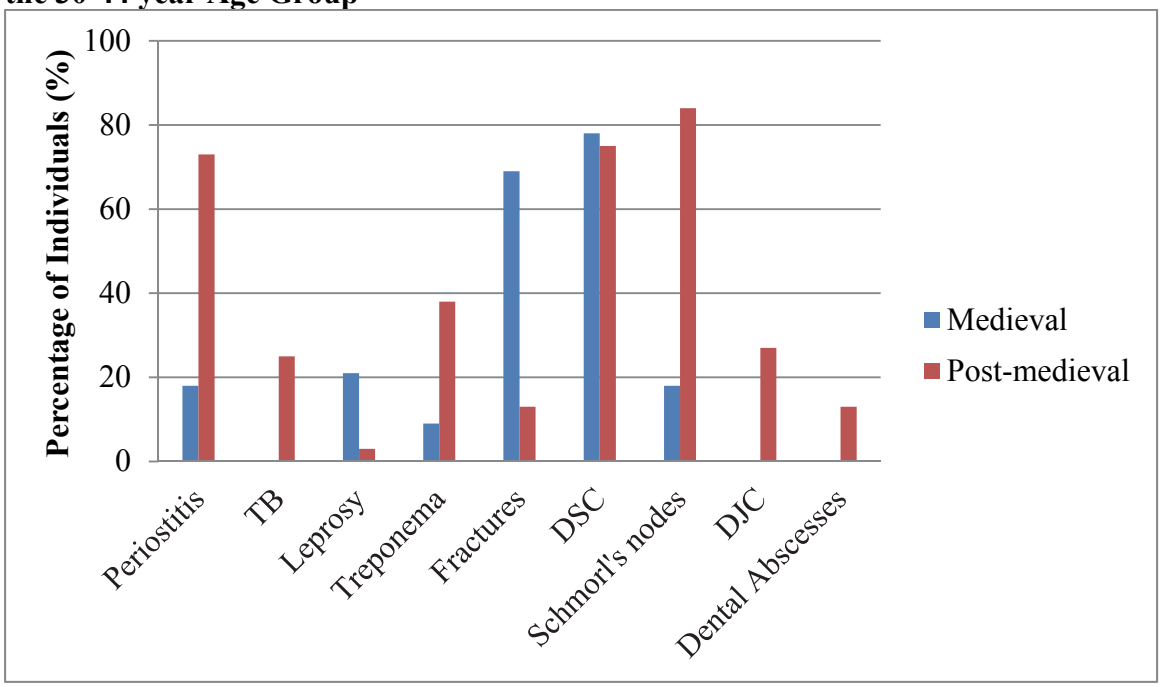
Overall, despite a lack of statistical significance, many pathological conditions were more prevalent in the post-medieval period, particularly visible in the middle adult age category, whereas in the youngest and oldest age categories this trend is less distinct (Figures 5.1 to 5.3). Also, in the post-medieval period there were some pathological conditions present that were absent in the earlier medieval period. In the youngest age group fractures and dental abscesses were only present in the post-medieval sample. In the middle age group TB, degenerative joint changes and dental abscesses were also only present in the post-medieval group. In the oldest age category all pathological conditions were represented in both the medieval and post-medieval groups except leprosy.

Figure 5.1 Percentage of Individuals with Pathological Conditions between Time Periods in the 18-29 year Age Group



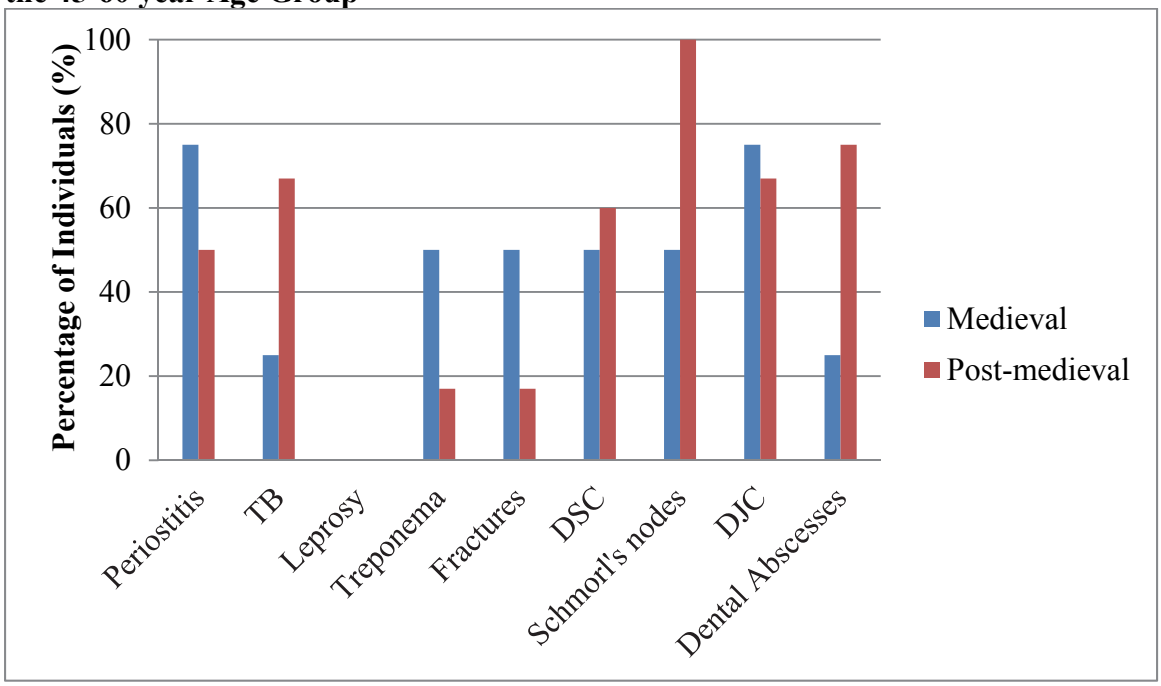
(DSC – degenerative spine changes; DJC – degenerative joint changes)

Figure 5.2 Percentage of Individuals with Pathological Conditions between Time Periods in the 30-44 year Age Group



(DSC – degenerative spine changes; DJC – degenerative joint changes)

Figure 5.3 Percentage of Individuals with Pathological Conditions between Time Periods in the 45-60 year Age Group



(DSC – degenerative spine changes; DJC – degenerative joint changes)

5.3.2 Stress Indicators

When analyzing the subadult sample, a Fisher's exact test was used to compare the prevalence of each stress indicator by severity category with time period and age. There were no significant differences observed between the four indicators when compared by time period and no significant differences when compared by age group except for EHL (χ^2=23.699; p=0.001). All subadult raw data is presented below in Tables 5.43 to 5.50.

Table 5.43 Subadult Cases of Cribra Orbitalia (CO) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	CO (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
Medieval (n=9)	0	3	3	1	0	7
Post-medieval (n=42)	12	11	4	1	1	29
Total (n=51)	12	14	7	2	1	36

Table 5.44 Subadult Cases of Porotic Hyperostosis (PH) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	PH (Severity Levels 0-4)					Total
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
Medieval (n=9)	6	0	1	0	0	7
Post-medieval (n=42)	23	4	1	1	0	29
Total (n=51)	29	4	2	1	0	36

Table 5.45 Subadult Cases of Enamel Hypoplastic Lesions (EHL) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	EHL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
Medieval (n=9)	5	0	1	1	7
Post-medieval (n=42)	10	3	6	10	29
Total (n=51)	15	3	7	11	36

Table 5.46 Subadult Cases of Harris Lines (HL) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	HL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
Medieval (n=9)	0	1	5	2	8
Post-medieval (n=42)	8	7	12	5	32
Total (n=51)	8	8	17	7	40

Table 5.47 Subadult Cases of Cribra Orbitalia (CO) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	CO (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
0-1 year (n=8)	2	1	0	0	0	3
2-4 years (n=5)	0	2	1	1	0	4
5-7 years (n=7)	1	2	2	0	0	5
8-13 years (n=24)	6	6	3	1	1	17
14-18 years (n=7)	3	2	1	0	0	6
Total (n=51)	12	13	7	2	1	35

Table 5.48 Subadult Cases of Porotic Hyperostosis (PH) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	PH (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
0-1 year (n=8)	3	0	0	0	0	3
2-4 years (n=5)	3	1	0	0	0	4
5-7 years (n=7)	4	1	1	0	0	6
8-13 years (n=24)	15	0	0	1	0	16
14-18 years (n=7)	3	2	1	0	0	6
Total (n=51)	28	4	2	1	0	35

Table 5.49 Subadult Cases of Enamel Hypoplastic Lesions (EHL) by Severity between Age Categories in the Black Friars Cemetery Sample

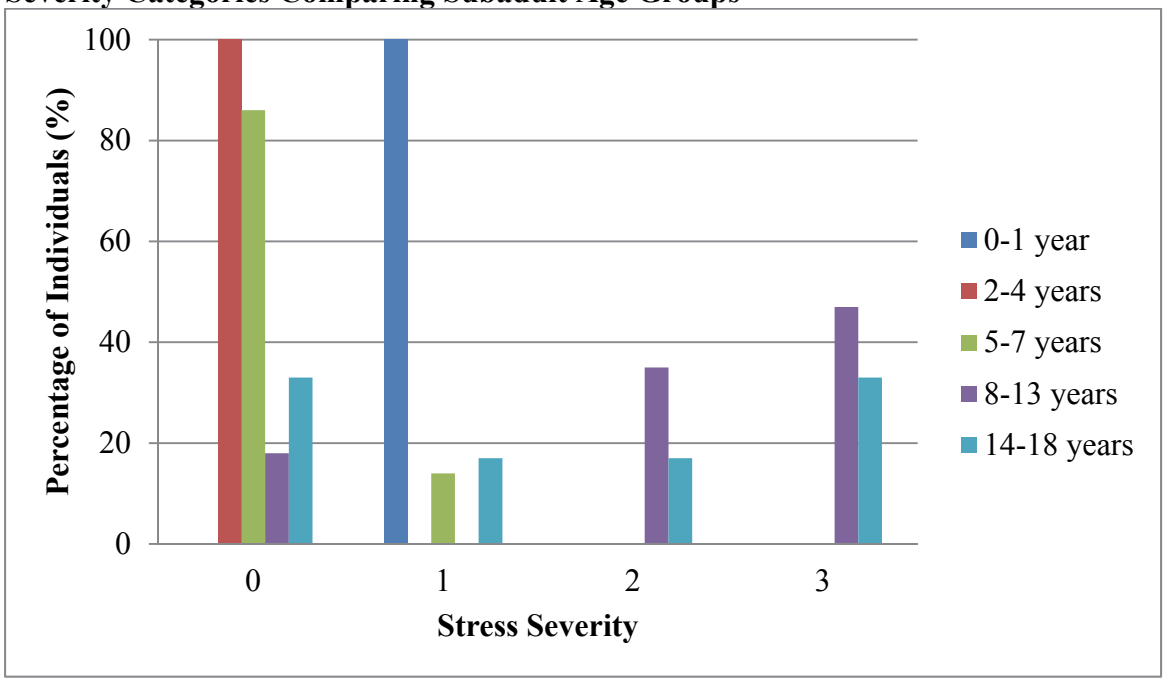
Age Category	EHL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
0-1 year (n=8)	0	1	0	0	1
2-4 years (n=5)	4	0	0	0	4
5-7 years (n=7)	6	1	0	0	7
8-13 years (n=24)	3	0	6	8	17
14-18 years (n=7)	2	1	1	2	6
Total (n=51)	15	3	7	10	35

Table 5.50 Subadult Distribution of Harris Lines (HL) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	HL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
0-1 year (n=8)	4	1	1	0	6
2-4 years (n=5)	0	1	2	1	4
5-7 years (n=7)	0	1	4	0	5
8-13 years (n=24)	3	4	8	3	18
14-18 years (n=7)	1	1	1	2	5
Total (n=51)	8	8	16	6	38

As shown in Figure 5.4, there is a trend where the older age categories not only show stress, but a higher severity than the younger age categories. However, in this example, missing dentition in the youngest age categories may in part be influencing these results.

Figure 5.4 Percentage of Individuals with Enamel Hypoplastic Lesions Across Stress Severity Categories Comparing Subadult Age Groups



For the adult sample, all stress indicators were compared to sex, age and time period using a chi-square test to assess any significant differences between severity categories. There were no significant differences between males and females or the medieval and post-medieval prevalence of these indicators; however, when looking at age, there was a significant difference in the prevalence of PH (χ^2=14.753; df=6; p=0.012) (Figure 5.5). In looking at PH, the oldest age category shows the most non-stressed or minimally stressed individuals, whereas the youngest and middle age categories show similar stress in the mild and moderate categories and only the youngest age groups shows the most severe level of stress. The youngest age group is also distinct in that it shows a similar frequency of individuals distributed across all four severity levels, whereas the middle and oldest categories cluster towards the milder end of the severity spectrum. All adult raw data is presented below in Tables 5.51 to 5.62.

Figure 5.5 Percentage of Individuals with Porotic Hyperostosis Across Stress Severity Categories Comparing Adult Age Groups

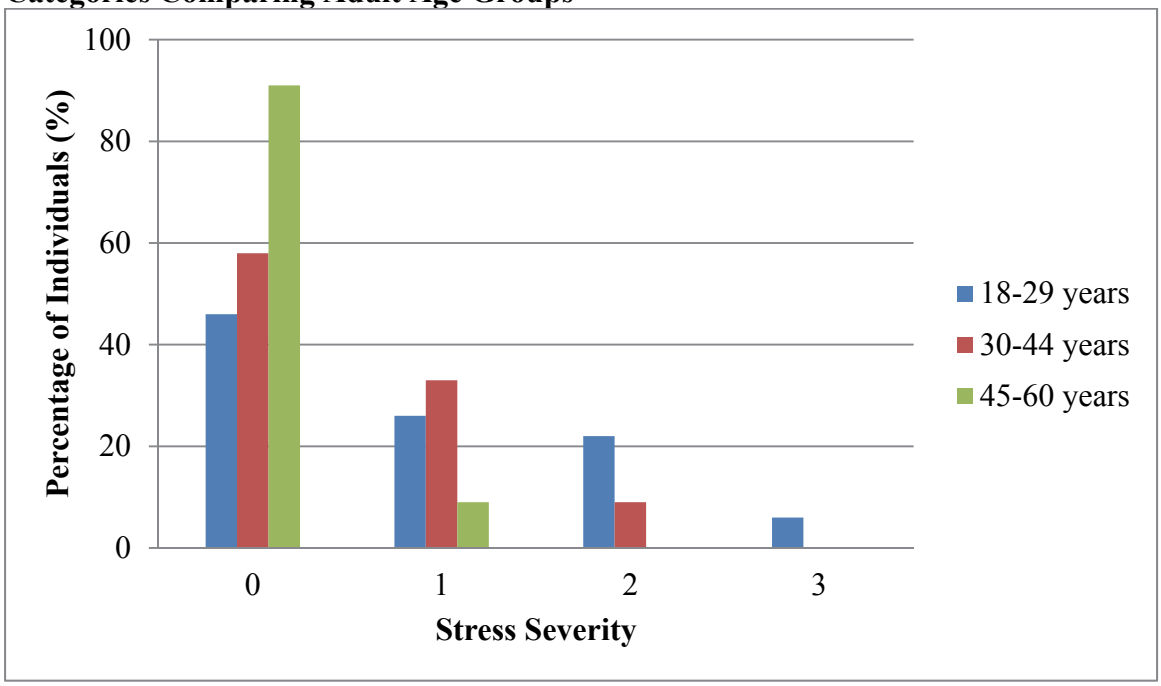


Table 5.51 Adult Cases of Cribra Orbitalia (CO) by Severity between Sexes in the Black Friars Cemetery Sample

Sex	CO (Severity Levels 0-4)					
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	Total (n)
Female (n=78)	55	12	7	3	1	78
Male (n=74)	59	9	6	0	0	74
Total (n=152)	114	21	13	3	1	152

Table 5.52 Adult Cases of Porotic Hyperostosis (PH) by Severity between Sexes in the Black Friars Cemetery Sample

Sex	PH (Severity Levels 0-4)					
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	Total (n)
Female (n=78)	46	24	6	2	0	78
Male (n=74)	40	20	13	1	0	74
Total (n=152)	86	44	19	3	0	152

Table 5.53 Adult Cases of Enamel Hypoplastic Lesions (EHL) by Severity between Sexes in the Black Friars Cemetery Sample

Sex	EHL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
Female (n=78)	19	17	32	9	77
Male (n=74)	22	10	23	14	69
Total (n=152)	41	27	55	23	146

Table 5.54 Adult Cases of Harris Lines (HL) by Severity between Sexes in the Black Friars Cemetery Sample

Sex	HL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
Female (n=78)	3	11	25	35	74
Male (n=74)	3	13	25	29	70
Total (n=152)	6	24	50	64	144

Table 5.55 Adult Cases of Cribra Orbitalia (CO) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	CO (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
Medieval (n=52)	42	4	4	1	1	52
Post-medieval (n=100)	72	17	9	2	0	100
Total (n=152)	114	21	13	3	1	152

Table 5.56 Adult Cases of Porotic Hyperostosis (PH) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	PH (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
Medieval (n=52)	35	10	7	0	0	52
Post-medieval (n=100)	51	34	12	3	0	100
Total (n=152)	86	44	19	3		152

Table 5.57 Adult Cases of Enamel Hypoplastic Lesions (EHL) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	EHL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
Medieval (n=52)	12	11	16	13	52
Post-medieval (n=100)	29	16	39	10	94
Total (n=152)	41	27	55	23	146

Table 5.58 Adult Cases of Harris Lines (HL) by Severity between Time Periods in the Black Friars Cemetery Sample

Time Period	HL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
Medieval (n=52)	0	10	12	24	46
Post-medieval (n=100)	6	14	38	40	98
Total (n=152)	6	24	50	64	144

Table 5.59 Adult Cases of Cribra Orbitalia (CO) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	CO (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
18-29 years (n=46)	35	5	2	3	1	46
30-44 years (n=96)	71	14	10	0	0	95
45-60 years (n=10)	8	2	1	0	0	11
Total (n=152)	114	21	13	3	1	152

Table 5.60 Adult Cases of Porotic Hyperostosis (PH) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	PH (Severity Levels 0-4)					Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	4 (n)	
18-29 years (n=46)	21	12	10	3	0	46
30-44 years (n=96)	55	31	9	0	0	95
45-60 years (n=10)	10	1	0	0	0	11
Total (n=152)	86	44	19	3	0	152

Table 5.61 Adult Cases of Enamel Hypoplastic Lesions (EHL) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	EHL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
18-29 years (n=46)	13	7	16	9	45
30-44 years (n=96)	23	19	39	11	92
45-60 years (n=10)	5	1	0	3	9
Total (n=152)	41	27	55	23	146

Table 5.62 Adult Cases of Harris Lines (HL) by Severity between Age Categories in the Black Friars Cemetery Sample

Age Category	HL (Severity Levels 0-3)				Total (n)
	0 (n)	1 (n)	2 (n)	3 (n)	
18-29 years (n=46)	2	8	15	19	44
30-44 years (n=96)	4	14	30	42	90
45-60 years (n=10)	0	2	5	3	10
Total (n=152)	6	24	50	64	144

A further analysis within each time period using a Fisher's exact test demonstrated that when comparing the prevalence of each stress indicator between the sexes, in the medieval period there was a significant difference between males and females for PH (χ^2=11.543; p=0.002) and EHL severity (χ^2=11.548; p=0.008), but in the post-medieval analysis there were no significant differences between the sexes.

Looking specifically at these differences shown in Figure 5.6 there were substantially more females in the first and second severity categories for EHL than males; however, males showed the greatest number in the most severe EHL category. Similarly, when looking at the trend for PH (Figure 5.7) there were more females in the mild stress category and more males in the most severe stress category. Also, in both examples, there were a far greater number of males who showed no evidence of EHL or PH at all.

Figure 5.6 Percentage of Individuals with Enamel Hypoplastic Lesion Across Stress Severity Categories Comparing Males and Females in the Medieval Group

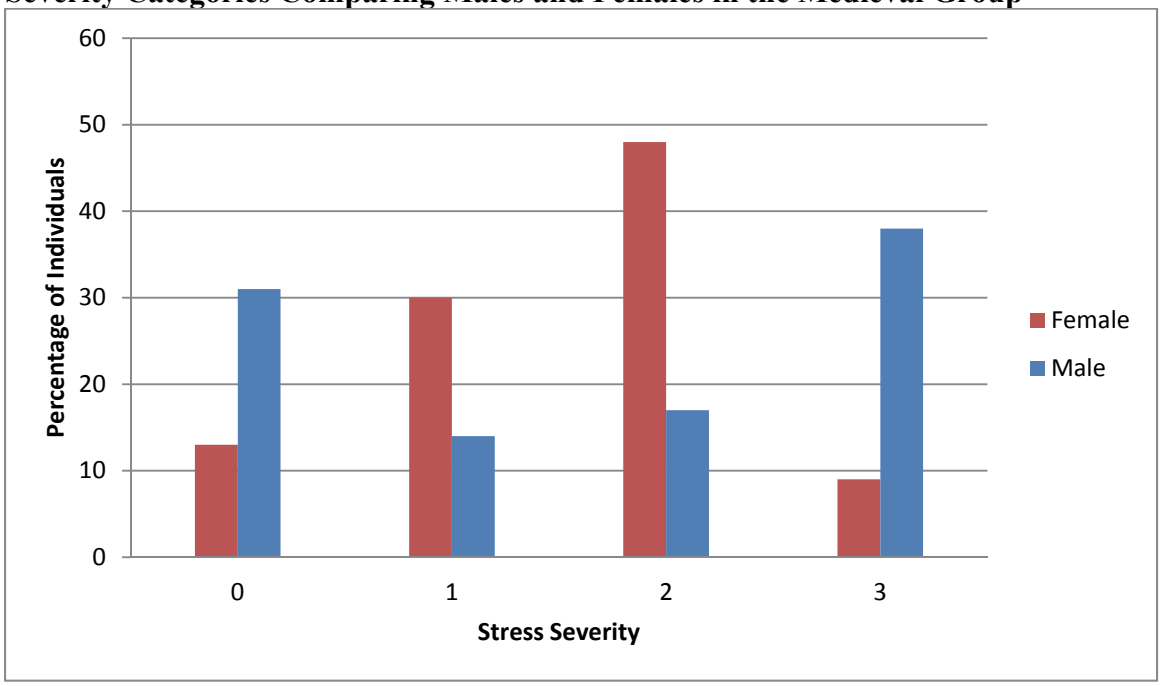
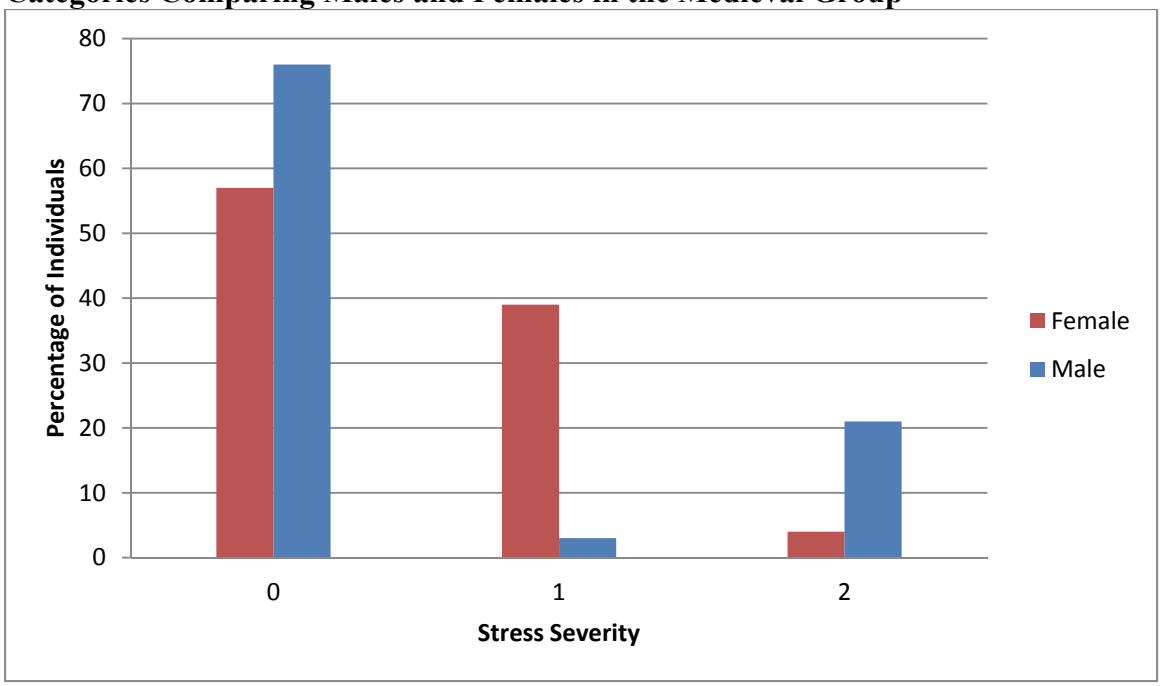


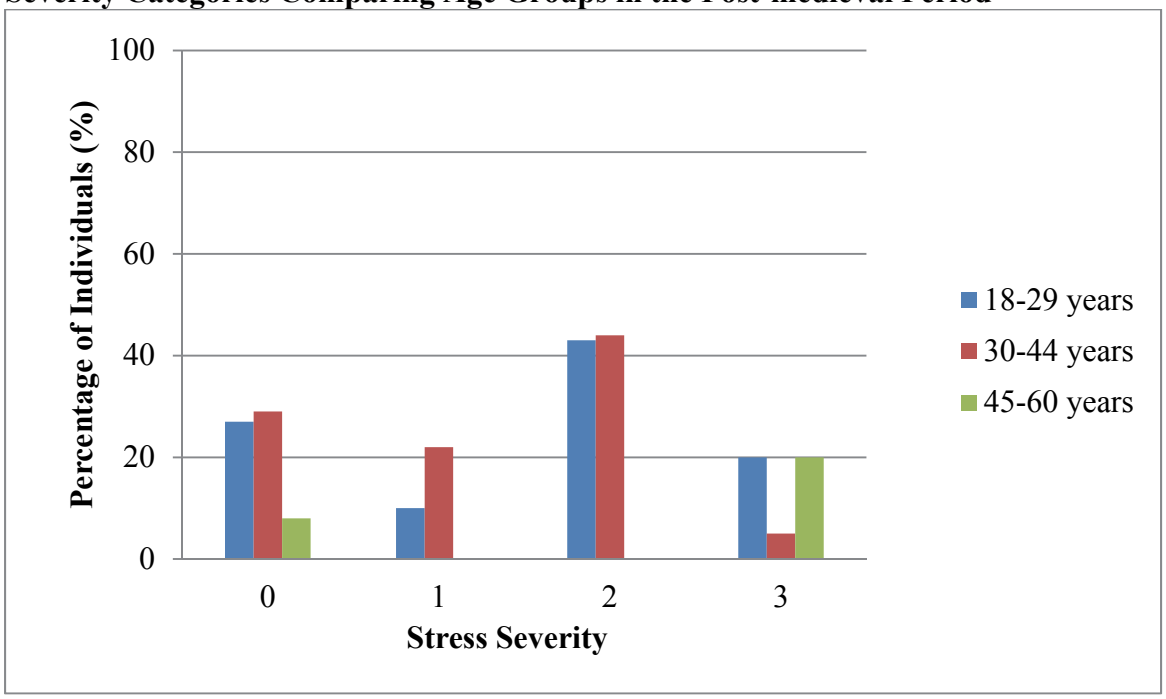
Figure 5.7 Percentage of Individuals with Porotic Hyperostosis Across Stress Severity Categories Comparing Males and Females in the Medieval Group



Looking at the distribution of stress indicators across age categories within each time period there were no significant differences in the medieval group; however, in the post-

medieval group when using a Fisher's exact test there was a significant difference in the prevalence of EHL across age category (χ^2=12.339; p=0.029) (Figure 5.8). By far, the 30-44 year category showed the highest frequency of EHL in the first two severity categories, surpassed in the highest severity category by the 18-29 year age group and the 45-60 year age group. This significance, however, may in part be associated with a small sample size, particularly in the oldest age category. Within this post-medieval grouping, the middle age category had nearly double the number of individuals (n=59) than the 18-29 year age group (n=30) and more than 10 times as many individuals as the 45-60 year age group (n=5).

Figure 5.8 Percentage of Individuals with Enamel Hypoplastic Lesion Across Stress Severity Categories Comparing Age Groups in the Post-medieval Period



Overall when analyzing the stress severity data some general trends emerged. As discussed, for the adults, there were no significant differences between severity categories based on time period (Figures 5.9 to 5.12); however, in both the medieval and post-medieval groups there was less disparity between the number of individuals occupying each severity category for EHL and HL than in CO and PH where the majority of individuals had no evidence of stress or

were in the lowest severity category (level 1). There was a slight increase in severity level observed across the majority of stress indicators into the post-medieval period, particularly in the middle severity category (level 2); however, in the most severe category (level 3) the number of individuals with EHL and HL decreases from the medieval to post-medieval period.

Figure 5.9 Percentage of Individuals with Cribra Orbitalia across Stress Severity Categories Comparing the Medieval and Post-medieval Periods

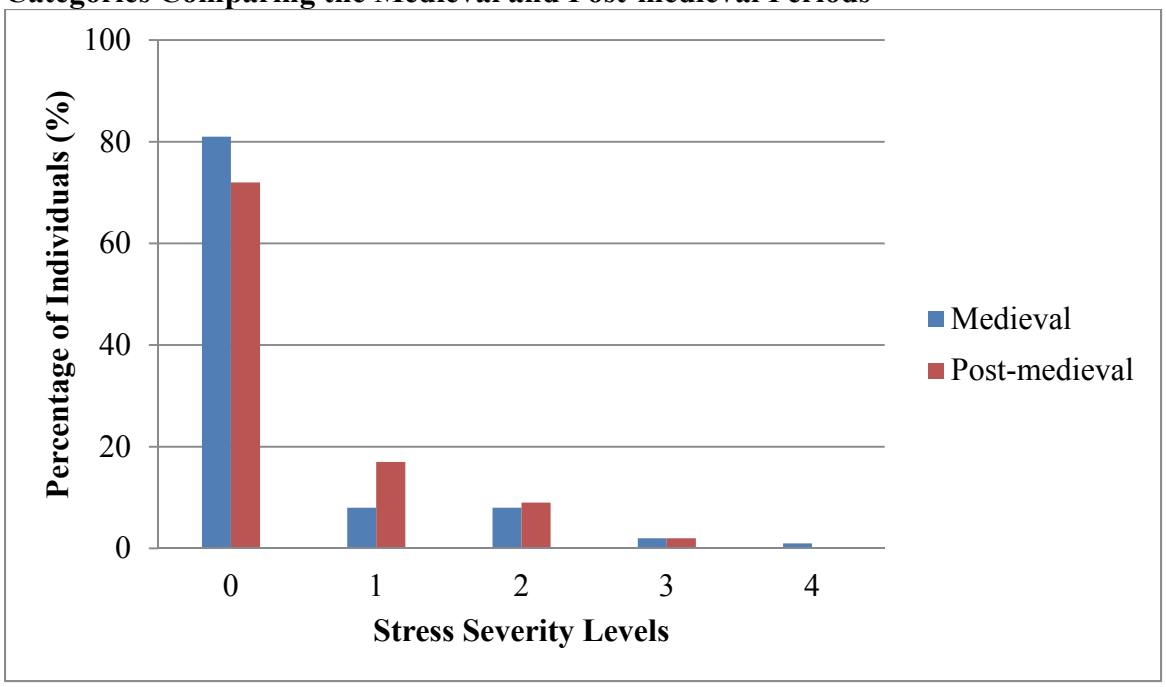


Figure 5.10 Percentage of Individuals with Porotic Hyperostosis across Stress Severity Categories Comparing the Medieval and Post-medieval Periods

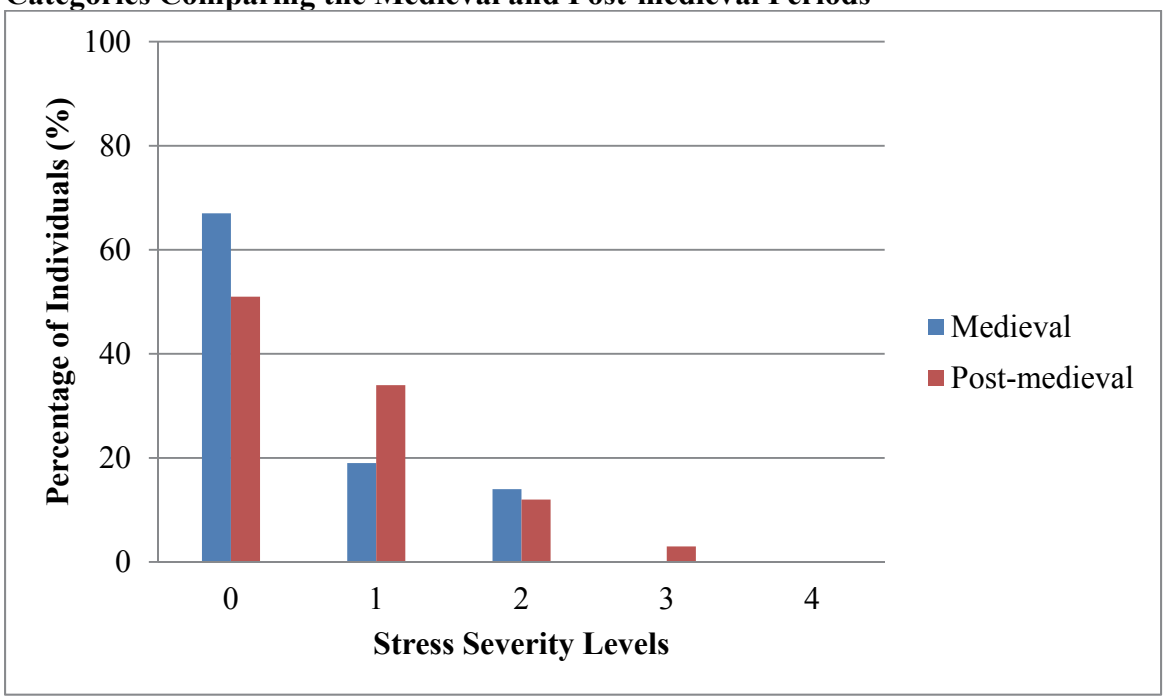


Figure 5.11 Percentage of Individuals with Enamel Hypoplastic Lesions Across Stress Severity Categories Comparing the Medieval and Post-medieval Periods

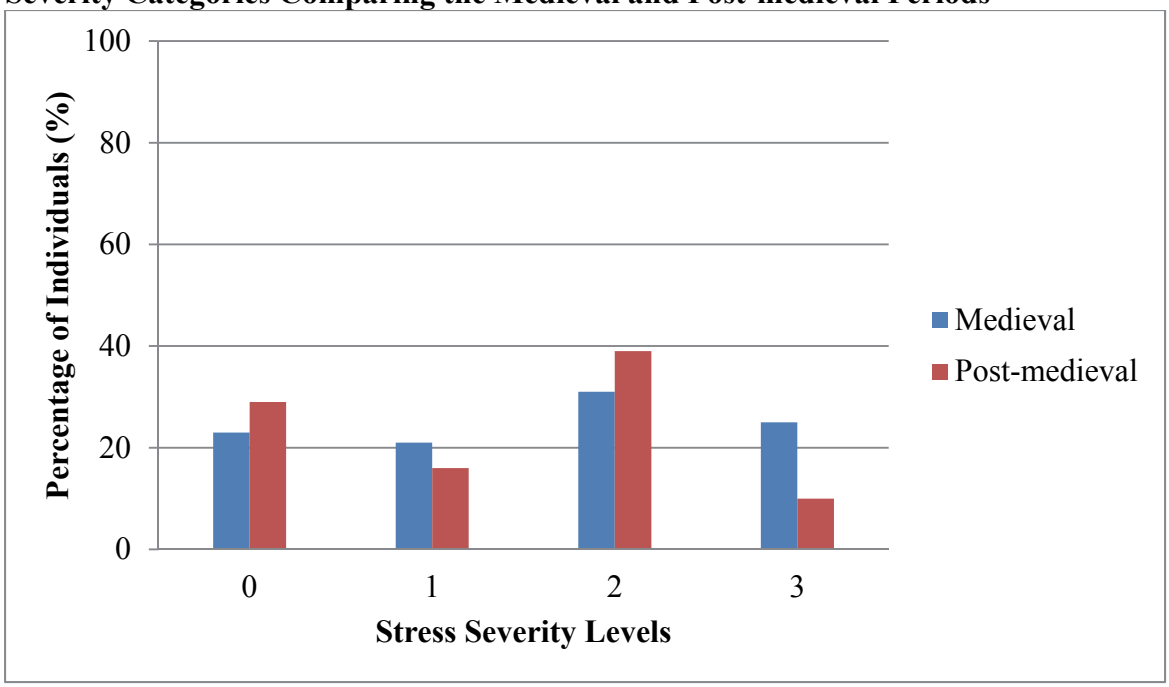
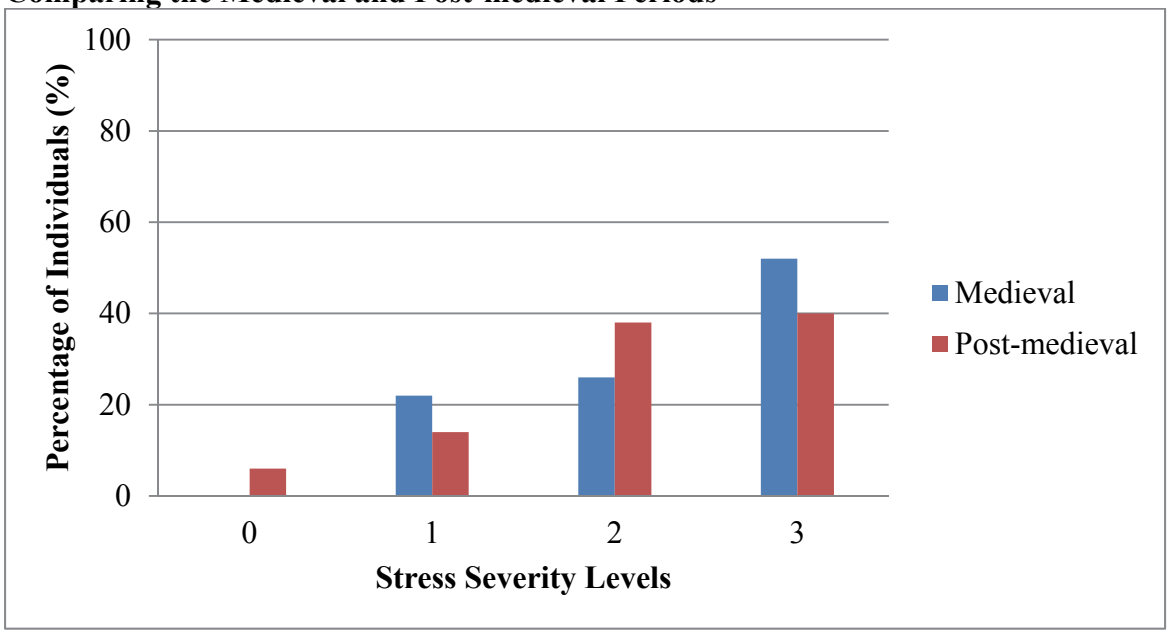


Figure 5.12 Percentage of Individuals with Harris Lines Across Stress Severity Categories Comparing the Medieval and Post-medieval Periods



Comparing the severity levels across each indicator divided by sex (Figures 5.13 to 5.16), the majority of males and females did not show evidence of CO or PH; however, this ratio was skewed when assessing EHL and HL where the majority of males and females occupied the higher severity categories (i.e. levels 2 and 3). In general, the females had more individuals in level 1 for all indicators except HL, in level 2 for all indicators except PH and in level 3 for all indicators except EHL. While not significantly different, the females tended to demonstrate a higher level of stress severity across the majority of indicators when compared to the males.

Figure 5.13 Percentage of Individuals with Cribra Orbitalia Across Stress Severity Categories Comparing Males and Females

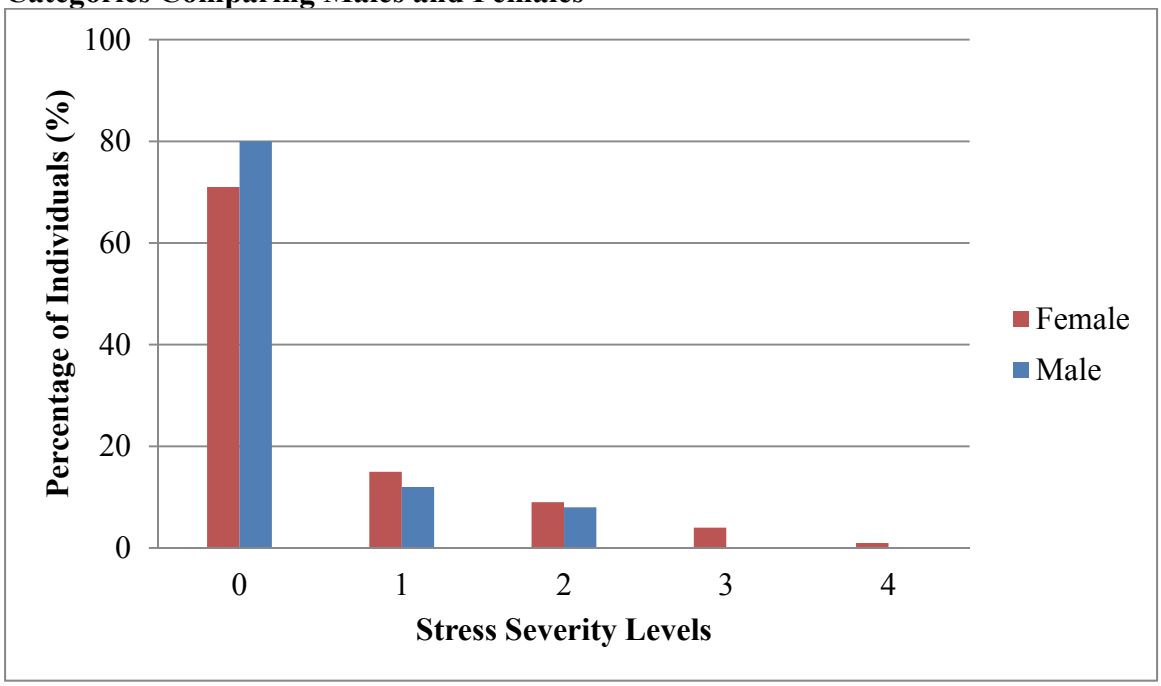


Figure 5.14 Percentage of Individuals with Porotic Hyperostosis across Stress Severity Categories Comparing Males and Females

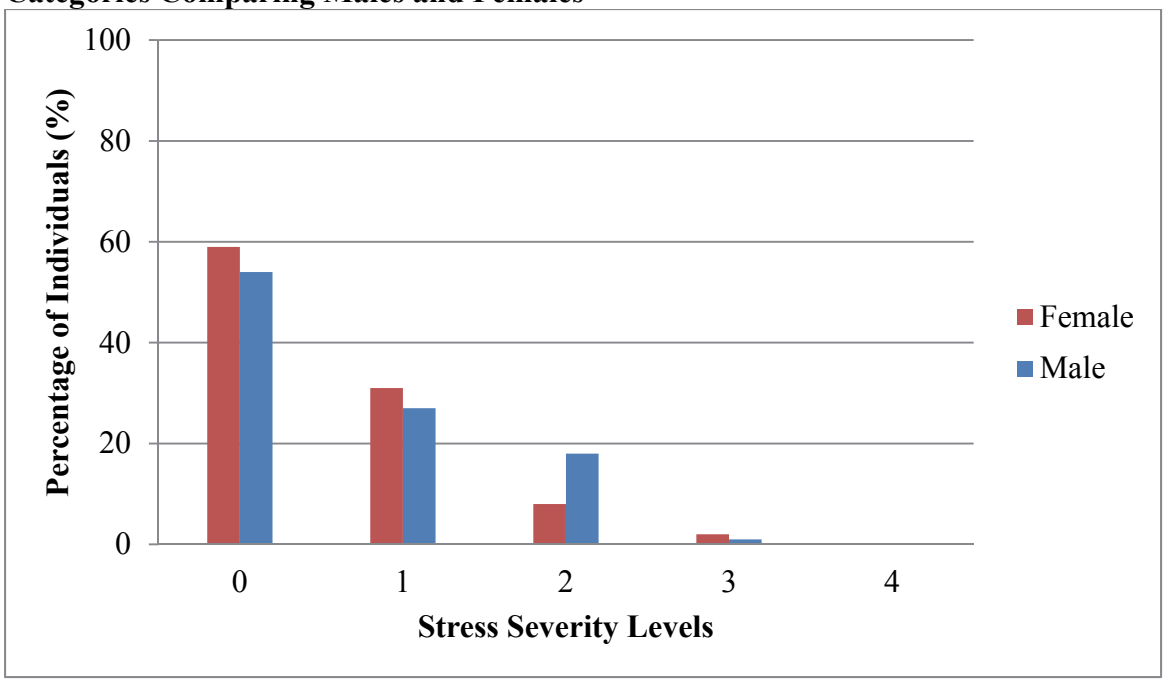


Figure 5.15 Percentage of Individuals with Enamel Hypoplastic Lesions across Stress Severity Categories Comparing Males and Females

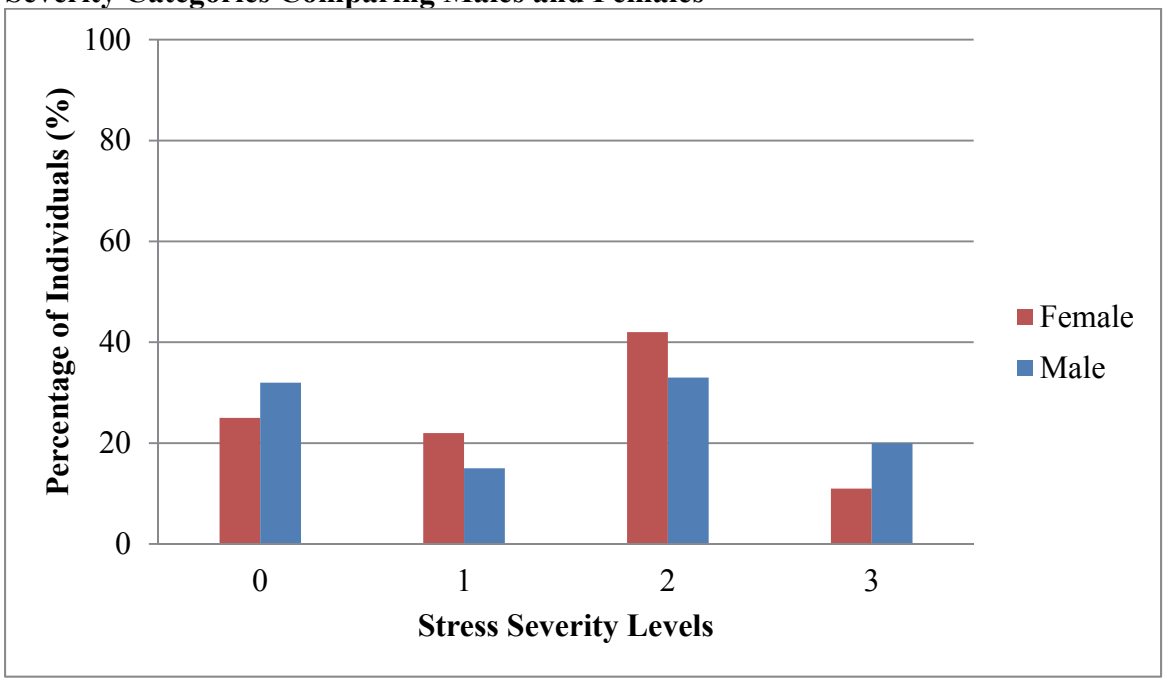
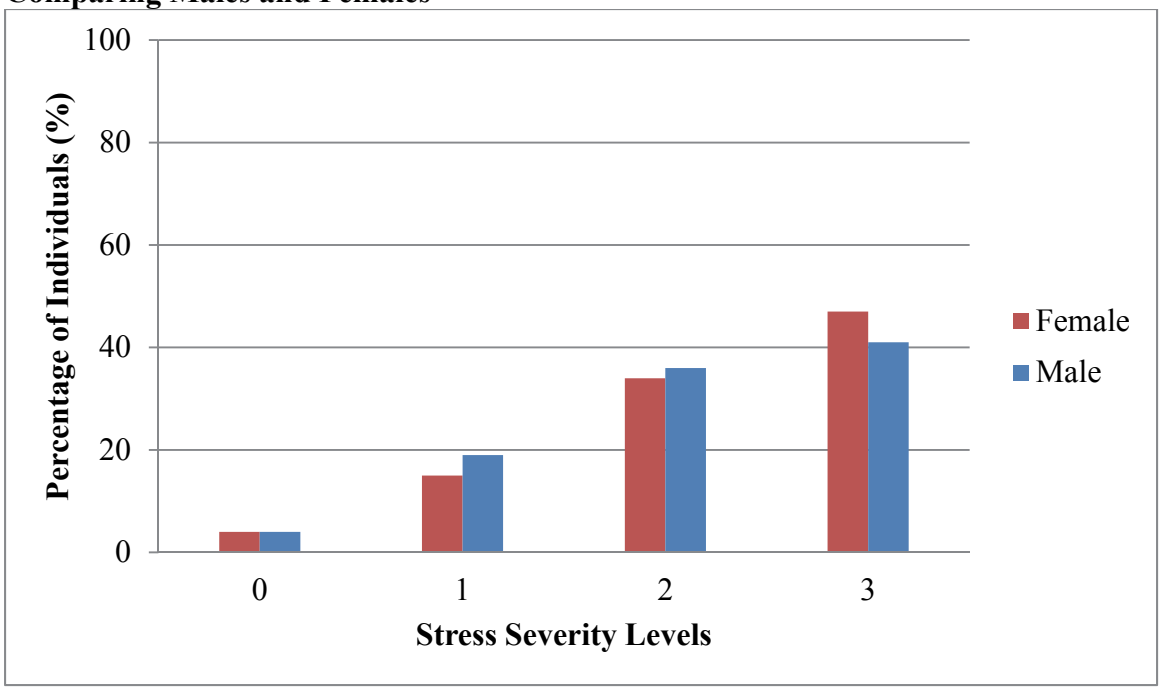


Figure 5.16 Percentage of Individuals with Harris Lines across Stress Severity Categories Comparing Males and Females



In looking at stress severity across the three adult age groups (Figures 5.17 to 5.20), CO and PH showed a relatively similar distribution across severity categories 1, 2 and 3 in the

youngest age group. In the middle and oldest adult age groups there were fewer individuals in the highest severity categories and continued to decline with age, as would be expected. For EHL there was far more fluctuation in stress severity between age groups with the least amount of individuals occupying the lowest severity category. For HL there was a clear trend in all three age groups where the majority of individuals occupied the highest severity categories, except for the oldest age group that showed a slight decline between levels 2 and 3.

Figure 5.17 Percentage of Individuals with Cribra Orbitalia across Stress Severity Categories Comparing Adult Age Groups

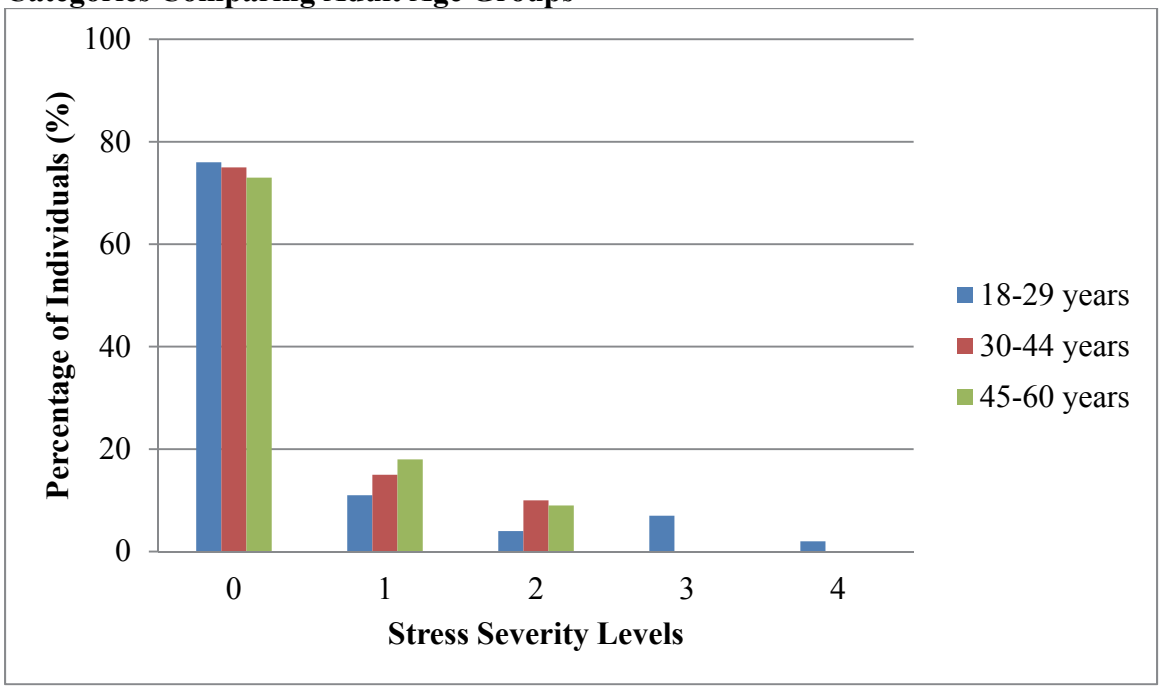


Figure 5.18 Percentage of Individuals with Porotic Hyperostosis across Stress Severity Categories Comparing Adult Age Groups

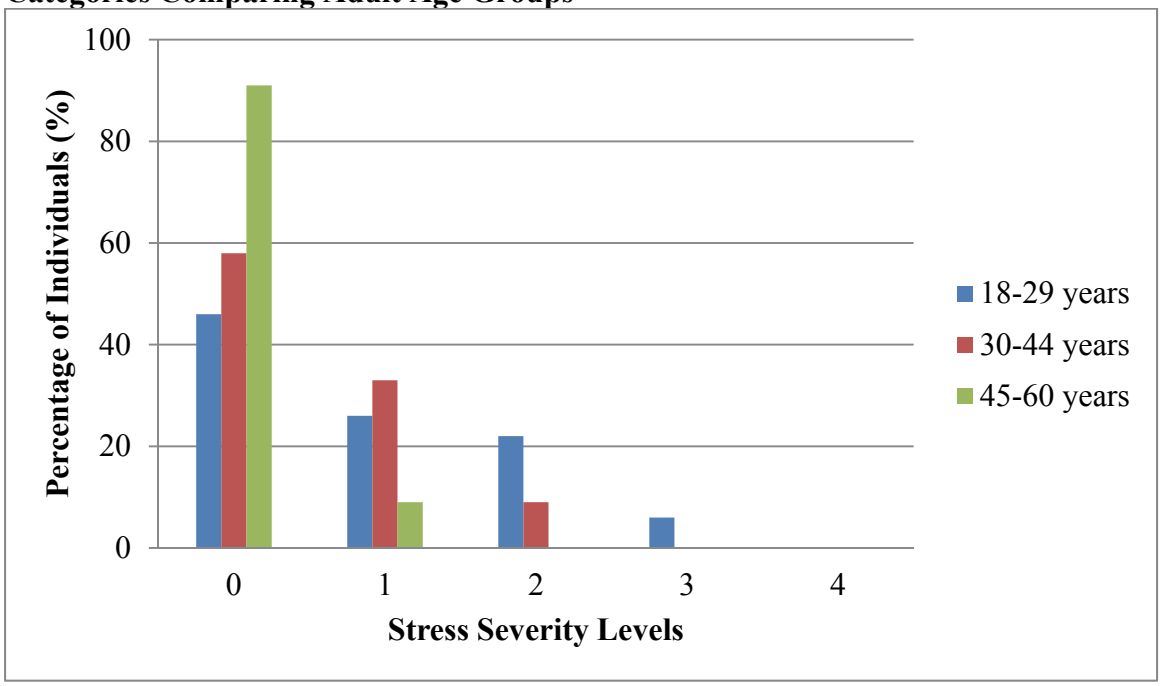


Figure 5.19 Percentage of Individuals with Enamel Hypoplastic Lesions across Stress Severity Categories Comparing Adult Age Groups

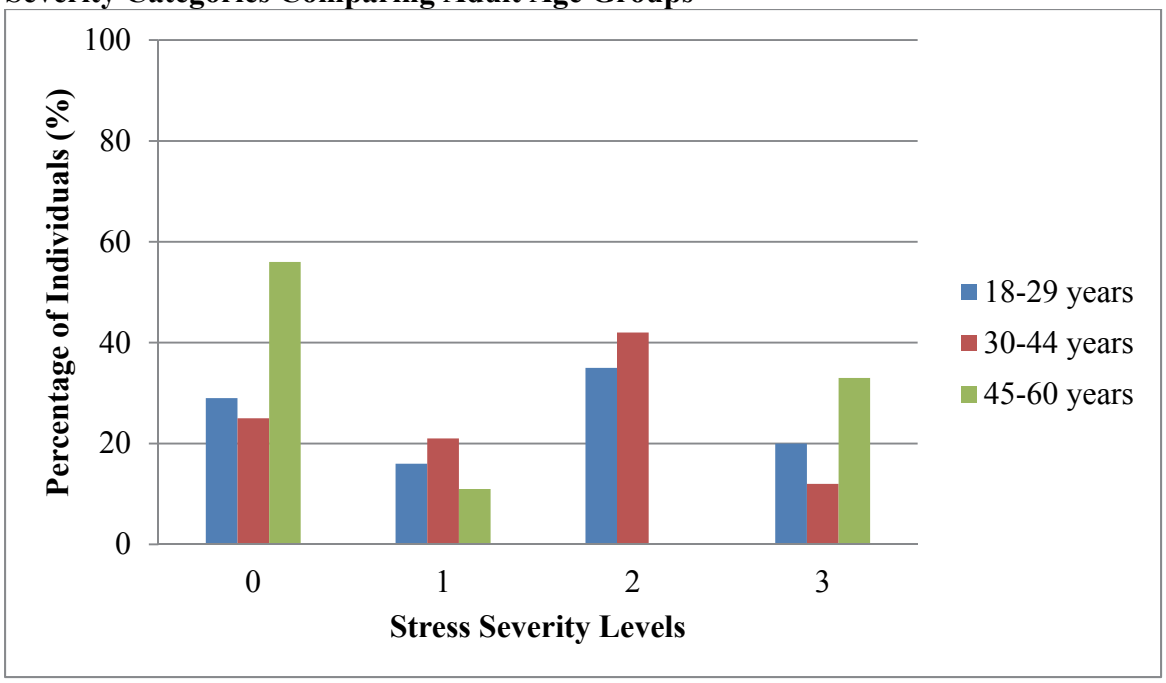
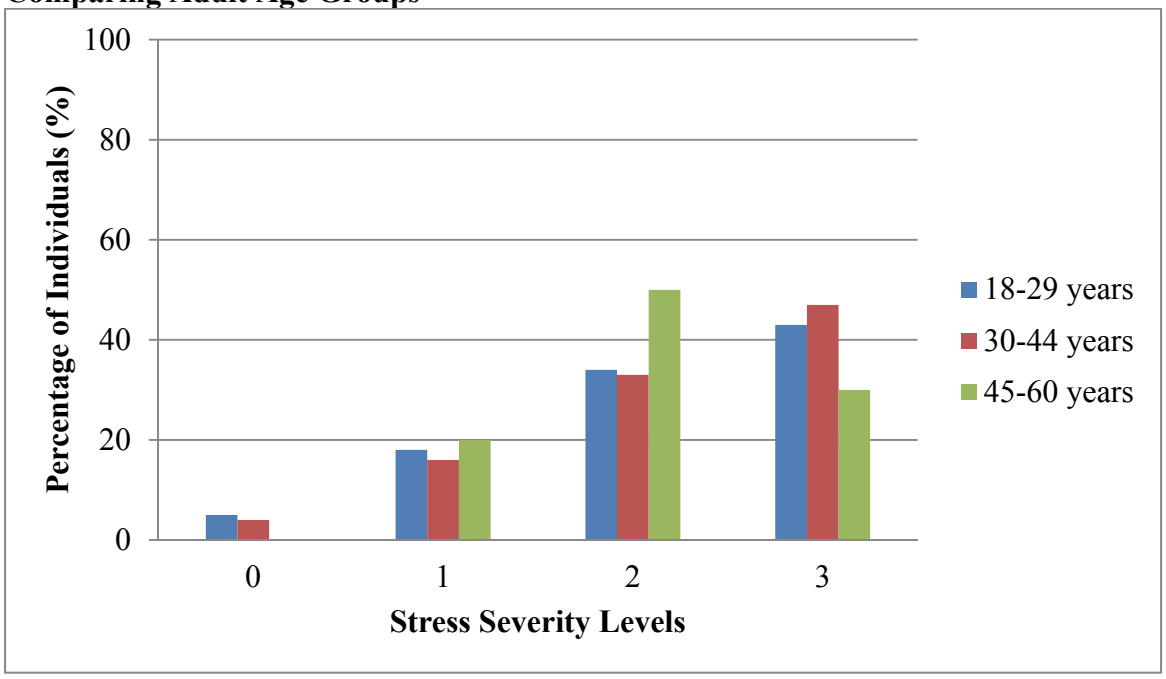


Figure 5.20 Percentage of Individuals with Harris Lines across Stress Severity Categories Comparing Adult Age Groups



For the subadult sample the data was also assessed by stress severity and compared by time period and age group (Figure 5.21 to 5.24). In the medieval grouping all individuals showed at least some evidence of CO and HL, whereas PH and EHL were only scored within the higher severity categories (i.e. levels 2, 3 and 4). In the post-medieval group, over 40 percent of individuals showed no evidence of CO. The distribution of PH was similar between time periods with a slight increase in severity in the post-medieval group. Similarly, EHL were also more prevalent in the post-medieval group and at higher severity levels. The distribution of HL between time periods was relatively similar with a slight decrease in severity into the post-medieval period. Overall, the post-medieval period showed more consistent stress across all four severity categories; however, individuals from the medieval period occupied the moderate and severity categories at a higher level across three of the four indicators (i.e. CO, PH, HL).

Figure 5.21 Percentage of Subadult Individuals with Cribra Orbitalia across Stress Severity Categories (0-4) between Time Periods

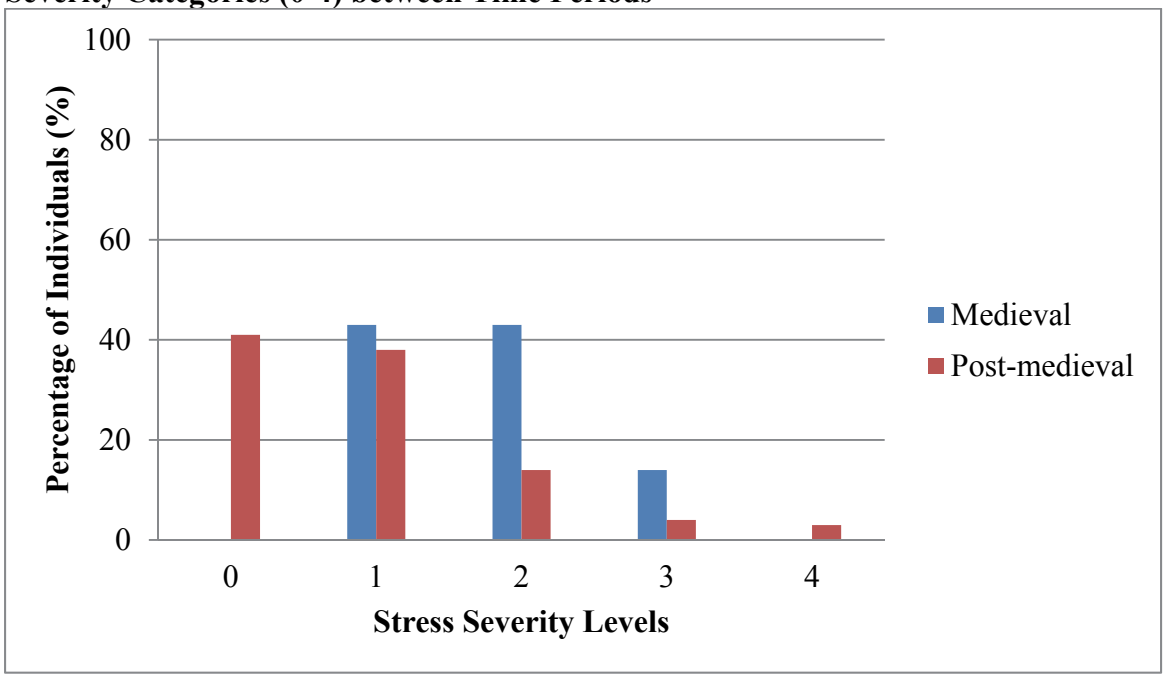


Figure 5.22 Percentage of Subadult Individuals with Porotic Hyperostosis across Stress Severity Categories (0-4) between Time Periods

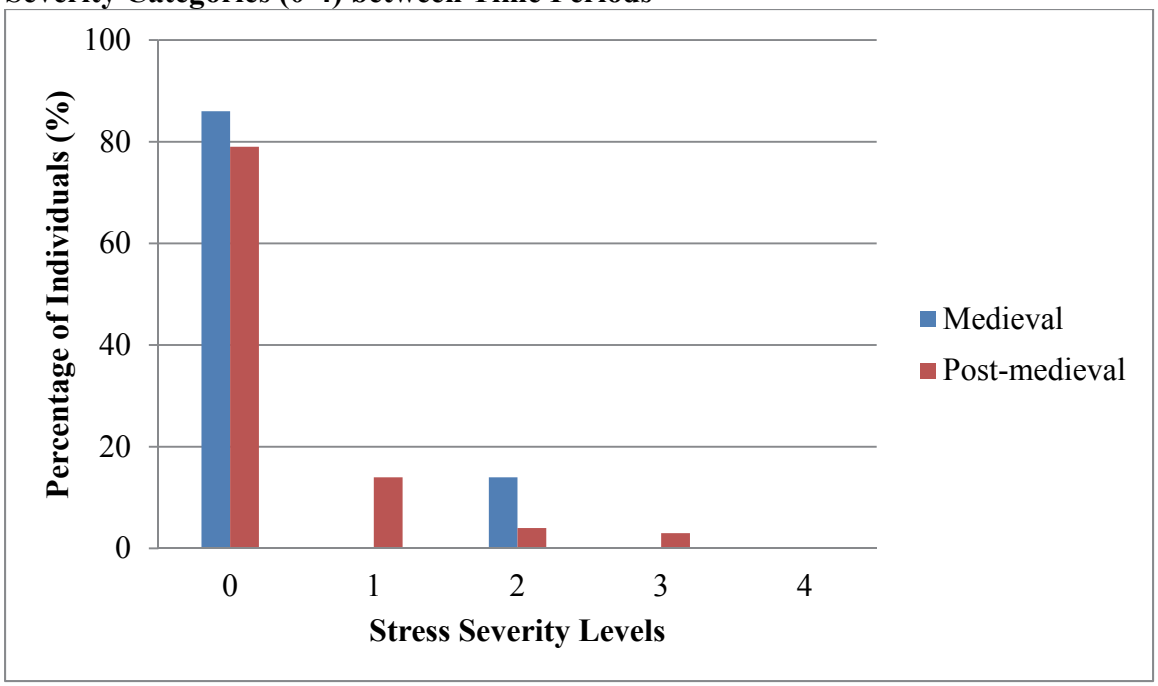


Figure 5.23 Percentage of Subadult Individuals with Enamel Hypoplastic Lesions across Stress Severity Categories (0-3) between Time Periods

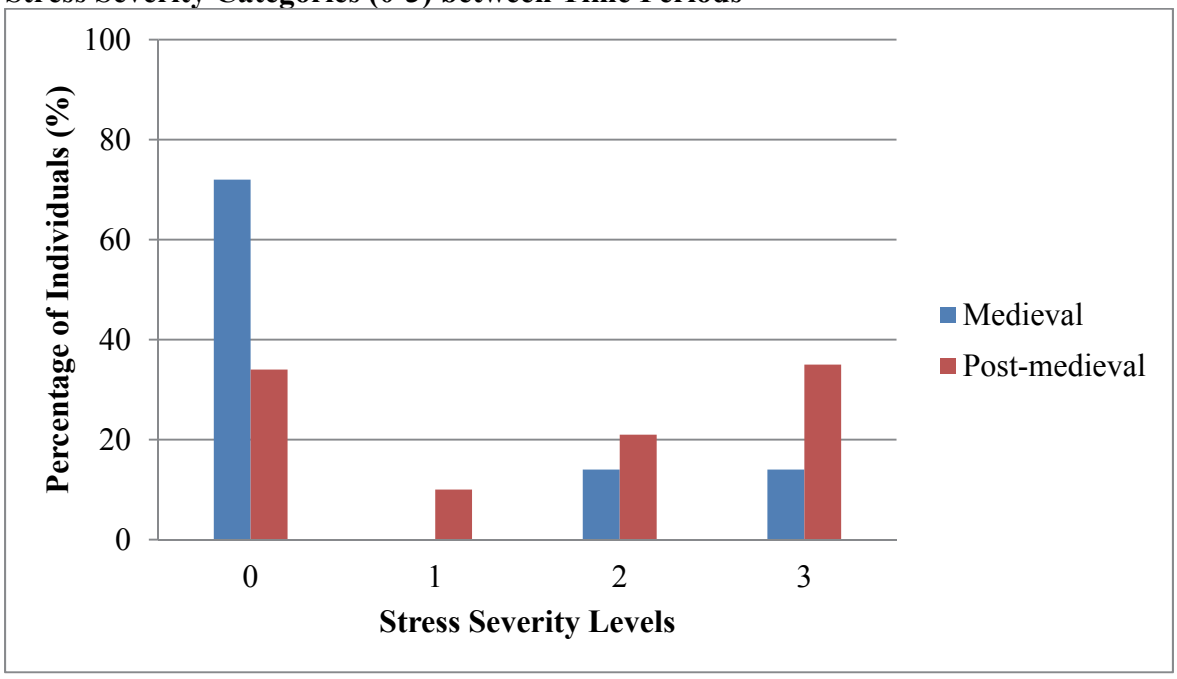
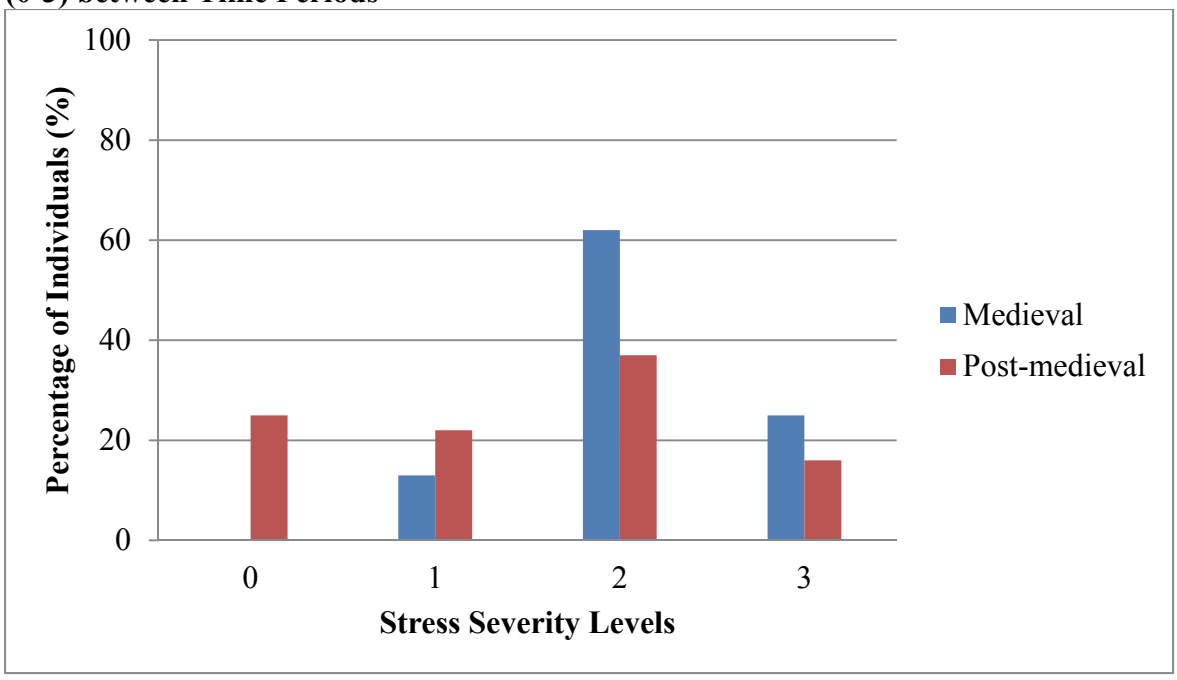


Figure 5.24 Percentage of Individuals with Harris Lines across Stress Severity Categories (0-3) between Time Periods



When analyzing the subadult data by age group and stress severity, the youngest age groups showed far fewer individuals (if any at all) in the highest severity categories. Arguably,

this was due to the time needed for stress to manifest in the skeleton; therefore, it was not expected that the 0-1 year individuals would show moderate or severe stress in most cases. There was a steady increase in the severity of EHL and HL within increasing age with many individuals showing moderate or severe stress for EHL in the 8-13 year category and moderate stress for HL in the 14-18 year category. Severe stress was only visible in CO and PH in the oldest subadult age categories. Overall, there was more diversity in the level severity in the older age groups with the most severe categories occupied by those with EHL and HL, similar to the adult group.

The relationship between each stress indicator was also analyzed using a Kendall's tau ranked correlation analysis testing both the adult and subadult groups, with no significant correlations between any of the four indicators which produced both positive and negative correlation coefficients.

This relationship analysis was further explored by calculating the sum of all stress indicator severity levels for each individual – the 'stress sum' score. For this analysis the amalgamation of the stress indicator scores provided a numerical representation of aggregated stress to provide a simple visual tool for assessing overall severity for each individual. While the nature of this data does not necessarily allow for a full understanding of the relationship between those with a higher level of severity in one indicator over another, it does provide a summary analysis of the general trends emerging in the data. Only individuals with a score for all four stress indicators were included. When comparing these stress sum scores in the adult group using a Kendall's tau-b analysis, there were no significant differences between time period (τ =-0.040; p=0.589), sex (τ =-0.076; p=0.313), or age categories (τ=-0.111; p=0.138). In all three comparisons the distribution of individuals clustered around the middle range of stress severity

with the fewest individuals occupying the lowest and highest ranges of stress (Figures 5.25 to 5.27). In looking at time period, there were no obvious differences between the medieval and post-medieval period calculated stress sums, similar to the male and female comparison. However, when assessing the data by sex, the females had slightly more individuals with a higher stress score than the males, but this comparison was not significantly different. When assessing the distribution of individuals by age group there was an interesting trend that continued through the three age categories (Figure 5.27). In the youngest age group (18-29 years) more individuals scored towards the higher range of stress severity. In the middle age group (30-44 years) this trend shifted where more individuals had a lower stress score. Finally in the oldest age group (45-60 years), no individuals showed the highest possible stress score, but rather occupied the lower end of the stress spectrum; however, the sample size in this oldest age category was small (n=8) and may in part, have influenced this outcome.

Figure 5.25 Distribution of Adult Stress Sum based on Time Period

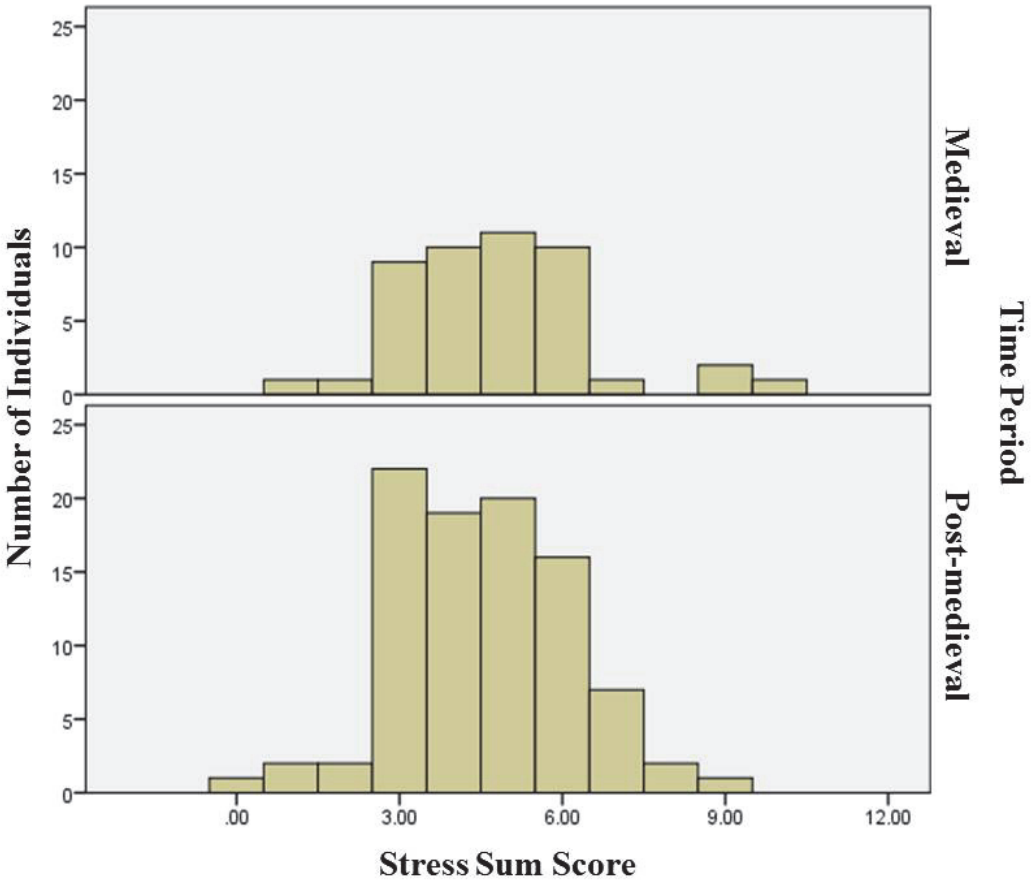


Figure 5.26 Distribution of Adult Stress Sum based on Sex

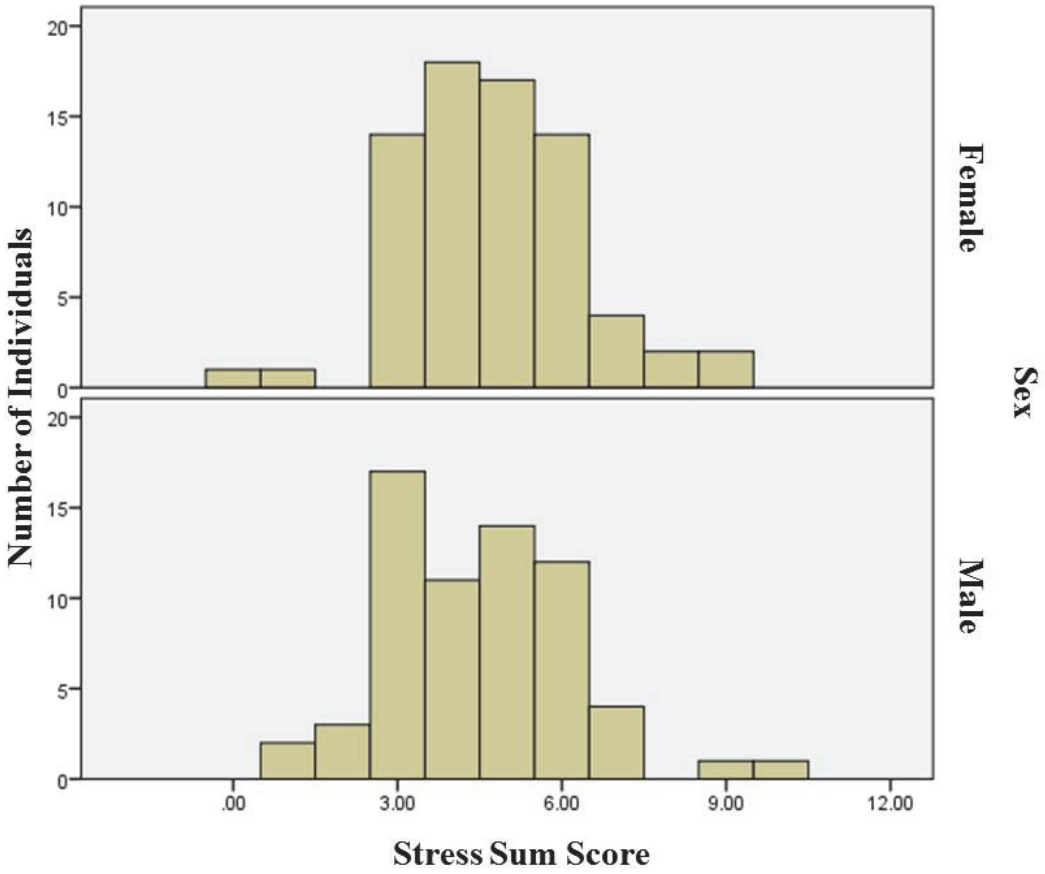
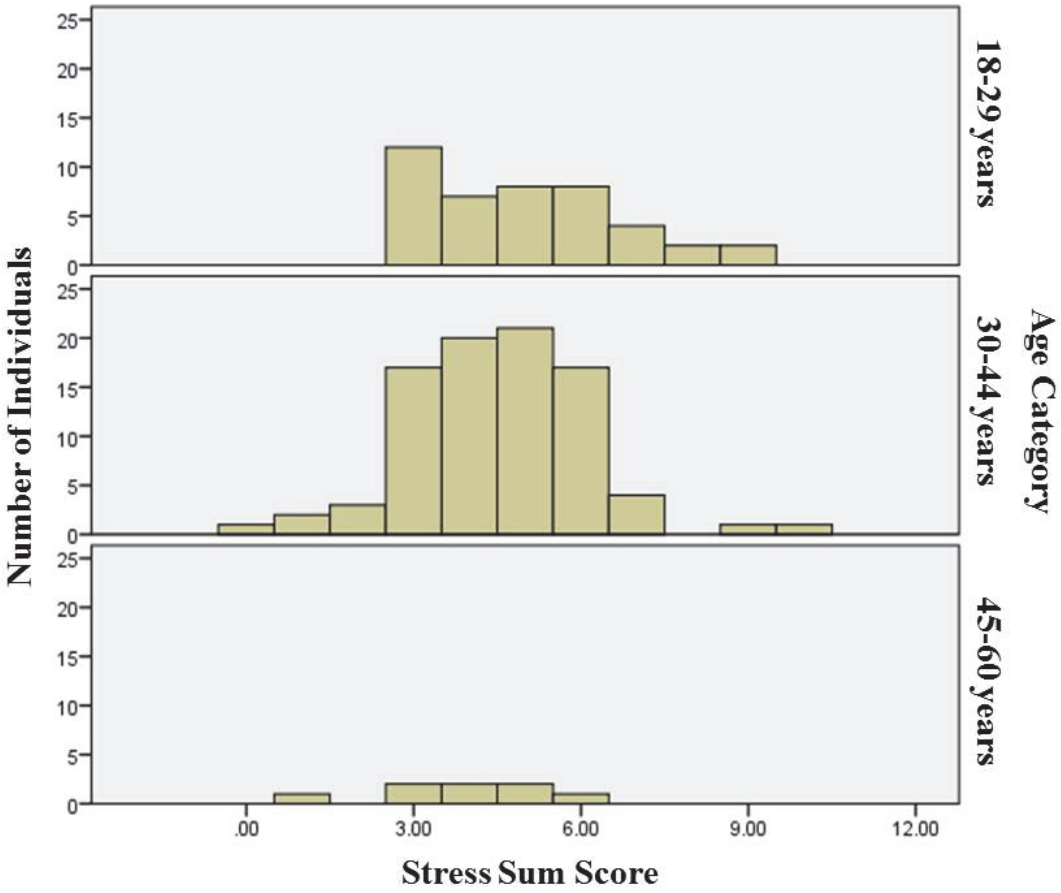


Figure 5.27 Distribution of Adult Stress Sum based on Age Category



Because the subadult sample was already reduced with the further omission of those without data for each of the four stress indicators, no further comparison analysis was completed. It was expected that the lack of correlation between any of the subadult stress indicators, similar to the adults, would provide a similar pattern of distribution across time period and age group. With an increased sample size a further analysis of the subadult data would be possible.

5.4 GROWTH AND DEVELOPMENT-BASED ANALYSES

5.4.1 Body Size Indicators

All body size indicator data were analyzed using a two-way ANOVA with the data divided by time period and sex. Because the focus of this analysis was on those individuals falling outside the 95 percent confidence interval, only the variables from individuals falling both

above and below the confidence interval were analyzed. The females showed a much higher proportion of variables falling below the confidence interval with the mean value ranging between 0.2666 and 0.3501, whereas in the male group the mean value ranged from 0.2055 to 0.2666. Additionally, moving from the medieval into the post-medieval period there was an increased proportion of variables falling below the confidence interval (Table 5.63). The two-way ANOVA results showed that both sex and time were significantly affecting the differences observed; however, their interaction effect was not significant suggesting the patterns visible between males and females over each time period were occurring in parallel (Table 5.64; Figure 5.28).

Table 5.63 Two-way ANOVA Results for Mean Proportion of BSI Variables Falling Below the 95% Confidence Interval

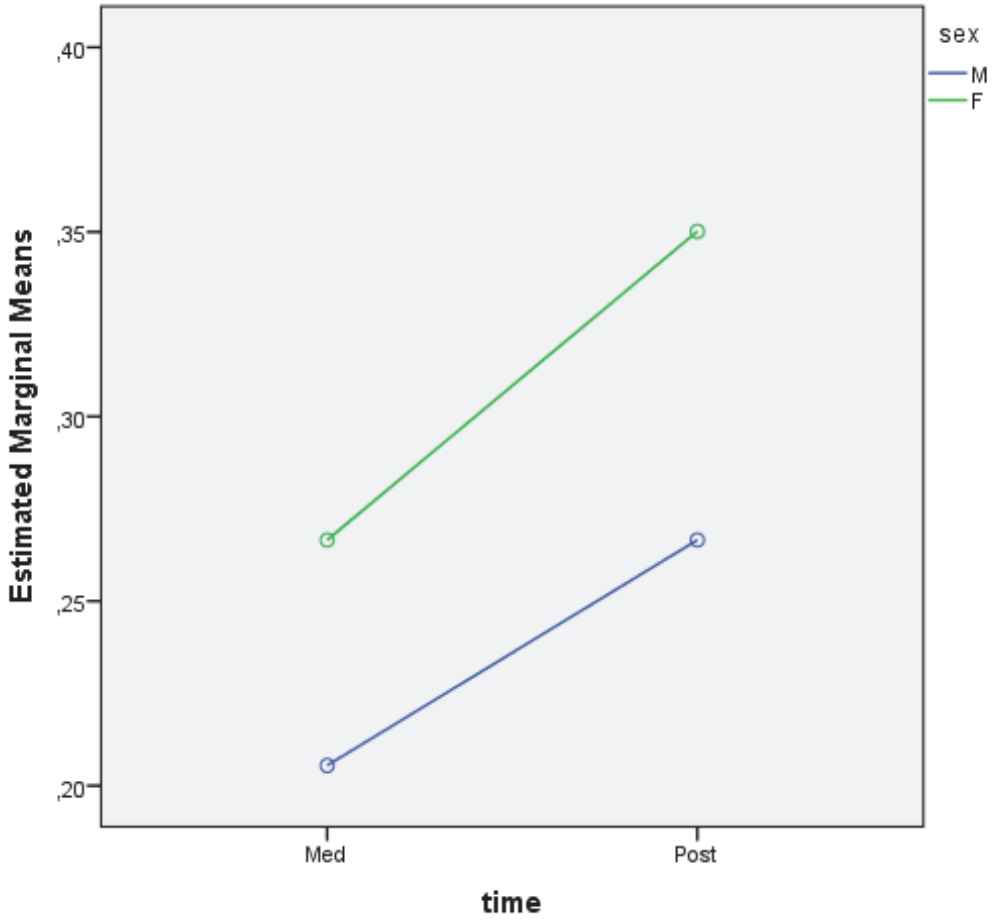
Time	Sex	Mean	Std. Deviation
Medieval	Male	0.2055	0.15228
	Female	0.2666	0.16932
	Total	0.2460	0.16622
Post-medieval	Male	0.2666	0.16932
	Female	0.3501	0.22402
	Total	0.3151	0.20696
Total	Male	0.2460	0.16622
	Female	0.3151	0.20695
	Total	0.2883	0.19508

Table 5.64 Two-way ANOVA Results with Independent Effects for BSI Variable Frequencies Below the 95% Confidence Interval

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	5.581[a]	3	1.860	52.248	0.000	0.066
Intercept	145.105	1	145.105	4075.367	0.000	0.646
Time	2.561	1	2.561	71.938	0.000	0.031
Sex	2.561	1	2.561	71.938	0.000	0.031
Time*Sex	0.062	1	0.062	1.741	0.187	0.001
Error	79.400	2230	0.036			
Total	270.628	2234				
Corrected Total	84.981	2233				

a. R squared = 0.066 (Adjusted R Squared = 0.064)

Figure 5.28 Plotted Two-way ANOVA (Estimated Marginal Means) for BSI Variables Below the 95% Confidence Interval



A similar pattern also emerged when looking at the frequency of variables falling above the confidence interval. The females showed more variables above the confidence interval with the mean value ranging between 0.2857 to 0.3111 and the male mean value between 0.1815 and 0.2857 (Table 5.65). For both males and females there was also an increase in the frequency of variables falling above the confidence interval when moving from the medieval into the post-medieval period. As independent effects, both time and sex were significantly influencing the frequency of BSI variables affected (Table 5.66). The interaction effect of time and sex was also significant suggesting the visible frequency patterns were not occurring in parallel as shown in Figure 5.29. These results suggest that when moving from the medieval into the post-medieval period the males were more affected than females in regards to the frequency of variables falling above the confidence interval.

Table 5.65 Two-way ANOVA Results for Mean Proportion of BSI Variables Falling Above the 95% Confidence Interval

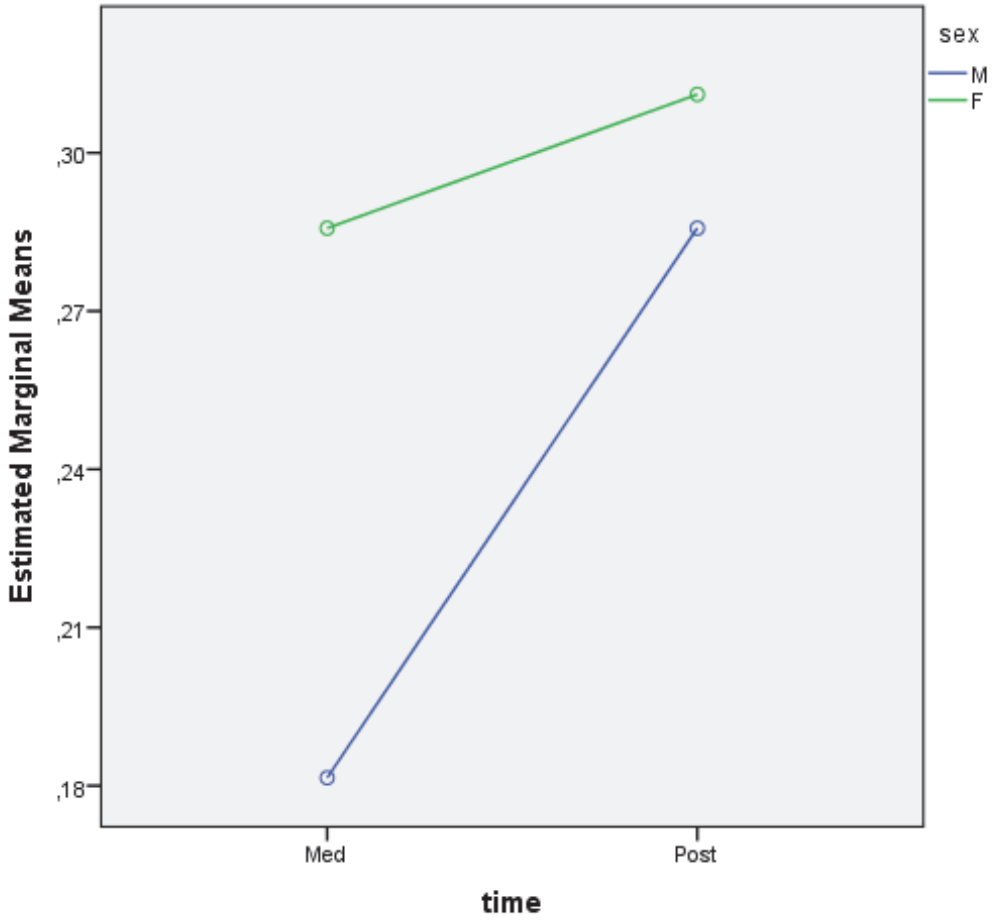
Time	Sex	Mean	Std. Deviation
Medieval	Male	0.1815	0.15173
	Female	0.2857	0.18591
	Total	0.2506	0.18185
Post-medieval	Male	0.2857	0.18591
	Female	0.3111	0.23292
	Total	0.3004	0.21475
Total	Male	0.2506	0.18185
	Female	0.3004	0.21475
	Total	0.2811	0.20404

Table 5.66 Two-way ANOVA Results with Independent Effects for BSI Variable Frequencies Above the 95% Confidence Interval

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3.635[a]	3	1.212	30.244	0.000	0.039
Intercept	138.599	1	138.599	3460.004	0.000	0.608
Time	2.055	1	2.055	51.313	0.000	0.022
Sex	2.055	1	2.055	51.313	0.000	0.022
Time*Sex	0.761	1	0.761	18.996	0.000	0.008
Error	89.328	2230	0.040			
Total	269.500	2234				
Corrected Total	92.963	2233				

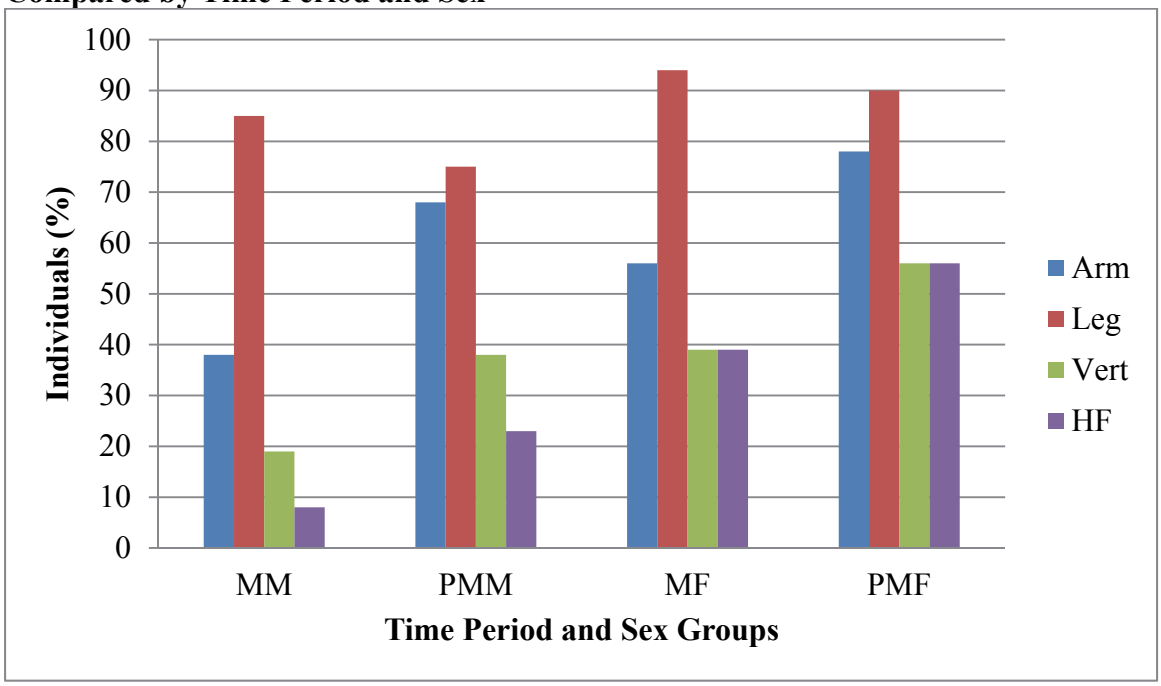
a. R squared = 0.039 (Adjusted R Squared = 0.038)

Figure 5.29 Plotted Two-way ANOVA (Estimated Marginal Means) for BSI Variables Above the 95% Confidence Interval



Looking specifically at the variables falling below the confidence interval, the data were divided into body sections maturing during specific periods (i.e. arms, legs, vertebrae, hands and feet) to determine how stress was manifesting across the body. For each time period and sex group a tally of which body sections fell below the confidence interval were converted into percentage values for cross-group comparison. Figure 5.30 shows that for all four groups the leg variables fell below the confidence interval the most followed by the arms, vertebrae and hands and feet. However, when looking at the nature of the data over half the BSIs selected for males and females were from the arms and legs; therefore, it is likely that even the smallest stress disruption would have been captured across one of the many elements observed. For the vertebrae and hands and feet however, there were minimal variables to observe and it is possible that small fluctuations in size may not have manifested in the particular elements studied. In the male group, the vertebral variables fell below the confidence interval much more than the hand and foot variables, whereas in the female groups there was no difference between these two body sections. The female groups also had more individuals with multiple body sections being affected with nearly 40% of the medieval group and over 50% of the post-medieval group falling below the confidence interval in all four body sections.

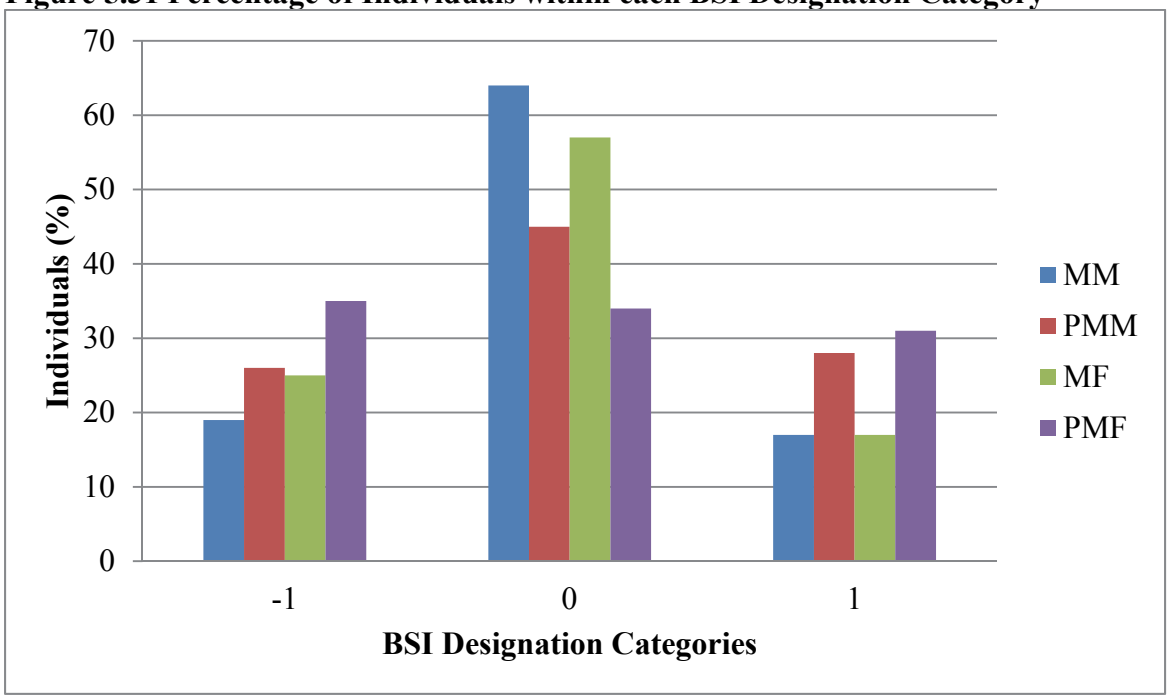
Figure 5.30 Percentage of Individuals with Body Sections below the Confidence Interval Compared by Time Period and Sex



(MM = medieval males; PMM = post-medieval males; MF = medieval females; PMF = post-medieval females)

As shown in Figure 5.31, when considering the general BSI trend within each time period divided by sex, the majority of individuals were clustered within the confidence interval. The medieval males showed the least amount of fluctuation in their BSI designations with the lowest number of individuals above and below the confidence interval. The post-medieval females showed the greatest fluctuation between the categories with the highest proportion of individuals both above and below the confidence interval. The medieval females showed the largest number of individuals within the confidence interval and slightly more individuals below the confidence interval than above. Finally, the post-medieval males had a nearly equal number of individuals above and below the confidence interval and almost 50 % of the group falling within the confidence interval.

Figure 5.31 Percentage of Individuals within each BSI Designation Category



(MM = medieval males; PMM = post-medieval males; MF = medieval females; PMF = post-medieval females)

To compare BSI variable frequencies with stress lesions, all severity data were converted into two categories (i.e. present, absent) for ease of analysis. Using a one-way ANOVA each stress indicator was compared to the BSI frequencies both above and below the confidence interval. As shown in Tables 5.67 and 5.68, there were no significant differences in the frequency of BSIs falling above or below the confidence interval for individuals with or without stress indicators present.

Table 5.67 One-way ANOVA Results for BSI Frequencies Below the 95% Confidence Interval and Stress Indicators

Stress Indicators	F-value	df	P-value
Cribra Orbitalia	0.066	1	0.797
Porotic Hyperostosis	0.072	1	0.789
Enamel Hypoplastic Lesions	1.395	1	0.239
Harris Lines	0.826	1	0.365

Table 5.68 One-way ANOVA Results for BSI Frequencies Above the 95% Confidence Interval and Stress Indicators

Stress Indicators	F-value	df	P-value
Cribra Orbitalia	0.655	1	0.419
Porotic Hyperostosis	0.099	1	0.754
Enamel Hypoplastic Lesions	0.787	1	0.377
Harris Lines	0.075	1	0.785

5.4.2 Cortical Bone Thickness

Cortical thickness was first analyzed by comparing the left and right side thickness averages based on orientation (ML and AP) using a paired samples t-test. For the adults there was a statistically significant difference between the left and right AP view (t=-2.837; df=31; p=0.008), but no difference in the ML view (t=-0.839; df=135; p=0.403). When comparing the ML and AP averages between time periods using an independent samples t-test there were no significant differences (ML view t=1.256; df=142; p=0.211; AP view t=-0.126; df=122; p=0.900). Assessing the data within the medieval and post-medieval groups divided by sex there were no significant differences between the ML and AP average in the males (medieval ML view t=0.523; df=21; p=0.607; post-medieval ML view t=-1.470; df=42; p=0.149; medieval AP view t=-1.283; df=3; p=0.290; post-medieval AP view t=-1.955; df=9; p=0.082) or females (medieval ML view t=-0.696; df=17; p=0.496; post-medieval ML view t=-0.220; df=52; p=0.827; medieval AP view t=0.086; df=4; p=0.936). However, there was a significant difference between the left and right AP average for the post-medieval females (t=-2.636; df=12; p=0.022) with the left side average smaller than the right.

Cortical thickness was also compared to pathology in both orientations. For this analysis, degenerative spine and joint changes as well as Schmorl's nodes were omitted as they are more reflective of age and activity than specific pathological conditions. An independent samples t-test was completed for each of the six remaining pathological conditions in both orientations. The

presence of leprosy and treponema were significantly different when compared to cortical thickness in the ML view and tuberculosis in the AP orientation (Tables 5.69 and 5.70).

Table 5.69 Independent Samples t-test Results for ML Cortical Thickness and Pathological Conditions

Pathology	t-value	df	P-value
Periostitis	-0.980	142	0.329
Tuberculosis	0.781	142	0.436
Leprosy	5.443	7.209	0.001*
Treponema	-3.136	142	0.002*
Fractures	1.157	142	0.249
Dental Abscesses	0.073	141	0.942

* significant at a 95% confidence interval

Table 5.70 Independent Samples t-test Results for AP Cortical Thickness and Pathological Conditions

Pathology	t-value	df	P-value
Periostitis	-1.354	122	0.178
Tuberculosis	2.048	122	0.043*
Leprosy	1.419	122	0.158
Treponema	-1.182	122	0.240
Fractures	1.090	122	0.278
Dental Abscesses	-1.148	121	0.253

* significant at a 95% confidence interval

Cortical thickness was also compared to each stress lesion using a one-way ANOVA. There were no significant differences between ML or AP cortical thickness in any of the severity categories (Tables 5.71 and 5.72).

Table 5.71 One-way ANOVA Results for ML Cortical Thickness and Stress Indicators

Stress Indicators	F-value	df	p-value
Cribra orbitalia	0.317	4	0.866
Porotic hyperostosis	0.797	3	0.497
Enamel hypoplastic lesions	1.236	3	0.299
Harris lines	1.567	3	0.200

Table 5.72 One-way ANOVA Results for AP Cortical Thickness and Stress Indicators

Stress Indicators	F-value	df	p-value
Cribra orbitalia	1.065	4	0.377
Porotic hyperostosis	0.870	3	0.459
Enamel hypoplastic lesions	2.093	3	0.105
Harris lines	1.730	3	0.165

In looking at the fluctuation in cortical thickness between severity categories (Figures 5.32 and 5.33), the ML orientation shows a slight pattern across the stress indicators where cortical thickness is reduced with increasing severity; however, this trend is not consistent. Whereas cortical thickness remains relatively stable for CO and PH in all severity categories, cortical thickness decreases as EHL and HL severity increases. However, in the most severe stress category cortical thickness for EHL and HL increases, in contrast to this observed pattern. In the AP orientation there is no discernable pattern between cortical thickness and stress severity.

Figure 5.32 Cortical Thickness Average in the ML Orientation Compared to Stress Severity Categories

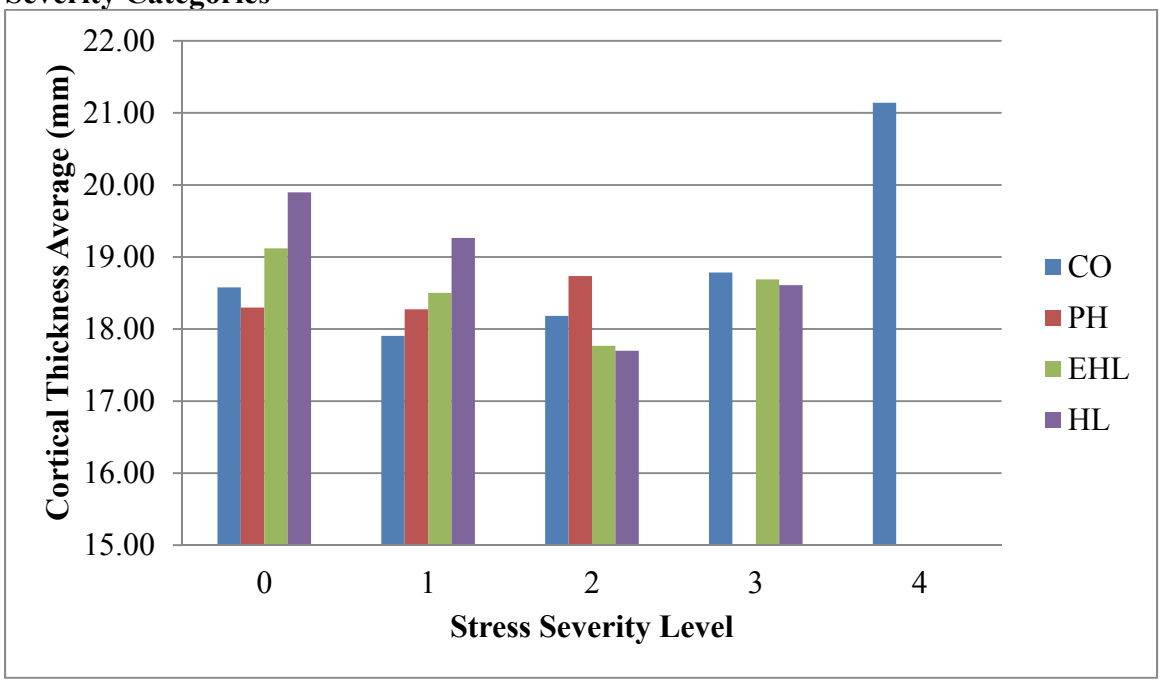
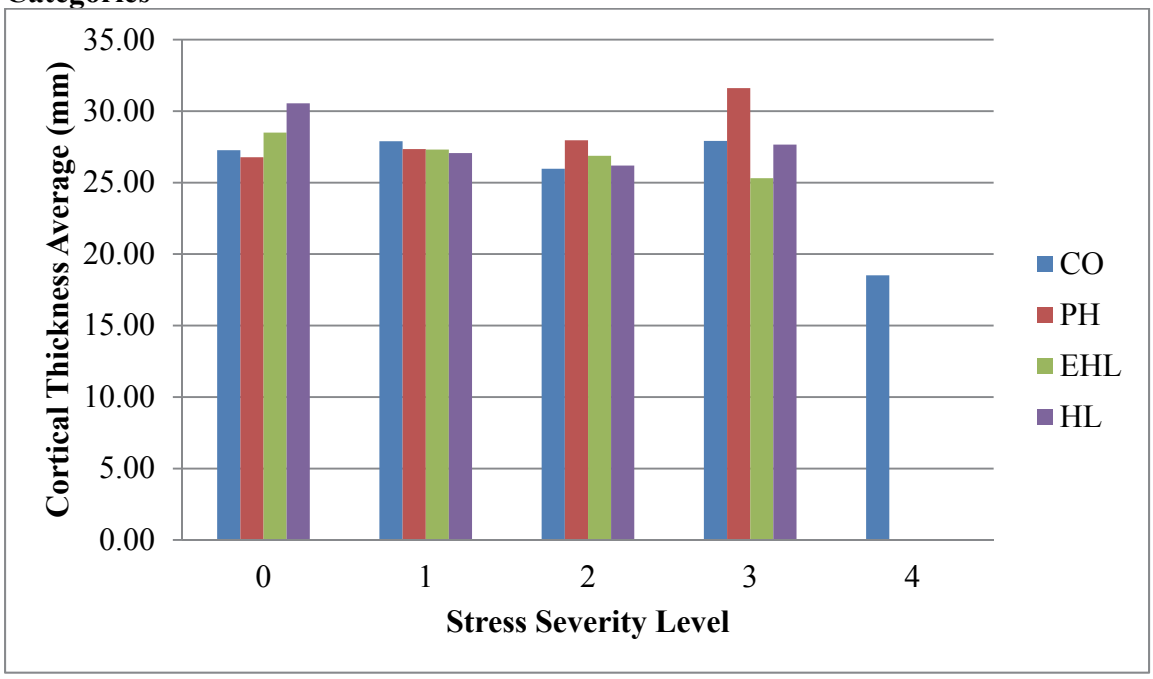


Figure 5.33 Cortical Thickness Average in the AP Orientation Compared to Stress Severity Categories



5.5 BIOCHEMICAL ANALYSES

5.5.1 Osteocalcin

A comparison of the mean osteocalcin levels between the two femur and clavicle dilutions (1:1 and 1:4) was analyzed using a paired samples t-test with both showing statistically significant differences between the 1:1 and 1:4 dilution results (femur t=2.880; df=19; p=0.010; clavicle t=3.044; df=19; p=0.007). The 1:4 dilution had less osteocalcin, however, this reduction in the mean osteocalcin level was not necessarily unexpected as an increased dilution would have made it more difficult for the ELISA kit to identify the target protein. When assessing the correlation between the osteocalcin levels of the 1:1 and 1:4 dilutions for each bone, all osteocalcin levels were highly correlated (Table 5.73) suggesting that despite statistical differences in the mean osteocalcin levels of the 1:1 and 1:4 dilution results, the osteocalcin levels remained consistent. Because the 1:4 dilution allowed for the capture of outliers produced

in the 1:1 dilution all remaining analyses were completed using the 1:4 dilution only (Table 5.74).

Table 5.73 Correlation between the 1:1 and 1:4 Osteocalcin Dilutions for the Femur and Clavicle

		Femur 1:1	Femur 1:4	Clavicle 1:1	Clavicle 1:4
Femur 1:1	Pearson Correlation	1	.938**	.809**	.774**
	Sig. (2-tailed)		.000	.000	.000
	N	20	20	20	20
Femur 1:4	Pearson Correlation	.938**	1	.765**	.773**
	Sig. (2-tailed)	.000		.000	.000
	N	20	20	20	20
Clavicle 1:1	Pearson Correlation	.809**	.765**	1	.962**
	Sig. (2-tailed)	.000	.000		.000
	N	20	20	20	20
Clavicle 1:4	Pearson Correlation	.774**	.773**	.962**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	20	20	20	20

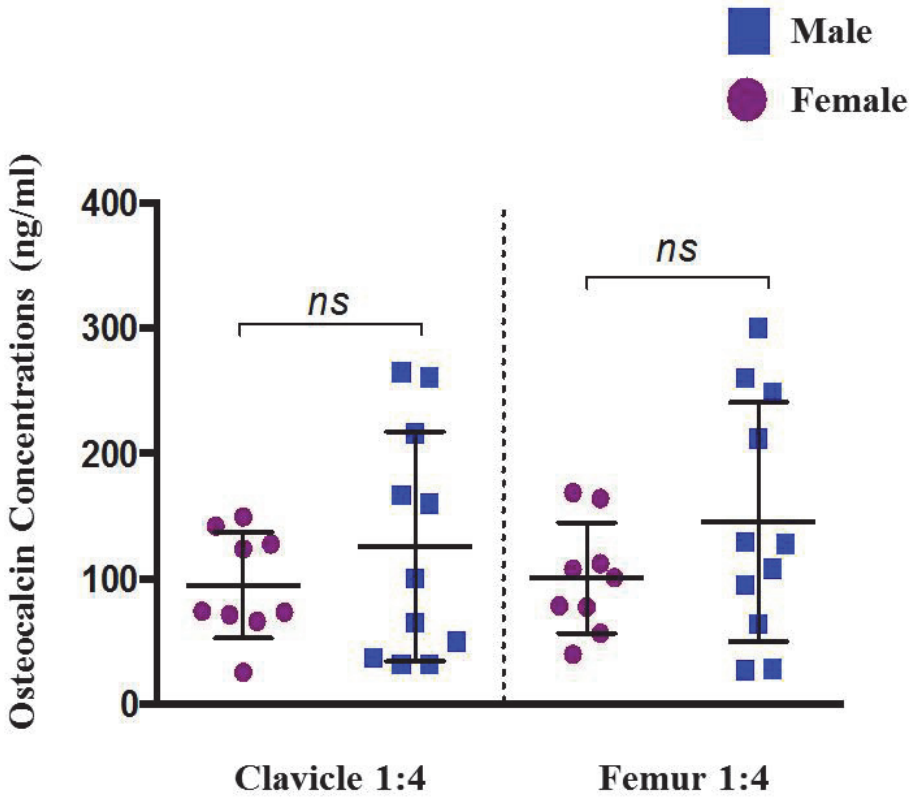
**. Correlation is significant at the 0.01 level (2-tailed).

Table 5.74 Osteocalcin Levels (ng/ml) for the Femur and Clavicle at 1:1 and 1:4 Dilutions

Individual	Femur 1:1	Femur 1:4	Clavicle 1:1	Clavicle 1:4
SBT81 GR513	159.2631	211.9808	75.03089	100.5954
SBT81 GR9	87.06953	78.47035	114.246	149.6363
SBT81 GR661	253.8341	260.4196	290.8625	260.7017
SBT81 GR313	50.42609	56.35345	100.6089	71.60475
SBT81 GR562	267.572	164.3239	167.5938	142.1643
SBT81 GR307	31.91991	28.07047	25.15279	32.26626
SBT81 GR378	149.8737	108.3201	264.2268	216.1523
SBT81 GR297	99.90307	95.30891	60.17099	50.24117
SBT81 GR457	366.4075	300.3326	307.5064	264.9299
SBT81 GR316	48.89608	39.82423	23.18932	25.41656
SBT81 GR208	179.0387	168.8958	160.1675	123.9141
SBT81 GR10	139.6174	101.0605	96.02565	66.12962
SBT81 GR241	141.9531	127.7056	36.41956	37.09395
SBT81 GR306	90.92723	63.86218	93.38669	64.99688
SBT81 GR193	308.6302	248.1174	246.6095	166.6851
SBT81 GR344	31.0902	27.18872	32.25212	31.57093
SBT81 GR550	145.0106	129.219	169.9521	160.1836
SBT81 GR131	78.68467	77.51607	87.41986	73.36707
SBT81 GR279	147.3511	112.3151	181.5874	127.857
SBT81 GR447	153.8425	107.8582	80.25337	74.32656

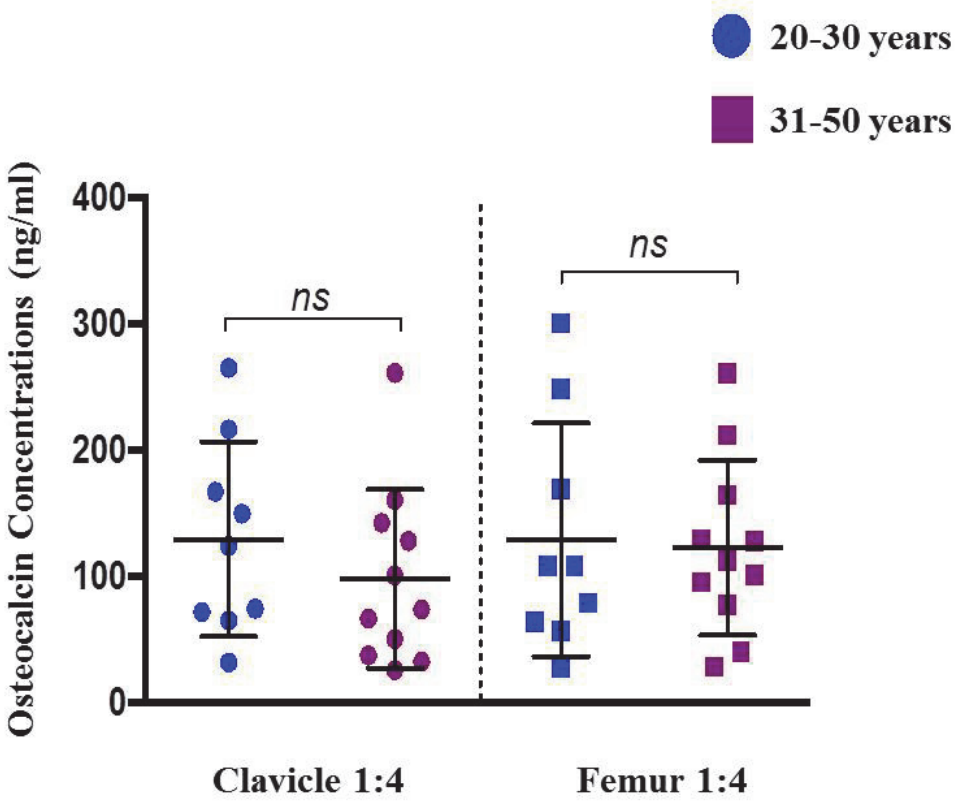
Comparing the femur and clavicle osteocalcin levels using a paired samples t-test there were no significant differences observed (1:4 dilution t=1.163; df=19; p=0.259). Similarly, when divided by sex, there were also no significant differences observed between the femur and clavicle levels (female 1:4 t=0.484; df=8; p=0.641; male 1:4 t=1.039; df=10; p=0.323) (Figure 5.34).

Figure 5.34 Mean Osteocalcin Concentrations at 1:4 Dilution Compared by Sex for the Femur and Clavicle



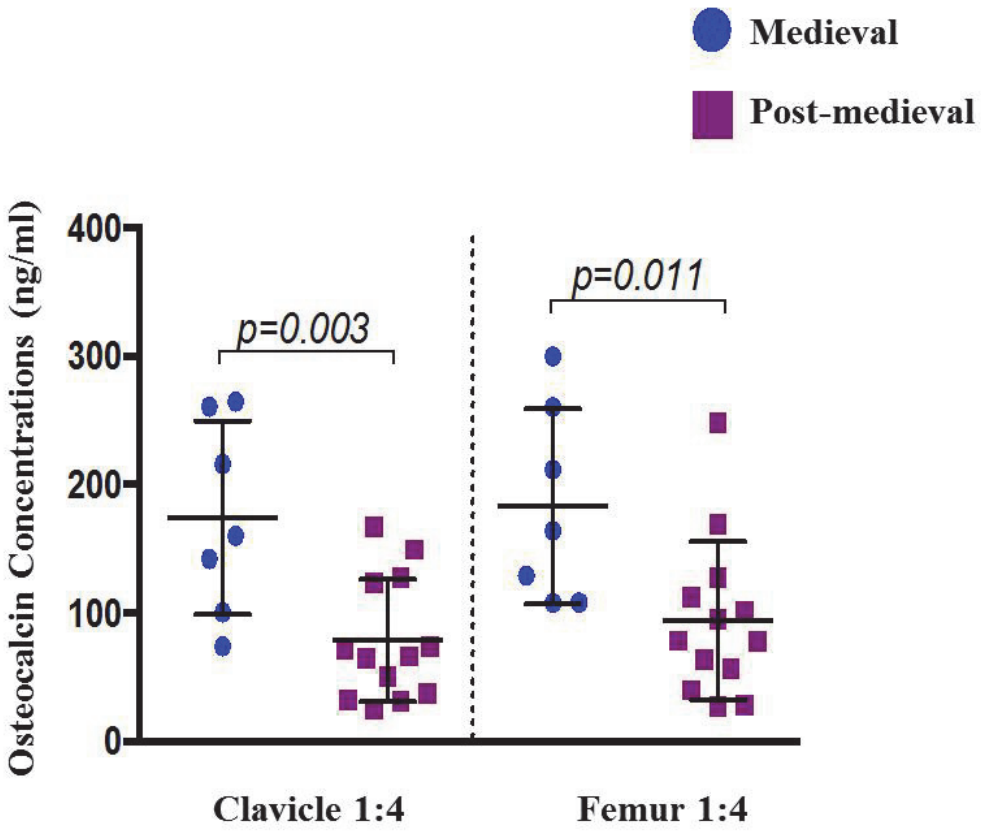
Osteocalcin levels were also assessed in relation to age with the data divided into two categories (20-30 years and 31-50 years). When comparing these age-specific data through a one-way ANOVA there were no statistically significant differences in the osteocalcin levels by age in the femur or clavicle (femur 1:4 F=0.030; p=0.981; clavicle 1:4 F=0.900; p=0.355). However, osteocalcin levels in the femur and clavicle did show a consistent decrease with age, as expected (Figure 5.35). When further comparing the age group data by sex using a one-way ANOVA, there were no significant differences between osteocalcin levels in either age category (female femur 1:4 F=0.015; p=0.906; female clavicle 1:4 F=0.368; p=0.563; male femur 1:4 F=0.015; p=0.905; male clavicle 1:4 F=0.547; p=0.479).

Figure 5.35 Mean Osteocalcin Concentration at 1.4 Dilution Compared by Age Group for the Femur and Clavicle



Differences by time period were also assessed using an independent samples t-test where there was a significant decrease in osteocalcin level from the medieval into the post-medieval period (femur 1:4 t=2.838; df=18; p=0.011; clavicle 1:4 t=3.490; df=18; p=0.003) (Figure 5.36).

Figure 5.36 Mean Osteocalcin Concentrations at 1:4 Dilution Compared by Time Period for the Femur and Clavicle



Using a one-way ANOVA, the osteocalcin data for both the femur and clavicle were compared to all pathological conditions as all are marked by skeletal change that may be reflected in fluctuating osteocalcin levels. Because some of these pathological conditions were not prevalent in this subsample of the Black Friars population (n=20), all data were analyzed together regardless of sex, time period or age grouping. While considering these variables may provide a more refined analysis of osteocalcin fluctuations, the small sample size did not allow for this. When assessing the pathological conditions and the variability in osteocalcin levels between those with and without these pathological markers, significant differences were only noted for degenerative spine changes in the clavicle sample (Table 5.74).

Table 5.75 Independent Samples t-test Results for the Comparison between Femur and Clavicle 1:4 Dilution Osteocalcin Concentrations and Pathological Conditions

Pathology	Bone/Dilution	t-value	df	p-value
Periostitis	Femur 1:4	2.041	18	0.056
	Clavicle 1:4	1.144	18	0.268
Tuberculosis	Femur 1:4	1.395	18	0.180
	Clavicle 1:4	1.441	18	0.167
Leprosy	Femur 1:4	-0.560	18	0.582
	Clavicle 1:4	-0.162	18	0.873
Treponema	Femur 1:4	0.581	18	0.569
	Clavicle 1:4	1.909	17.690	0.073
Fractures	Femur 1:4	1.688	18	0.109
	Clavicle 1:4	1.962	18	0.065
Degenerative Spine Changes	Femur 1:4	1.128	18	0.274
	Clavicle 1:4	2.439	18	0.025*
Schmorl's Nodes	Femur 1:4	-1.546	18	0.139
	Clavicle 1:4	-1.535	18	0.142
Degenerative Joint Changes	Femur 1:4	-0.798	18	0.435
	Clavicle 1:4	0.551	18	0.588
Dental Abscesses	Femur 1:4	1.578	18	0.132
	Clavicle 1:4	1.314	18	0.206

* significant at a 95% confidence interval

When looking at osteocalcin levels compared to the stress indicators, there were no significant differences in the osteocalcin averages between the severity categories (Table 5.75). In looking at the specific trends in the data, the clavicle and femur osteocalcin levels were similar between severity levels. However, the clavicle showed a more consistent trend where increasing stress severity was associated with decreasing osteocalcin levels, as expected. Figures 5.37 and 5.38 show the fluctuation of osteocalcin levels between severity level for the femur and clavicle 1:4 dilution. Looking closer at the data, it was clear that HL provided the most consistent trend for osteocalcin levels in both the femur and the clavicle, whereas EHL severity seems to have no real effect on osteocalcin levels. CO and PH showed the most inconsistency between osteocalcin levels and stress severity, particularly in the clavicle.

Table 5.76 One-way ANOVA Results for the Comparison between Femur and Clavicle 1:4 Dilution Osteocalcin Concentrations and Stress Indicators

Stress Indicators	Bone/Dilution	F-value	p-value
Cribra Orbitalia	Femur 1:4	0.118	0.948
	Clavicle 1:4	0.216	0.884
Porotic Hyperostosis	Femur 1:4	0.012	0.988
	Clavicle 1:4	0.346	0.712
Enamel Hypoplastic Lesions	Femur 1:4	0.067	0.977
	Clavicle 1:4	0.092	0.963
Harris Lines	Femur 1:4	2.838	0.071
	Clavicle 1:4	2.452	0.101

Figure 5.37 Mean Osteocalcin Concentrations in the Clavicle at 1:4 Dilution Compared to Stress Severity Categories

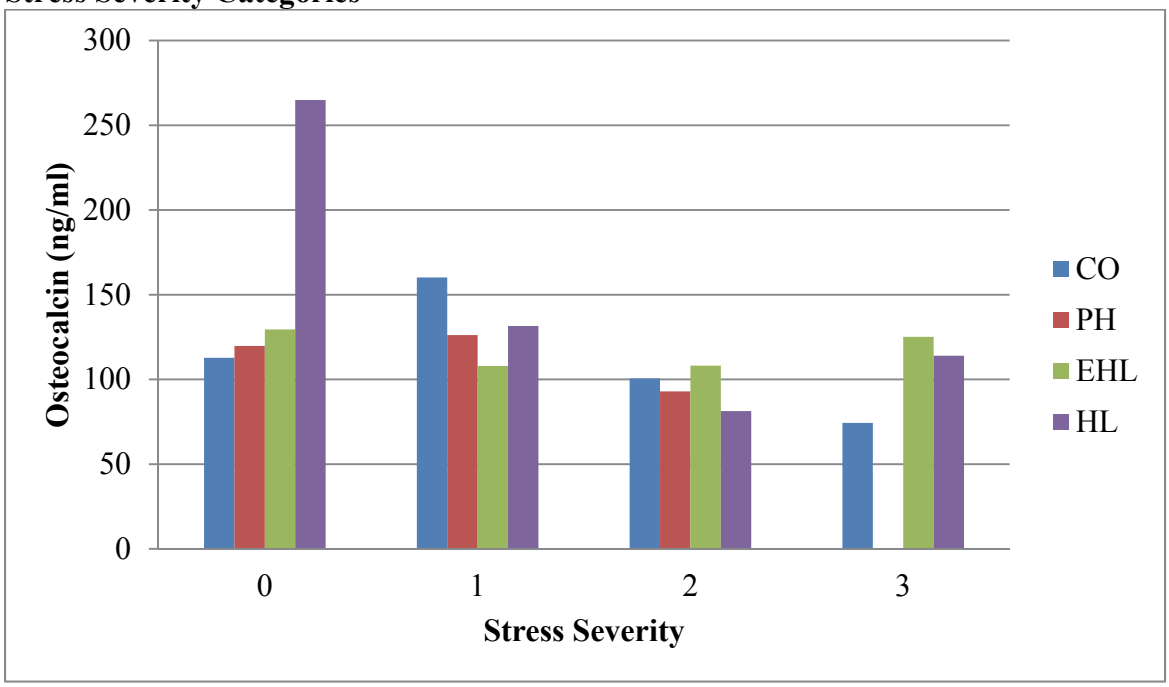
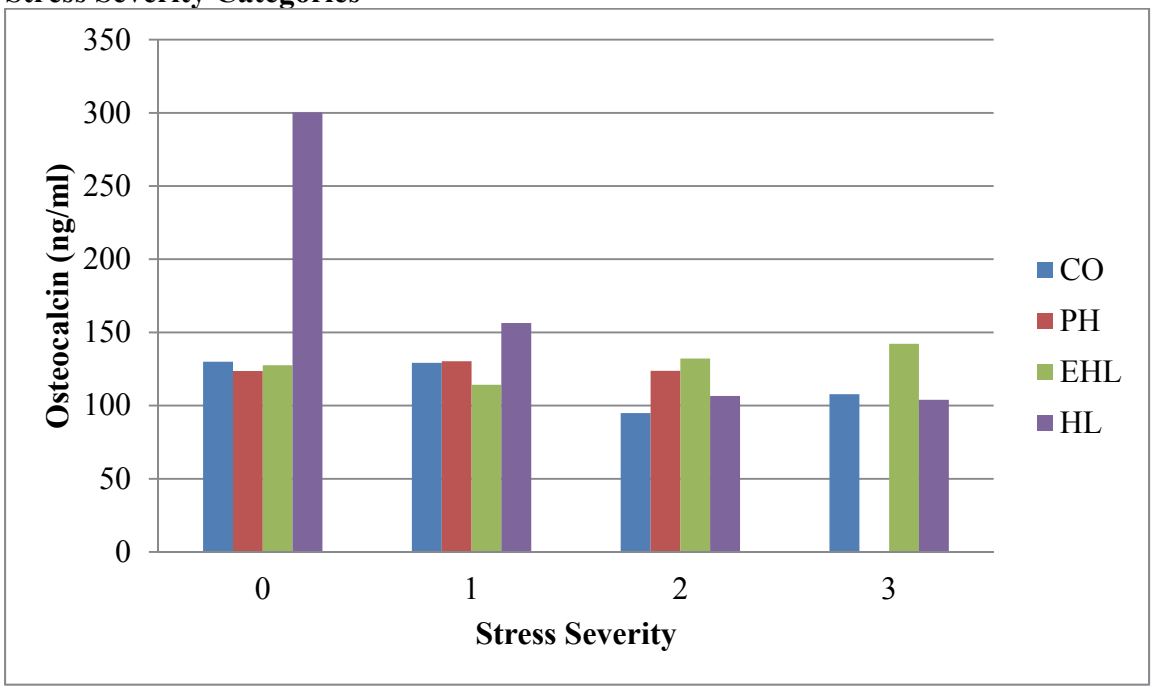


Figure 5.38 Mean Osteocalcin Concentrations in the Femur at 1:4 Dilution Compared to Stress Severity Categories



Because the sample size for osteocalcin analysis was only 20 individuals, it did not allow for a further break down of the stress severity data by sex, age group or time period. When reassigning the stress data into nominal categories (present or absent), it was possible to assess sex, age and time period in relation to osteocalcin levels. For all three variables there were no statistically significant differences in osteocalcin levels between those with and without CO, PH or EHL. As for HL, the femur 1:4 dilution showed a significant difference in osteocalcin levels between those with and without HL ($F=6.552$; $p=0.038$). It should be noted that the division of the HL data into nominal categories may have influenced this significant outcome as only one individual in the entire subsample of 20 was classified in the absent category. As such, the significance of these data may be reflecting this bias.

Osteocalcin levels were also compared to cortical thickness in both the ML and AP orientation to assess if any correlation was present. There were no significant correlations between the femur or clavicle at the 1:4 dilution when compared to either the ML or AP cortical

thickness averages. The relationship between osteocalcin and BSI designations was also analyzed. When comparing the frequency of BSI variables falling above and below the 95% confidence interval using a linear regression analysis, there was no significant trend in the data suggesting a relationship between BSI fluctuation and osteocalcin levels.

CHAPTER 6: DISCUSSION

6.1 INTRODUCTION

This chapter discusses the results of this study focusing on pathological conditions, stress indicators and biochemical data to better inform the understanding of medieval and post-medieval life in the city of Odense. More specifically, the implications of the results are discussed with respect to the four hypotheses and the overall assessment of stress in bioarchaeological populations.

6.2 PATHOLOGY

6.2.1 Pathological Patterns between the Sexes

When looking at the adult pathology results, the only conditions that were statistically shown to manifest differently between the sexes were those related to activity, specifically Schmorl's nodes and degenerative joint changes. The presence of these nodes more frequently in one sex than the other would suggest a division in labour between males and females and the type of mechanical loading they were exposed to during their lifetime. While Schmorl and Junghanns (1971) suggest that Schmorl's nodes may form as the result of localized trauma or high-activity during the growth and development period of life, more recent bioarchaeological studies associate these pathological changes with repeated stress to the spine from habitual activity (Weiss 2005; Fan 2006). The differences between males and females in the Black Friars population are not inconsistent with the bioarchaeological literature (Table 6.1), where the prevalence of Schmorl's nodes can vary greatly between populations and temporal periods.

Table 6.1 The Percentage of Adult Individuals with Schmorl's nodes from Medieval and Post-medieval Archaeological Assemblages

Skeletal Sample (Location)	**Time Period** (centuries)	**n**	**Males** (%)	**n**	**Females** (%)	**Reference**
Aberdeen (Scotland)	13^{th} - 16^{th}	18	72.0	6	67.0	Saluja et al. 1986
Nova Rača (Croatia)	14^{th} - 18^{th}	35	21.1	33	8.8	Šlaus 2000
Klostermarienberg (Austria)	16^{th} - 18^{th}		9.5		4.3	Üstündağ 2009
London St. Bride's Church (England)	18^{th} - 19^{th}	28	75.0	25	20.0	Saluja et al. 1986
Black Friars Medieval (Denmark)	13^{th} - 16^{th}	29	78.0	23	57.0	
Black Friars Post-medieval (Denmark)	16^{th} - 17^{th}	45	90.0	55	71.0	

The Aberdeen, Scotland sample most closely resembles the distribution of Schmorl's nodes in the Black Friars population with females showing less evidence of the pathology. However, in the post-medieval London, England, sample the percentage of males and females affected by Schmorl's nodes is greatly reduced compared to the contemporaneous Black Friars post-medieval sample, particularly in the females. If activity is the primary catalyst for the formation of these lesions, then the results suggest different activity patterns between the sexes. Üstündağ (2009) explains that while both males and females would have worked the agricultural fields in the post-medieval Klostermarienberg population, the different type of mechanical loading and twisting of the spine between men wielding a scythe and females using a sickle may have led to this discrepancy between the sexes, which may also be similar to what is happening in the Black Friars population. As Dyer (1989) explains, despite living in an urban environment many individuals had access to plots of lands in the countryside or garden plots within the city limits that required tending, in addition to small herds of livestock. For females that worked in domestic service (Dyer 1989), the responsibility of tending these small agricultural plots likely fell to them, increasing their daily labour. However, as Jacobsen (1983) discusses, domestic

service was not abundant during the medieval period forcing women to find work in different vocations requiring varying levels of physical labour.

Before AD 1550, the social and legal position of women in Denmark was based on her marital status. That is, those who were married to or were fathered by a merchant, draper, or craftsman had the opportunity to learn the trade or craft and become incorporated into the Danish workforce. However, there were strict statutes regulating how much work a woman could do and how many people she could hire to work for her (Jacobsen 1983). During the early medieval period the statutes put in place by merchant and craft guilds were much more lenient towards women. However, into the post-medieval period these statutes became stricter and greatly impeded a woman's ability to obtain and retain viable employment (Jacobsen 1983). As a result of these increasingly stifling regulations and legal ties to marriage titles, the majority of women were forced into heavy labour occupations such as laundry, dishwashing, or preparing flax for weaving (Jacobsen 1983). While there was some opportunity for women to work as brewers and alewives, by the time of the Reformation, these occupations became less abundant and lost much of their status (Jacobsen 1983). Jacobsen (1983:19) argues that, "as society became more organized, it became increasingly difficult for women to find lucrative careers that were legal". It is the result of these legal barricades that the majority of women likely worked wherever they could, including heavy manual labour that would have taken its toll physically. Additionally, as more women joined the workforce moving into the post-medieval period their time and energy would have been split between their daily work responsibilities and any domestic household work.

The overall high prevalence of Schmorl's nodes and degenerative joint changes in the Black Frias males, similar to other archaeological populations is likely related to the nature of

work during this time period. As Gallagher (2005) discusses, repetitive manual labour can compromise the synergy of the musculoskeletal system leading to increased tissue damage over time. For the Black Friars males, their increased and restrictive manual labour, typical of urban environments (i.e. craftsmen, building workers) (Jacobsen 1983; Dyer 1989) would have increased these degenerative changes during this transition from the medieval to post-medieval period. While females would have been exposed to differing types of heavy manual labour resulting in varying severities of degenerative changes for the time period, the higher levels of skeletal disruption seen in males was likely associated with repetitive activities, causing more localized and severe changes over time. Because both adult males and females were needed for manual labour in a variety of physical settings in a changing urban environment, it is not surprising that the Black Friars population shows compromised spine and joint health.

6.2.2 Pathological Patterns between Time Periods

In assessing the overall temporal trend, the post-medieval period shows more individuals with evidence of pathology (Table 6.2), although not significantly increased. The infectious diseases (TB, leprosy, treponema) associated with crowded living conditions and increased human contact (Roberts and Manchester 2007), represent the influx of individuals moving into city centers like Odense in the late medieval and early post-medieval period. The lower prevalence of leprosy compared to TB and treponema is likely related to the timeline of the disease in Denmark. As discussed by Boldsen and Mollerup (2006), leprosy was a disease of the early medieval period, dropping significantly after AD 1400. While the Black Friars skeletal sample spans both the medieval and post-medieval period, only 10 percent of the skeletal assemblage likely belongs to those buried before AD 1400 (Boldsen and Mollerup 2006;

Mollerup and Boldsen 2010); therefore, this lower frequency of the disease represents both the nature of the skeletal sample and the known timeline of the disease in Denmark.

Table 6.2 Percentage of Black Friars Adults with Pathological Conditions across Time Periods

Time Period		Perio.	TB	Lep.	Trep.	Fracs.	DSC	SN	DJC	Den. Abs.
Medieval	n	29	9	1	14	5	26	34	14	1
	%	56.0	17.0	2.0	27.0	10.0	52.0	68.0	27.0	2.0
Post-medieval	n	70	26	3	36	14	59	79	27	14
	%	70.0	26.0	3.0	36.0	14.0	59.0	79.0	27.0	14.0

(Perio. – periostitis; TB – tuberculosis; Lep. – leprosy; Trep. – treponema; Fracs. – fractures; DSC – degenerative spine changes; SN – Schmorl's nodes; DJC – degenerative joint changes; Den. Abs. – dental abscesses)

When compared to biomolecular studies, contemporaneous populations from southern Germany (AD 1400-1800) and Hungary (AD 1500-1600), reported that TB was present in 19.6 and just under 40 percent of individuals, respectively (Djurić-Srejić and Roberts 2001; Pósa et al. 2015). Both the medieval and post-medieval Black Friar samples fall within range of these two comparative samples, however it should be noted that the true prevalence of TB in the Black Friars sample may be higher as skeletal lesions occur in only three to five percent of individuals (Roberts and Manchester 2007). Conversely, because those who comprise the cemetery sample are the non-survivors, it is expected that the frequency of lesions would be elevated compared to those unaffected by the disease making a true estimate of prevalence difficult when using macroscopic observations alone. The advantage of biomolecular studies then, is their ability to capture both infection prevalence *and* disease prevalence, whereas macroscopic studies only allow for the observation of disease prevalence once skeletal changes have occurred, skewing estimates of mycobacterial diseases like TB.

The increase in cases of treponema between the medieval and post-medieval period is not surprising when compared to the known spread of the disease at the end of the 15th century (Walker et al. 2015). While debate continues as to the origins of treponemal disease (Harper et al. 2011), evidence suggests that in the early medieval period treponemal disease was already present in Europe but in a more benign form that then lead to the rise of the venereal strain into the 16th century (Stirland 1994; Cole and Waldron 2010; Schwarz et al. 2013). Because only 10 percent of the Black Friars medieval sample makes up the period before AD 1400, it is likely that the majority of individuals affected by treponema within this temporal period represent the later part of the medieval period and the venereal strain of the disease. However, there is possibility that the non-venereal early medieval strain of the disease is also present in these remains. The increase in treponemal disease in the post-medieval period was likely influenced by urban development and population crowding that fostered the spread of the more virulent venereal form of the disease; a pattern seen across western Europe at this time. When compared to the work of Schwarz (2009) who also studied the Black Friars population, the prevalence of treponema is elevated in this study with 31. 5 percent of adults affected, compared to the 16.7 percent reported by Schwarz (2009). This discrepancy may be the result of three factors: 1) which skeletal remains were chosen for each study; 2) how the pathological data was collected; and 3) new diagnostic criteria. Because pathology for this study was scored as "possible or present" the individuals making up this distribution may have had other conditions that resembled early treponemal changes, such as increased periostitis on the lower legs (Ortner 2003; Roberts and Manchester 2007). While all attempts were made to ensure that any pathological diagnoses were consistent, there is a possibility that this study inflates the true number of treponemal cases present in the Black Friars sample. However, it should still be noted

that the increased shift in prevalence between the medieval and post-medieval period remains consistent with the literature. Similarly, those pathologies related to increased activity (fractures, Schmorl's nodes, degenerative spine and joint changes), also increase in frequency into the post-medieval period suggesting more mechanical loading and repetitive activity during this period of urban expansion.

Compared to the study by Milner et al. (2015) and their extensive look at fractures in three medieval Danish populations, the frequency of fractures in the Black Friars sample is quite low with only 24 percent of individuals being affected, whereas Milner et al. (2015) showed 47 percent of individuals had evidence of healed fractures. This trend may in part be influenced by the amalgamation of data in the Milner et al. (2015) study. While their three study populations mainly represented urban environments, the Tirup individuals likely still participated in far more agricultural activities than the Black Friars individuals predisposing them to different types of injuries that may or may not have resulted in increased fractures. Additionally, the co-occurrence of different pathological conditions may have masked some of the skeletal changes associated with fractures, particularly the final stages of bone remodelling potentially leading to an underestimate of the fracture prevalence in the Black Friars sample. Despite this difference in fracture frequency, the pattern between these two studies is the same where males experience more fractures than females likely a result of occupational differences (Milner et al. 2015). Further to this, these fracture patterns allow for a closer examination of selective mortality (Boldsen et al. 2015). For the Black Friars population, the average age-at-death for males with fractures during the medieval period was 41.8 years, but only 32.7 years in the post-medieval period. In looking at the type of fractures incurred by males, the post-medieval fractures were more severe, including unhealed hip fractures and humeral head fractures, whereas the medieval

males experienced phalanx and clavicle fractures. The nature of these injuries and their impact on mobility likely affected the post-medieval males to a greater extent than the medieval males increasing their risk of death. Similarly, Boldsen et al. (2015) looking at three medieval Danish samples demonstrated that males who sustained cranial fractures and showed evidence of healing had a much higher risk of dying than those without fractures present. For females with fractures, the average age-at-death was 32.5 years in the medieval period and 35.2 years in the post-medieval period. The female pattern is opposite to that of the males where those in the post-medieval period with fractures lived slightly longer. Of the post-medieval women with fractures, 50 percent were Colles' fractures, possibly associated with early osteoporotic changes. However, as Agarwal et al. (2004) and Brickley and Howell (1999) discuss, aging trabecular bone in archaeological samples is not compromised in a similar way as modern female populations. While half of the post-medieval fractures in Black Friars females are suspiciously osteoporotic in nature, these injuries more likely reflect the economic necessity for women to work later into adulthood putting them at higher risk for fractures. A limitation of this analysis however, is the representativeness of the sample and fracture misclassification. Whereas Milner et al. (2015) and Boldsen et al. (2015) argue that fracture analysis should only be completed when like elements are present between all individuals; fracture analysis for this study was completed using all individuals possible. Additionally, there is also a risk that perimortem fractures were classified as antemortem; however, because all pathological conditions were categorized as absent or possible/present, the results are ultimately an estimate of the general trends present in this population.

Periostitis is formed as a result of periosteal inflammation and identified archaeologically by its distinct striations and plaque like structure on the surface of cortical bone (Roberts and

Manchester 2007). In the Black Friars sample the prevalence of periostitis also increased from the medieval into the post-medieval period (56% to 70%) and was likely associated with increased traumatic injuries or more substantial systemic stress, particularly in diseases such as leprosy and treponema where the preliminary skeletal change is periostitis (Ortner 2003; Roberts and Manchester 2007). In assessing the frequency of periostitis minus those individuals suffering from leprosy or treponemal disease the medieval and post-medieval frequencies were reduced to 29 and 32 percent, respectively (Table 6.3). Looking at comparative populations, there is large range of variation in the presence of periostitis which is not unexpected as its etiology is broad and overlaps with multiple conditions (see Ortner 2003; Roberts and Manchester 2007). Shown by Šlaus (2000), the adult frequency of periostitis in the medieval Nova Rača sample is 17.6 percent suggesting the pathological load experienced by the Black Friars medieval and post-medieval groups was much higher. However, when compared to the early medieval Stenjevec sample from Croatia, the frequency of periostitis is comparable (Šlaus 2002). The higher frequency of periostitis in the Black Friars sample is likely associated with increased systemic stress from new pathogens (e.g. the Black Death, venereal treponema) and acute trauma from the dangers of urban dwelling and occupation which was exacerbated from the medieval into the post-medieval period (Dyer 1989). Discussed by Hanawalt (1998), 61 percent of accidents for women in the medieval period were associated with their domestic work and village life, where daily activities such as doing laundry, fetching food and water, starting fires, tending domestic animals, put these women more at risk for fracture and injury. Only 36 percent of men had accidents in the home, but they were at an increased risk when moving from their domestic home to their place of employment where there were dangers associated with horseback riding, walking and carting (Hanawalt 1998). Similar to the elevated risk of accidents for women in the

home, men experienced more accidents at work where four percent of deaths were associated with construction and carpentry (Hanawalt 1998). Fractures and injury associated with work, both within and outside the domestic sphere would have been a real risk for the Black Friars population, particularly in the post-medieval period as urban expansion and development would have introduced new dangers.

Table 6.3 Percentage of Adult Individuals with Periosteal Lesions in Comparative Archaeological Assemblages

Skeletal Sample (Location)	**Time Period** (centuries)	**n**	**Periostitis** (%)	**Reference**
Nova Rača (Croatia)	$14^{th} – 18^{th}$	12	17.6	Šlaus 2000
Stenjevec (Croatia)	$10^{th} – 12^{th}$	15	26.0	Šlaus 2002
Black Friars Medieval (Denmark)	13^{th} - 16^{th}	15	29.0	
Black Friars Post-medieval (Denmark)	16^{th} - 17^{th}	32	32.0	

The post-medieval period also shows an increased frequency of dental abscesses, particularly in the older adult individuals. As discussed by Boldsen (1998a), dental disease was common in populations of the middle ages. In Boldsen's (1998) examination of the Danish Tirup medieval skeletal assemblage he demonstrated a similar distribution of dental abscesses with higher frequencies present in the oldest adult age groups, but cautions that attrition more than age may be to blame for these patterns. Similarly, DeWitte and Bekvalac (2011) also demonstrated higher levels of periodontal disease in the older adults from their urban medieval St. Mary Graces, London sample (AD 1350-1538) but suggest similar to Boldsen (1998a) that age is not necessarily the only contributing factor. Focusing on the relationship between periodontal disease and the presence of periostitis, DeWitte and Bekvalac (2011) discuss how periodontal disease and other indicators of ill-health occurring in tandem may represent underlying susceptibility to illness in immunochallenged individuals. In the Black Friars sample, a similar

trend was evident where more individuals in the post-medieval period show evidence of multiple pathologies likely reflecting an increased susceptibility to illness when particular pathologies interact within the biological system.

6.2.3 Pathological Patterns between Age Categories

In the youngest adult age group (18-29 years), the medieval and post-medieval frequencies of pathology are relatively equal; even for pathology only present in the post-medieval sample, less than 20 percent of individuals were affected. This similarity is likely the result of comparable exposure to pathogens during the subadult years which were shown to be relatively similar between the medieval and post-medieval periods and will be further discussed. Also, because these individuals represent those who did not survive, it is not surprising that they demonstrate a relatively similar distribution of pathological conditions that likely contributed to their premature death. While Bogin (2001) argues that late adolescence and early adulthood is the healthiest and most buffered period of biological development, these individuals in the Black Friars sample represent those who were the unhealthy within their community and do not represent the normal patterns of health we would expect to see. Moving into the middle adult age category, there were more discrepancies between time periods with the post-medieval group showing higher frequencies of infectious disease such as treponema, TB and also periostitis. In the youngest age category, the frequencies of TB and leprosy are similar in the medieval and post-medieval periods; however, within the two subsequent age groups TB levels rise but, the frequency of leprosy decreases. This pattern is not dissimilar to that discussed in the literature and the concept of cross-immunity between these diseases (see Manchester 1984; Lietman et al. 1997). However, the coexistence of both diseases is possible (see Donoghue et al. 2005), suggesting that both intrinsic and extrinsic factors (i.e. establishment of leper hospitals with

separate burials grounds for those with the disease (Roffey 2012)) likely played a role in the gradual decline of leprosy and increase in TB in skeletal samples such as the Black Friars. In the oldest age category, TB is at its highest frequency and leprosy is absent in both the medieval and post-medieval samples. TB prevalence is the highest in the post-medieval category for all three age groups reflecting the population density requirements of the disease and the relative ease of its spread through respiratory mechanisms (Cockburn 1963; Manchester 1984).

It is important to note that this discussion of pathology is based on *any* presence of skeletal disease and not reflective of disease severity. Therefore, in some cases individuals included in this pathological analysis may show differing degrees of disease progression from initial localized skeletal involvement to tertiary wide-spread changes. While this type of classification is not sufficient for a succinct pathological analysis, it does provide the relevant information needed for this study which is a basic examination of those who were at some point during their life affected by disease that left skeletal evidence. Additionally, because disease takes time to manifest in the skeleton (Goodman and Armelagos 1989), it is expected that some individuals suffering from disease did not show skeletal disruption before death, or that the delay in manifestation may indirectly change the pathological frequencies within each specific age category based on the timeline of when physical lesions actually form. For example, treponemal disease is most often contracted in late adolescence or early adulthood but does not show skeletal changes until the tertiary stages of the disease between two to ten years later (Ortner 2003), potentially biasing the data towards the older age categories. However, timelines such as this, are based on the modern behaviour of these diseases and do not necessarily account for how the timeline of skeletal involvement may have changed between different strains of a disease or various mutations.

6.2.4 Subadult Pathology

Important to note is that while the study of the medieval and post-medieval subadult groups provides a snapshot into the living conditions of the Black Friars children, an analysis of all individuals within the population would provide the most robust data set for a subadult-specific analysis. While those who died in childhood represent the non-survivors of a population, the adults represent the children who did survive. The assessment of pathology in the subadult group therefore, focused on frequency by age with the assumption that the most pathological changes would be present in the older age groups of the non-survivors. This trend was demonstrated to some extent within the subadult sample; however, not consistently as all age groups appear to experience a similar level of stress. Because the pathological data was captured using a nominal scale, the severity of each condition was not assessed. This may have influenced the results of this analysis, where these pathological conditions may have been similarly present or absent within each of the age groups but at different levels of severity.

Periostitis was the most common pathological condition affecting the subadult sample with approximately 22 percent of individuals affected. The highest frequency of periostitis in the 8-13 year age category (55%), followed by the youngest age category from 0-1 year (27%). The overall prevalence of periostitis is similar to the distribution reported by Grauer (1993), where 14 percent of the subadult sample from the St. Helen-on-the-Walls showed evidence of periostitis. This 10^{th}-16^{th} century medieval sample represents the urban poor of York, England, where individuals were likely exposed to similar urban stressors as the Black Friars population. The timing of this high frequency of periostitis in the later phase of childhood before puberty is similar to the results reported by Ribot and Roberts (1996) for the rural and urban cemeteries of Raunds and Chichester, respectively. For the rural Raunds cemetery (8^{th}-10^{th} centuries), children

were equally affected with high frequencies of periostitis at the ages of three, seven and 12 years, where the urban Chichester cemetery (16^{th}-17^{th} centuries when cemetery was open to children) showed the highest levels of periostitis in the oldest subadult age group at 18 years (Ribot and Roberts 1996). Ribot and Roberts (1996) suggest that the high frequencies of periostitis likely reflect systemic stress rather than specific localized trauma. Despite the large age range between the subadult age groups most affected by periostitis in the Black Friars sample (i.e. 0-1 year; 8-13 years), systemic disturbance resulting in periostitis may be related to immunological changes during these distinct maturation periods. As Holt and Jones (2000) discuss, neonates (0-1 year) are not "immunologically naïve" (688), but rather experience a period of transition from maternal immunity to independent immune competence (West 2002), making this first year of life vulnerable to external insults and systemic disturbance. Conversely, while immunocompetence may be reached during the adolescent years, hormonal changes and behavioural shifts may introduce new challenges for the immune system during the final stage of childhood, possibly explaining this increased level of periostitis in the young adolescents from the Black Friars population.

6.3 STRESS LESIONS IN SUBADULTS

6.3.1 Patterns of Subadult Stress Lesions between Time Periods

In the Black Friars subadult sample there were no significant differences in the presence of stress indicators when dividing the data by time period. Table 6.4 illustrates the percentage of Black Friars subadults affected by each stress indicator compared to other contemporaneous subadult skeletal assemblages.

Table 6.4 Percentage of Subadult Individuals with Stress Indicators across Archaeological Assemblages

Skeletal Sample (Location)	**Time Period** (centuries)		**CO**	**PH**	**EHL**	**HL**	**Reference**
Raunds (England)	8^{th} - 10^{th}	n	39	12	43	28	Ribot and Roberts 1996
		%	58.0	17.0	49.0	42.0	
Chichester (England)	12^{th} - 17^{th}	n	40	10	32	33	Ribot and Roberts 1996
		%	67.0	15.0	38.0	32.0	
Wharram Percy (England)	10^{th} - 16^{th}	n	112		36	40	Mays 1995; Lewis 2002
		%	56.0		30.0	37.0	
St. Helen-on-the-Walls (England)	10^{th} - 16^{th}	n	49		31		Lewis 2002
		%	56.0		34.0		
Christ Church Spittalfields (England)	18^{th} - 19^{th}	n	63		22		Lewis 2002
		%	57.0		24.0		
Næstved (Denmark)	13^{th} - 16^{th}	n	36		23		Bennike et al. 2005
		%	60.0		42.0		
Æbelholt (Denmark)	12^{th} - 16^{th}	n	57		21		Bennike et al. 2005
		%	53.0		17.0		
Black Friars Medieval (Denmark)	13^{th} - 16^{th}	n	7	1	2	8	
		%	100.0	14.0	29.0	100.0	
Black Friars Post-medieval (Denmark)	16^{th} - 17^{th}	n	17	6	19	24	
		%	59.0	21.0	66.0	75.0	

As shown in Table 6.4, during the medieval period, a similar percentage of subadult individuals exhibited stress indicators regardless of geographic location. This similarity was likely influenced by two main factors: 1) food availability and 2) living conditions. In the early 14^{th} centuries wide spread famines affected much of Europe compromising the quality and quantity of crop yields (Jordan 1996; Fagan 2000; Hybel and Poulsen 2007). Because grain crops were the primary foodstuffs during the medieval period, particularly for children (Hybel and Poulsen 2007; Mays 2007), this famine would have directly affected these subadult populations, whether

through malnutrition or under nutrition. Aside from the post-medieval populations (i.e. Raunds, Christ Church, Black Friars post-medieval), all other skeletal samples demonstrate a similar prevalence of stress indicators with the Chichester and Black Friars medieval samples with 100 percent of the Black Friars subadults with evidence of CO and HL.

While the high percentage of individuals affected by CO in the Black Friars sample may be a function of the small sample size of this subgroup (n=7), the high level of CO at Chichester is likely due to the nature of this site as a hospital where arguably poorer overall health was the norm (Ribot and Roberts 1996). Despite the possible influence of a small sample size, the number of individuals affected by CO in the Black Friars medieval sample more likely reflects the reduced caloric intake or malnutrition experienced by these children as they began to engaged in adult activities as early as six years of age, with more intensive involvement and labour beginning between 10 and 12 years of age. This increase in activity for these subadults would have required an increased caloric intake and adequate nutrition to maintain healthy development and normal immune function (Hanawalt 2002; Gilchrist 2013). Goran et al. (1993) demonstrated that in a resting environment of low level activity, children between four and six years of age required on average 1379 +/- 290 kcal/day. Muldrew (2011) determined caloric intake for late and post-medieval English children to be between 943 kcal/day in the very impoverished and up to 2021 kcal/day for the average middle-class child. While the caloric intake of middle-class children met the daily requirements outlined by Goran et al. (1993), it does not account for the additional calories needed for physical work, particularly the protein energy needed from meat consumption. As Muldrew (2011) outlined children consumed approximately 1299 kcal/day in grain products, 383 kcal/day in dairy products and only 339 kcal/day in meat. With this low consumption of meat, which at times may have been absent from

the diet, children would have been deprived of an iron and B-12 rich diet possibly contributing to the increased prevalence of CO in the medieval period. Hoffman (2001) argues that meat was always present in the medieval diet. However, grain-based foods dominated the caloric intake in these populations, particularly children who were fed less meat and more plant-based foods (Mays 2007). Further to note is that despite the shifting nature of economic exports out of Denmark from grain to cattle during the late and post-medieval periods, these resources were primarily for export and did not likely contribute to a drastic shift in the national Danish diet.

The relationship between CO and PH has been well-explored in the literature (e.g. Oxenham and Cavill 2010) with arguments that both indicators of stress are associated with similar dietary etiologies (Walker et al. 2009). However, Stuart-Macadam (1989) demonstrated that despite high co-prevalence of CO and PH in the Romano-British Poundbury sample, they do not always manifest in tandem. Stuart Macadam (1989) and Wiggins (1991) argue that CO may represent the milder form of this stressor as changes in bone thickness occur first in the orbital roof followed by the posterior vault where PH manifests (Caffey 1937). In looking at the Black Friars subadult data (Table 6.4), it is clear that the medieval period is dominated by CO and the post-medieval period PH. Interestingly, in both the medieval and post-medieval subadult groups PH is not present in any individuals without CO, possibly demonstrating that CO is the primary skeletal change to occur followed by PH as the more severe or advanced lesion type. Looking specifically at dietary trends during the medieval period, Hoffman (2001) outlines that the dietary level of iron- and B12-rich meat would have been highest during the century after the Black Death with the increased expansion of cattle rearing and higher per capita income. However, this did not last into the post-medieval Reformation period where meat consumption declined and did not resume its previous height until into the late 19th century (Hoffman 2001). If

truly tied to anemia (see Chapter 3, section 3.6) from low levels of iron and B12 in the diet, the switch from mild stress during the medieval period to more severe stress in the post-medieval period may explain the CO and PH patterning within the subadult sample.

The number of individuals with HL is also high in both the Black Friars medieval and post-medieval samples compared to other contemporaneous subadult populations (see Table 6.4). This disparity may be occurring for one of two reasons: 1) image capture techniques and 2) severity scores. For all subadult individuals HL data were captured by radiographic technologists and then analyzed using the MIMICS medical imaging software contributing to a better visualization and identification of these lines (Scott and Hoppa 2015). With this improved visualization through standardized capture methods, more obscure or previously invisible lines were able to be captured for analysis based on the mechanics of bone remodelling and how that remodelling affects the radiographic process. The etiology of these skeletal disruptions also contributes to how these data are analyzed, specifically the previously unequivocal connection of these lines to periods of stress. Recently it has been suggested that due to a lack of correlation with other indicators of stress (see Alfonso et al. 2005; Alfonso-Durruty 2011; Papageorgopoulou et al. 2011), HL may represent normal periods of growth stasis that occur during skeletal maturation. Because growth is a highly regulated process (Eveleth and Tanner 1990), it is expected that if HL do represent normal growth then all subadults individuals, who have yet to undergo substantial skeletal remodelling, would show evidence of these lines. However, in the Black Friars sample eight subadults have no visible HL with their ages at death ranging between birth and 18 years of age. This stands in contrast to the expectation that lines would be visible in at least the three individuals that are in their adolescent years and also likely for the fourth individual who died during late childhood as all of them would have passed

through normal periods of growth fluctuation and stasis. As Steckel (2005) argues, in order for HL to form there needs to be sufficient stress to illicit a skeletal response and based on the target-seeking nature of growth (Eveleth and Tanner 1990), it is unlikely that normal growth fluctuations would have the capacity to halt growth enough to create a HL. Assuming then that HL do represent periods of stress endured during the subadult years of life, the data suggest that the Black Friars subadults were likely exposed to a variety of insults capable of stalling growth, including disease, dietary deficiencies and psychological stress which will be discussed (Garn et al. 1968; Marshall 1968; Blanco et al. 1974).

The comparatively low level of EHL in the Black Friars medieval subadults likely reflects the greater resilience of teeth over bone during periods of stress (Garn et al. 1965). However, this discrepancy may also be related to the type of stress affecting these individuals and how it is able to manifest in particular skeletal tissues. The stress load experienced by the Black Friars subadults during the medieval period demonstrates fluctuations between each indicator suggesting different stressors at differing levels of severity were likely affecting these subadults. Moving into the post-medieval period, however, the number of individuals with EHL increases substantially possibly indicating that the cause of stress may have remained the same, but the intensity of the stress was more biologically disruptive. This greater biological disruption may not have necessarily affected mortality in these individuals, but it does demonstrate a change in stress between the medieval and post-medieval period. Because this increase in EHL also matches the increase in PH, it is likely that the higher prevalence of both stress indicators during the post-medieval period is closely tied with severity over specific cause. As Dyer (1989) argues in reference to British trends, dietary shifts and the changing urban environment with increased pollution greatly compromised individual health in the 16th century.

6.3.2 Patterns of Subadult Stress Lesions between Age Groups

Similar to the pathological results, the subadult individuals did not show great variability between age groups and the severity of stress, except for EHL. In the oldest age categories, EHL were not only more prevalent, but also more severe than in the youngest age categories. This discrepancy is likely driven by two factors: 1) the nature of lesion formation and 2) methodological limitations. Enamel formation for all deciduous teeth begins in utero (Mahoney 2012). Therefore, any defects may represent conditions before and shortly after birth, whereas all permanent enamel defects represent only conditions later in life after birth. Permanent teeth also have a longer period of enamel formation than deciduous dentition and therefore, have the ability to capture longer periods of skeletal disruption (Schaefer et al. 2009). Methodologically, macroscopic observation of deciduous teeth are a challenge as these tooth crowns are small in size and their thinner enamel makes any hypoplasias more difficult to see than on the thicker permanent dentition where disturbances in the perikymata are more obvious (Grine 2005; Hassett 2014). However, normal variation of the tooth surface, more visually apparent in permanent dentition, can also cause additional challenges in the accurate identification of these enamel defects (Schwartz et al. 2001).

In looking at the prevalence of stress indicators and age-at-death estimates in the subadult group, mortality appears to be tied with the presence of multiple stress indicators. Individuals were assessed based on having one, two or three indicators present (no subadult individuals had all four indicators present) with the average age-at-death falling from 12.1 years (1 indicator), to 10 years (2 indicators) and finally 9.3 years (3 indicators). However, when assessing the average age-at-death for each stress indicator at varying levels of severity this pattern is less distinct (Table 6.5). A one-way ANOVA of this data showed no significant differences between the

average age at death and stress severity level except for EHL (F=4.174; p=0.013). Those with increased severity had an older average age at death; however, this may be an artifact of the time it takes for severe EHL to form in the dentition.

Table 6.5 Subadult Average Age-at-death Estimates (years) in Different Stress Severity Categories

Stress Indicator		Level 0	Level 1	Level 2	Level 3	Level 4
CO	n	12	14	7	2	1
	years	10.8	8.9	9.1	8.0	8.5
PH	n	29	4	2	1	0
	years	9.0	10.4	10.5	12.0	
EHL	n	15	3	7	11	
	years	7.3	9.0	12.9	11.6	
HL	n	8	8	17	7	
	years	6.5	8.8	8.1	12.4	

In this comparison it is clear that average age-at-death distributions fluctuate much more based on stress severity, which may be a reflection of the arbitrary severity levels chosen for this study. For example the severity difference between level 1 and 2 may represent a different magnitude of biological change than that occurring between levels 2 and 3 or 3 and 4. Further, this comparison does not consider the interactions between these indicators and how the presence of one specific lesion may help or hinder an individual's immune system when met with subsequent stress. Perhaps then the number of *different* indicators present is more reflective of severity rather than the various physical changes seen *within* each specific stress indicator. Because biological variability and individual frailty affect how stress will manifest within the skeleton (Wood et al. 1992; Boldsen 2007), the varying degrees of physical change for each stress indicator may in part be dictated by these inherent biological differences. As Elenkov et al. (1999) discuss, when stress occurs glucocorticoids selectively suppress aspects of the immune

system and the pattern of these changes is based on the acute or chronic nature of the stress. Perhaps then, when stress is acute, it remains contained within a specific tissue type, reflecting a finite level of stress. Conversely, when stress is chronic the immunosuppressive function of glucocorticoids may fluctuate between different tissue types resulting in the formation of multiple stress indicators across multiple tissues. While the immune system certainly plays a role in the manifestation of skeletal changes associated with stress it does not function in isolation as there are a variety of factors that affect the mechanisms of the endocrine system. Essentially, the extrinsic stressors of daily living during the medieval and post-medieval periods would have influenced the intrinsic responses that lead to skeletal change with those intrinsic responses mediated by individual frailty, exposure, sex, age, etc. While the immune system and the influence of glucocorticoids may be responsible for the physical changes associated with stress, arguably it is the extrinsic factors (i.e. harsh working environments, lean calorie intake, exhaustion, etc.) that drive the extent of endocrine involvement and how substantial the resultant skeletal disruption will be.

6.4 STRESS LESIONS IN ADULTS

6.4.1 Adult and Subadult Stress Lesion Comparison

For the adult sample (Table 6.6), the distribution of individuals with each type of stress lesion was relatively similar to the distribution of the subadult sample except for CO and EHL (Table 6.4). The frequency of CO in the adult sample (10-30%) was much less than that present in the subadult sample (59-78%) likely reflecting the selective mortality of this indicator where those suffering from CO in the subadult years are less likely to survive into adulthood; therefore, those in adulthood show less evidence of the pathology. Conversely for EHL, the adult sample showed much higher prevalence (68-87%) compared to the subadult sample (29-66%), likely

reflecting the time it takes for manifestation. The CO and PH data in the adults differs from the subadults in that more individuals showed evidence of PH over CO. If PH is considered the more severe type of cranial lesion, similar to the prevalence of EHL, it may take time for this lesion to manifest and as such is more obvious in the adult sample. While CO and PH are mainly associated with the childhood period, they may still be active into adulthood as the cortical bone continually remodels throughout life (Kular et al. 2012). This persistence of CO and PH into the adult years, similar to the subadults, is likely associated with unmet caloric requirements that may have led to conditions such as iron-deficiency anemia.

In the early post-medieval period Muldrew (2011) estimates that the baseline caloric rate for average males and females was 2100 kcal/day and 1900 kcal/day, respectively. In addition to this baseline, however, are the caloric requirements needed for manual labour. For example, males employed in the building industry working for 10 hours a day would require approximately 6300 kcal/day, whereas women employed doing heavy housework for 10 hours a day would require 4900 kcal/day (Muldrew 2011). Based on Muldrew's (2011) estimates for average middle class males and females in the 1600s, males would only be consuming 3950 kcal/day and females 3215 kcal/day resulting in considerable caloric deficits. Similar to the subadults, meat made up only a small portion of the diet and as such, these individuals would have been deprived of specific nutrients such as iron and B12 possibly contributing to the elevated presence of PH into the adult years. As discussed in Chapter 2, Section 2.6.3, after the Reformation religious fasting became less prominent in the lives of parishioners essentially allowing for non-regulated food consumption on all calendar days (Albala 2011). However, it cannot be assumed that the abandonment of fasting was immediate or complete for all individuals, as shifts in religious practice took time and in most cases were merely adapted to the

more lenient viewpoints proposed by the Protestant church (Albala 2011). While this diminished adherence to fasting would have arguably improved the nutritional status of the post-medieval population, it is likely that the medieval population experienced a similar leniency towards the fast as children, the ill and manual labourers were given dispensation and allowed to eat full meals during holy periods. While this compliance to religious fasting adds an additional consideration to the overall nutritional status of the Black Friars cemetery population it likely did not greatly influence the overall health trends visible before or after the Reformation, as nutritional hardships were already commonplace.

In the adult sample, all individuals were assessed for HL based on the methodology presented by Scott and Hoppa (2015), whereas the subadult data was captured using standard AP orientation, as discussed in Chapter 4, Section 4.5.3. The elevated prevalence of HL in the adult sample is not surprising as the ML orientation provides better visualization of HL, based on cortical thickness and known patterns of remodelling in the tibia (Scott and Hoppa 2015). Similar to the subadults, some adult individuals did not show evidence of HL (n=6). While the argument can be made that any HL could have remodelled out of the tibia, all six individuals fell within the youngest and middle adult age categories where complete obliteration of these lines is not likely as individuals within the oldest age category still showed evidence of HL. Further, the slow remodelling rate of the tibia increases the likelihood that HL will remain visible into later adulthood. The high number of individuals with HL in both sex groups and time periods suggest that growth faltering was a common occurrence for the Black Friars population. However, it is important to note that these individuals also experienced growth recovery to some extent as HL are not radiologically visible until growth has resumed (Mays 1995).

Because the adult individuals represent the survivors of their population, it is evident that they did not pass through childhood unscathed. When inspecting the data based on severity, there is a clear trend where increased severity leads to a lower average age-at-death for CO and PH (Table 6.7); therefore, while these adult individuals may represent the survivors of childhood, they were still likely affected by increased mortality and predisposed to increased morbidity as a result of these early life stress events. Statistically, there is a significant difference between the average age at death between severity levels for PH ($F=4.530$; $p=0.005$), but not for the other indicators (CO $F=1.888$; $p=0.116$; EHL $F=0.141$; $p=0.935$; HL $F=0.840$; $p=0.474$). However, this age data is an amalgamation of all adults and may be influenced by sex and time period, potentially skewing the results. Likely, more severe CO contributes to an increased risk of dying similar to PH based on their similar etiology and the data presented; however this is not true for EHL and HL. For both of these stress lesions severity does not seem to increase the risk of dying which may be due to the nature of these lesions where formation occurs only after a period of recovery. Therefore, those with higher frequencies of EHL and HL represent individuals who showed the most recovery and were not necessarily predisposed to an increased risk of dying. This pattern is in contrast to the non-survivor subadults where there appeared to be no direct relationship between stress severity and increased mortality; however, this may be a product of their foreshortened lives where any negative biological outcomes associated with particular stressors did not have time to manifest and affect the average age at death statistics.

Table 6.6 Percentage of Adult Individuals with each Stress Indicator by Sex and Time Period

Black Friars Groups		CO	PH	EHL	HL
Medieval Males	n	3	7	20	27
	%	10.0	24.0	69.0	100.0
Post-medieval Males	n	12	27	27	40
	%	27.0	60.0	68.0	93.0
Medieval Females	n	7	10	20	19
	%	30.0	43.0	87.0	100.0
Post-medieval Females	n	16	22	38	52
	%	29.0	40.0	70.0	95.0

Table 6.7 Adult Average Age-at-Death Estimates in Different Stress Severity Categories

Stress Indicators		Level 0	Level 1	Level 2	Level 3	Level 4
CO	n	114	21	13	3	1
	years	33.8	34.6	37.3	25.2	29.5
PH	n	86	44	19	3	
	years	35.5	33.5	30.3	23.8	
EHL	n	41	27	55	23	
	years	33.9	34.0	33.2	34.2	
HL	n	6	24	50	64	
	years	33.5	33.8	35.5	33.0	

6.5 BODY SIZE INDICATORS (BSIs)

As Johnston (1969) states, "the major components of variation among adults is due to variations in the growth process" (335); therefore, similar to the stress indicators, the BSI data provides a window into the formative years of life that may or may not influence overall heath during the adult years. As an individual matures, biological processes promote linear growth in the skeleton but the rate of that linear growth is not constant between individuals (Johnston 1962) or within an individual as shown in the BSI data. Mensforth (1985) argues that it is the

study of these fluctuations and the rate of growth that can provide a measure of stress within a population and the heterogeneous nature of skeletal growth provides an opportunity to observe and track growth fluctuations at different periods of maturation (Vercellotti et al. 2011).

6.5.1 BSI Patterning between the Sexes and Time Periods

The BSI data, similar to the pathological and stress data, demonstrated more stress within the female group, particularly in the post-medieval period. When assessing the variables, the females showed more fluctuation both above and below the confidence interval. As discussed, variables falling below the confidence interval represent poor growth during a particular maturation period; conversely those falling above the line reflect accelerated growth during the maturation period. Therefore, the observed fluctuation in the post-medieval female group likely represents periods of poor growth and the resultant catch-up growth achieved after these periods of growth retardation or stasis. Because catch-up growth may only act upon skeletal elements that have not reached adult maturity, it is not surprising that while the majority of female variables fell below the confidence interval, there were also ones above the confidence interval, likely those maturing at a later period and could still be influenced by the mechanisms of catch-up growth (Prader et al. 1963; Tanner 1986). While some individuals experiencing catch-up growth may fall within the limits of the confidence interval, these periods of acceleration are usually rapid and as a result may push the growth of these variables beyond the population average. The importance of assessing catch-up growth cannot be overstated in studies of growth and development. As discussed by Clark (1988) and Clark et al. (1986), it is imperative to study both periods of early and late maturation to gain a complete understanding of individual growth velocity and deviation. Also, it is likely that all individuals within this population were experiencing some level of stress. Thus, the confidence interval determined for each variable

pairing may not necessarily represent optimal growth. Individuals experiencing catch-up growth may surpass the population-specific confidence interval as their body attempts to restore the normal trajectory of growth expected under optimal conditions.

When comparing the medieval and post-medieval male groups, the proportion of variables falling below and within the confidence interval was similar; however the post-medieval males had a higher proportion of variables falling above the confidence interval. While the females are more likely to fluctuate around the confidence interval, the males show a more consistent trend between time periods with the only real discrepancy visible in those variables falling above the confidence interval. These results are surprising considering that variables falling above the confidence interval likely represent periods of rapid catch-up growth where stress has subsided long enough in order to not only resume growth but accelerate it in elements that have yet to reach adult maturity. While some individuals may have been fortunate enough to experience catch-up growth through improved living conditions, this trend in the post-medieval male group could also be associated with a temporal shift in overall body size. Boldsen and Søgaard (1997) argue that the average height of males and females was constant throughout the medieval and into the post-medieval period with average male height at 167.1cm and females at 156.0cm. Because stature is closely associated with body size, it is likely that if average height remained stable through this period body size would have also remained as such. Therefore, the BSI pattern observed likely reflects cultural disparity between males and females and access to resources. Because females show more fluctuation around the confidence interval it is likely that they were faced with increased hardships into the post-medieval period including differential access to food, housing, jobs, health care, etc.

When comparing the number of skeletal elements affected by this growth disruption (i.e. legs, arms, vertebrae, hands and feet), the leg variables were most affected for both males and females and in both time periods. Because the majority of variables used in this study were from the leg this trend was not surprising, additionally the faster maturation of the leg bones (e.g. femur) may predispose these elements to skeletal changes where catch-up growth cannot occur after the stress has passed. When assessing the data by time period, the medieval males and females showed less disruption in the other skeletal elements (i.e. arms, vertebrae, hands and feet). However, in the post-medieval period arm variables were almost disturbed as much as the leg variables illustrating a more widespread disruption of skeletal growth than that seen in the medieval period. Because the maturation of the arms begins earlier than the legs (Schaefer et al. 2009), this post-medieval shift likely demonstrates prolonged stress during the maturation years, perhaps more systemic in nature than what was experienced in the medieval period. Further, within these temporal periods, the females in particular had more overlapping skeletal elements that fell below the confidence interval. In the medieval period only 40 percent of females had all four body sections affected, whereas in the post-medieval period over 50 percent of females had all four body sections affected. These results suggest that the stress experienced by the post-medieval females was more severe and longer in duration, affecting multiple variables maturing across the entire period of growth and development and likely contributed to poorer overall health.

This pattern of disruption in the leg variables closely resembles the work of Jantz and Owsley (1984) where the Arikara population showed higher levels of disruption in leg growth over arm growth. Tanner (1978) and Humphrey (1998) discuss the variability in skeletal maturation and the periods in which stress may affect different skeletal elements. It is not

surprising that specific regions are targeted by stress, or may have a predisposition to disruption. Disruption may also be identified through changes in body proportions; whereas catch-up growth has the ability to diminish size disparity resulting from stress episodes, element proportions are exacerbated by this process leaving a lasting indication of stress (Boldsen 1998b). Even secular changes in overall skeletal size has demonstrated that the upper and lower limbs do not follow a similar proportion shift (Jantz and Jantz 1999), making a stronger case that despite a similar physiological make-up, different regions of the skeletons react differently to biological changes, particularly stress.

The elevated growth disruption experienced by the Black Friars post-medieval females over any other group was likely tied to the poor living conditions in urban Odense. While Mays et al. (2008) demonstrate that urban living conditions did not greatly affect the growth of subadults until the Industrial Revolution, the biological hardships for young adolescent women, when growth was still occurring, may have drastically impacted their maturation and later life health status. While the similar age-at-death and distribution of stress lesions and pathologies between males and females does not suggest a large cultural disparity between the sexes, the impact of menarche and early pregnancy would have hindered many young females within these populations. Because menarche occurs at the end of the female adolescent growth spurt (Eveleth 2008), iron requirements are already elevated and must be continually met throughout life if menstruation remains normal (Hallberg 2001). During the medieval period estimates suggest that first menstruation occurred between 13-14 years of age (Amundsen and Diers 1973); however, the onset of menstruation was highly dependent on nutritional status (Eveleth 2008). Those girls living in poor conditions with inadequate nutrition likely experienced later menarche into the 15th year of life (Eveleth 2008). The benefit of BSI data is that it provides growth information for the

final stages of skeletal maturation (i.e. nine to 20 years) and as such can be used to assess the increased biological stress that menstruation and puberty may have brought to these young women.

Additionally, early pregnancy during the final years of growth and development would have also been a burden for young women as growth and pregnancy both require increased levels of dietary iron and other essential nutrients (Hallberg 2001). While the majority of iron requirements can be met through dietary supplementation, the second and third trimester of pregnancy require more iron than can be absorbed through diet alone; therefore, iron stores within the body are needed to meet demand (Hallberg 2001). It is unlikely that the majority of women during the post-medieval period, with inconsistent food sources and reduced meat consumption (Hoffman 2001), were able to meet the iron and nutrient demands associated with later stage pregnancy. The onset of menstruation did not necessarily mean immediate pregnancy for young women; however, when pregnancy did occur it would have created additional biological strain for these women as their bodies adapted to both the demands of pregnancy and the energy requirements needed for the final stages of physiological growth.

6.6 CORTICAL THICKNESS

6.6.1 Cortical Thickness and Orientation

In looking at cortical thickness patterns for the Black Friars population, there were less consistent trends than the BSI data in regards to overall growth and development fluctuations. The medial and lateral measures of cortical thickness (captured in AP radiographic orientation) provided more consistent data over the anterior and posterior cortical measurements, likely related to the bone structure of the anterior and posterior tibia. As the anchor for the tibialis anterior muscle, the anterior crest of the tibia is under constant muscle strain during foot and

ankle movement. Similarly, the posterior tibia is the origin point for the tibialis posterior muscle which provides stability in the leg and also controls foot and ankle movement (Marieb and Hoehn 2013). Cortical thickness in these regions of the tibia may be highly influenced by mechanical strain with resultant remodelling of the cortical tissue. The medial and lateral cortical bone of the tibia, however, is somewhat insulated from these mechanical influences that are associated with the primary muscle attachment sites for the lower leg and foot. Therefore, the medial and lateral cortical bone may be more reliable when assessing acute or chronic stress. Focusing then on the medial and lateral cortical measurements, the results of this study tentatively reflect higher levels of stress in the post-medieval female group as cortical thickness discrepancies between the left and right tibiae could be associated with fluctuating asymmetry. Defined as the deviation of skeletal formation from symmetrical development, specifically in the long bones, asymmetry can become exacerbated with prolonged stress (Palmer and Strobeck 1986; Leung et al. 2000). Bioarchaeological research into fluctuating asymmetry has been associated predominately with handedness (Steele 2000), but has also been considered evidence of "developmental instability" (Albert and Greene 1999; DeLeon 2007:520), associated with environmental shifts (excessive heat or cold), nutritional deficiencies, excessive noise, prenatal chemicals, and diabetic fetal environments (Albert and Greene 1999; DeLeon 2007).

6.6.2 Cortical Thickness and Pathology

In assessing the relationship between cortical thickness and pathology, the increase in thickness associated with conditions such as leprosy, treponema and tuberculosis likely represent the normal skeletal response associated with the initial onset of these diseases (Ortner 2003) and is therefore not unexpected. Conversely however, the post-medieval females showed a decrease in cortical thickness when fractures were present. This relationship however, likely reflects the

natural decrease in cortical thickness with age (Parfitt 1984) and the increase prevalence of fractures associated with conditions such as osteoporosis (Cummings et al. 1985). In the post-medieval group, the average age of the women with fractures was 35.2 years. This suggests that while some of these women may have shown a decrease in cortical thickness due to early osteoporotic changes, the younger women likely showed reduced cortical bone when fractures were present due to the energy diverted away from normal bone remodelling while a bone callous is formed and maintained.

6.7 OSTEOCALCIN

6.7.1 Osteocalcin Levels and Sex Steroids

Osteocalcin is regulated by the osteoblast cells and their ability to produce new bone tissue (Christenson 1997; Rauch 2008). However, the function of osteoblasts cells is greatly influenced by glucocorticoids that are secreted as part of the stress response system (Cooper et al. 1999) and can indirectly influence osteocalcin levels. As mentioned briefly in Chapter 3 Section 3.5.2, osteocalcin levels are closely associated with sex, more specifically sex steroids. Estrogens have been clinically shown to increase the circulation of glucocorticoids in the body, whereas androgens reduce the number of circulating glucocorticoids (DeSilva 1999). This increase and decrease of circulating glucocorticoids has a direct effect on the ability of the osteoblast cells to synthesize osteocalcin. This pattern of fluctuation was clearly demonstrated in the Black Friars population with males showing increased levels of osteocalcin and females showing decreased levels of osteocalcin. While there are other contributing factors affecting the overall level of osteocalcin in these male and females groups (e.g. age), the influence of circulating sex steroids is primarily responsible for this observed pattern. A consistent decrease of osteocalcin with age was also demonstrated in these data where factors such as slower bone

remodelling and decreased growth hormone secretion (Vanderschueren et al. 1990; Ingram et al. 1994) contribute to the diminished function of osteoblast cells and consequently the level of osteocalcin captured in the skeletal tissues.

6.7.2 Osteocalcin Levels and Skeletal Elements

As presented in Chapter 5, Section 5.5.1, there were no significant differences between the clavicle and femur osteocalcin levels compared across the 20 individuals. However, the clavicle levels of osteocalcin appeared to be more consistent than the femur osteocalcin levels when compared to the pathological and stress data. Once synthesized, 60-90 percent (Neve and Corrado 2011) of osteocalcin is retained in the skeletal tissues, protected overtime by the hydroxyapatite. Once incorporated into the skeletal tissues, osteocalcin remains present until the remodelling process occurs whereby a basic multicellular unit controlled by osteoclasts and osteoblasts begin to remove old bone and deposit new bone (Beaupré et al. 1990; Kular et al. 2012). The duration of this remodelling phase can vary extensively between bone types due to different mechanical and metabolic processes that dictate this process (Parfitt 2010; Kular et al. 2012). The heterogeneity of bone also plays an important role in this rate of turnover where appendicular turnover is prompted more by mechanical influences and axial turnover is dictated more by metabolic requirements (Parfitt 2010). As Parfitt discusses (2002), cortical bone is made up of three distinct layers: endosteal, intracortical and periosteal with the periosteal and endosteal bone experiencing the most turnover. The approximate mean age of these outer layers of cortical bone are between 0.5 – 4.0 years (Parfitt 2010) illustrating the variability of cortical composition and the period of skeletal incorporation that these osteocalcin samples may represent. Because the cortical bone of the axial skeleton is much thinner than the appendicular skeleton (Parfitt 2010), there is faster turnover over of the cortical bone, approximately 4 percent per year (Parfitt

1983; Parfitt 2002; Parfitt 2010). Considering the remodelling properties of bone, it is clear that the data collected from the femur and clavicle for this study likely represent different periods of osteoblast activity and therefore, variable levels of osteocalcin due to regional differences of bone location and overall cortical thickness. The higher rate of bone remodelling in the clavicle would therefore represent more recent osteocalcin levels while the femur likely represents slightly earlier levels of osteocalcin incorporation. However, overlap between the samples is likely.

The differences in glucocorticoid receptor prevalence may also influence which type of bone is more negatively affected (Weinstein et al. 1998; Henneicke et al. 2011). These glucocorticoid receptors are responsible for transducing the glucocorticoid signal into all surrounding cells and their presence in the body is tissue-specific. Therefore, the magnitude in which the effects of glucocorticoids are incorporated biologically depends heavily on these receptors (Bamberger et al. 1996). These receptors are present in all bone cells (LaCorte et al. 2010); however, some skeletal tissues may be more vulnerable to the effects of glucocorticoids based on the number of receptors they house. Using rabbit femora, Eberhardt et al. (2001) demonstrated that the trabecular bone of the femur is particularly vulnerable to the effects of glucocorticoids. The authors concluded that this trend was due in part to the large size of the femur and the number of glucocorticoid receptors present in the bone. This would suggest that osteocalcin levels would show more fluctuation in the femur over the clavicle. However, the heavy mechanical loading of the femur promotes faster bone remodelling and the loss of accumulated osteocalcin data, introducing very specific limitations for the bone best used for osteocalcin analysis. Additionally, research by Prigodich and Uesely (1997) and more recently by Chen et al. (2015) has explored the relationship between osteocalcin and type-1 collagen. This

not only has implications for the bone mineralization process (Chen et al. 2015), but also influences how osteocalcin can be extracted and detected in archaeological remains. Each collagen molecule has one binding site for osteocalcin; therefore, osteocalcin and collagen form a 1:1 complex (Prigodich and Uesely 1997). As such, the amount of collagen preservation in bone directly influences osteocalcin preservation and should be similar across all skeletal elements regardless of remodelling rates, potentially explaining the close similarity between the osteocalcin levels recorded in the clavicle and femur samples.

6.7.3 Osteocalcin Levels and Pathological Conditions

When considering the effect of bone heterogeneity, remodelling and collagen preservation on osteocalcin levels, there is also a need to consider the nature of the pathological and stress data collected. While the stress indicators represent conditions of childhood that remain visible in adult tissues, the pathological conditions more likely represent chronic ailments. Therefore, the demonstrated relationship between decreasing osteocalcin levels and degenerative spine changes reflect a condition affecting these adult individuals near the time of death. With this pathology there is a characteristic deconstruction of the spinal elements with subsequent disorganized bone formation (e.g. osteophyte lipping) (Ortner 2003). While this reactionary bone formation may temporarily increase osteocalcin levels, the long term destruction of bone and lack of proper remodelling would eventually lead to the disruption of normal osteocalcin synthesis.

6.7.4 Osteocalcin Levels and Stress Indicators

Enamel hypoplastic lesions and HL are confident markers of childhood stress, whereas CO and PH may continue into adulthood and must be considered when analyzing osteocalcin levels. In the Black Friars sample, individuals with evidence of CO or PH showed lower

osteocalcin levels, possibly representing the persistence of these lesions into adulthood, as it is unlikely that the subadult years were captured in these bone samples. The formative process of stress lesions may also need to be considered in osteocalcin analysis. Enamel hypoplastic lesions and HL are characterized by distinct periods of growth stasis, whereas CO and PH form throughout the growth period. These dynamics affect osteocalcin synthesis in different ways. For EHL and HL, osteocalcin levels likely decrease significantly while growth is stalled due to osteoblast inactivity. However, once the stress had passed and growth resumed, synthesis can rebound quickly to normal levels. For CO and PH, however, because the growth disruption process occurs on a continual basis and is characterized by bone porosity due to dysfunctional osteoblastic activity, these indicators may create long-term disruptions in osteocalcin levels. The lack of correlation between osteocalcin levels and diminished BSI growth is also likely influenced by when these osteocalcin levels were incorporated into the bone. If stress was endured through the entire period of skeletal maturation any reduction in osteocalcin levels would only be reflected for a finite amount of time before being remodelled. Alternatively, these osteocalcin fluctuations may be masked through rapid catch-up growth recovery which would saturate the tissues with osteocalcin during this accelerated period of growth, possibly explaining the pattern visible in this small sub-sample of the Black Friars population.

6.7.5 Osteocalcin Levels and Time Period

A significant outcome of this pilot study was the comparison of osteocalcin between time periods where there was a clear reduction in the femur and clavicle levels from the medieval into the post-medieval period (see Figure 5.36). Because of the small sample size the sex and age group data were combined and of course could have influenced the result. However, when the distribution of males and females and age groups across time period were statistically compared

there were no significant differences. This suggests that the pattern observed is reflective of real differences between the medieval and post-medieval periods and not significantly influenced by known differences between sex or age groups. As discussed throughout this chapter, there were many hardships endured by the Black Friars population between the medieval and post-medieval period most highly influenced by food quality and supply and the increasing strain of the growing urban environment of Odense. These lower levels of osteocalcin in the post-medieval period suggest these individuals were exposed to higher levels of circulating glucocorticoids associated with increased stress. When looking at this post-medieval osteocalcin data compared to the pathological, stress lesion and BSI data, all suggest a similar trend where increased hardships were faced by the post-medieval individuals in the Black Friars population. While the macroscopic skeletal data does allude to this temporal shift, it is this biochemical data that truly substantiates these results.

6.7.6 Osteocalcin Levels and Psychological Stress

The effect of psychosocial stress on overall health is also a consideration in the study of archaeological populations. While difficult to extract from skeletal remains, actual or perceived psychological stress can signifcantly affect overall health. Known as the immunosuppression model, the onset of stress depresses the immune system making the body more vulnerable to disease, in part through the release of glucocorticoids (Miller et al. 2002; Segerstrom and Miller 2004). The impact of psychological stress on glucocorticoid release has been thoroughly discussed in the literature (see Miller et al. 2007). However, the fluctuation of these steroids and their resultant impact on biological tissues is highly dependent on the duration of the stress, the type of threat and the emotions associated with the stress (Miller et al. 2007). With the onset of psychological stress, cortisol levels rise rapidly to help the individual adapt, but fall as the stress

becomes more distant and removed from thought (Miller et al. 2007). The type of psychological threat is also an important factor regulating glucocorticoid release as each type of stress may "pose different adaptational demands" (Miller et al. 2007:27). While there is a general trend to assume that the biological response to stress is homogenous and non-specific, in reality the biological response evolved and adapted to improve coping and survival (Weiner 1992; Kemeny 2003). The emotions attached to different types of stressors also contribute to the biological response, where traumatic stress with threat to physical well-being elicits an elevated glucocorticoid/endocrine response compared to other benign factors (Miller et al. 2007). Individual variability also contributes to how psychological stress is internalized. For example, Kirschbaum and colleagues (1992) examined the differences in cortisol levels between males and females when public speaking. Cortisol levels were greatly increased in males over females even when the threat was only perceived and had not yet taken place (Kirschbaum et al. 1992). Not only do these increased levels of steroids associated with psychological stress affect personal health, they also have the ability to influence fetal growth and development. Studying cortisol levels in individuals from the womb through to adolescence, Jones and colleagues (2006) demonstrated that those exposed to higher levels of glucocorticoids from maternal stress while in the womb had differential responses to later life stress. Despite the difficulty in extracting this type of information from archaeological remains "social stressors, whether they are acute or chronic, reliably activate the HPA (hypothalamic-pituitary-adrenal) axis" (Miller et al. 2007:36) requiring appropriate attention for how this may influence skeletal tissues and interpretations of health.

Regardless of time period, the Black Friars population was exposed to both chronic and acute psychological stressors including periods of famine, climate change, disease outbreak and

warfare. However, these psychological stressors may have been short-lived as prolonged periods of the same psychological stressor effectively begins to desensitize the biological system Dienstbier 1989; Gunnar and Vazquez 2001; Miller et al. 2007). For example, the psychological stress associated with unreliable food resources in the Black Friars sample likely did not contribute to a drastic increase in circulating glucocorticoids as famine was commonplace during this period and arguably expected. While the physical toll of these chronic nutritional stresses would have been inevitable the associated psychological upset would not have drastically influenced the skeletal outcome.

As an acute psychological stressor, the Black Death during the 14th century, likely had wide-spread consequences as individuals who managed to survive were burdened with the mental stress of caring for those who were sick and mourning for those who had died (Meinlschmidt and Heim 2005; Nicolson 2004). Additionally, the threat of subsequent outbreaks would have also placed a psychological burden on these populations, contributing to increased levels of stress. Another source of psychological stress particularly in the post-medieval period would have been the outbreak of the Danish civil war (Count's War) at the dawn of the Reformation. Spread across the whole of Denmark, this war saw the battle between the old Catholic faith and the new Protestant masses (Lockhart 2004). While warfare and political unrest was not new in Denmark, arguably, the Count's War was the largest wide-spread war in Denmark during the period of the Black Friars cemetery and no doubt lead to various levels of psychological stress, particularly once the war reached the island of Fyn in the summer of AD 1535 (Lockhart 2004). As Miller et al. (2007) outline, psychological stressors that threaten the physical self lead to increased cortisol levels, and in some cases of post-traumatic stress disorder (see Yehuda 2000). Therefore, the psychological stress and resultant glucocorticoid levels would

have been vastly different for those participating in the war as soldiers, those left behind in Odense, and those burdened with injury or the loss of loved ones at the war's end. While the analysis of psychological stress in archaeological remains is a near impossible task, the impact of mental health must not be overlooked, particularly when studying biochemical fluctuations associated with stress. Interestingly, the caveats of studying psychological stress can also be considered in the wider context of the physical stress markers already discussed, where the tissue distribution and physical manifestation of various stress indicators have likely evolved to help maximize our ability to cope with stress.

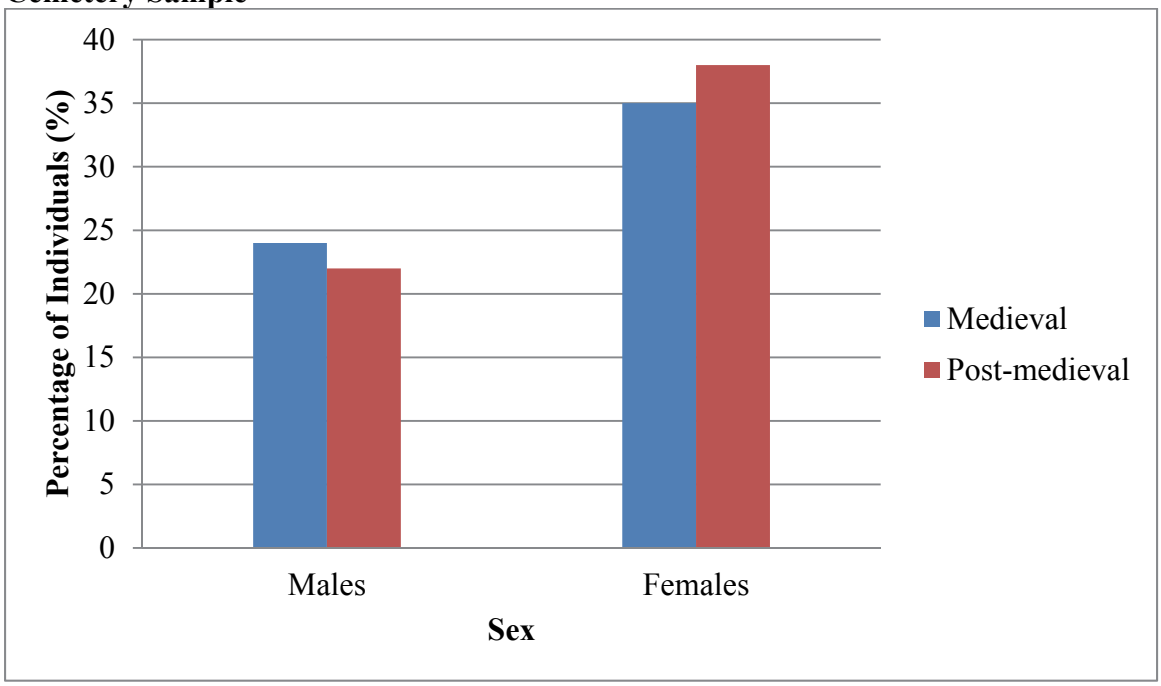
Despite the successful extraction of osteocalcin for this study, there is a need to measure the amount of crystallinity present in these samples to determine how much of the protein has been lost and how that compares to what has been successfully extracted. This is not to say the osteocalcin levels obtained for this analysis are not true reflections of what were present in the bone samples acquired, but rather without a clear understanding of the diagenetic processes influencing the rate of protein degradation over time these results must be considered cautiously and preliminary in nature.

6.8 MORTALITY

6.8.1 Adult Mortality

For the medieval males and females, the average age-at-death was 34.8 and 32.4 years, respectively. In the post-medieval sample, the average age-at-death for males was 34.6 years and 33.7 years for females. While these age-at-death averages are not noticeably different between the sexes, when looking at the average age distribution and the number of individuals dying before 30 years, the females show a sharp increase compared to the males (Figure 6.1).

Figure 6.1 Percentage of Males and Females dying before 30 years in the Black Friars Cemetery Sample



While the differences in biological frailty between males and females have shown a general trend towards increased male susceptibility, cultural and social factors also influence these biological frameworks (Buchanan 1975; Waldron 1983; Högberg et al. 1987; Klein 2000). For women, childbirth and complications arising from pregnancy (Wells 1975) are likely driving this disparity as the sharp increase in female mortality dissipates after the childbearing years (Boldsen 1984; Högberg et al. 1987). In addition to the biological disparities between men and women, the urban environment of medieval and post-medieval Denmark also likely contributed to this increased female mortality. Urban Odense at the beginning of the 17th century was estimated to have a population of 5000-6000 (http://museum.odense.dk/viden-om/historie/odense-historie/odense-bys-historie), and would have been characterized by the many challenges of urban dwelling, including increased pathogen exposure, high population density, poor sanitation and unreliable water supply (Dyer 1989; Landers 1993). With women at an increased susceptibility to infectious disease at the time when they gave birth (Wells 1975;

Högberg et al. 1987), these urban environments likely exacerbated the dangers of childbearing and considerably challenged any natural immunity females may have had over males (Klein 2000). Discussed by Palubeckaitė et al. (2002), the urban post-medieval Subačiaus Lithuanian population, similar to the Black Friars sample, experienced what the authors describe as an environment of chronic stress elevating levels of morbidity, but not drastically affecting overall mortality. Looking at the shift in mortality from the medieval Black Friars sample into the post-medieval period there is a similar trend of rising morbidity evidenced through an increase in pathological conditions, stress lesions and growth disruptions. For the females in particular, life expectancy was slightly higher in the post-medieval period despite this increased morbidity, possibly reflecting a biological shift where individuals exposed to more stress earlier in life were better equipped to withstand later stressors in adulthood.

6.8.2 Subadult Mortality

Opposite to the adult mortality data, the Black Friars subadults in both the medieval and post-medieval groups had the same average age-at-death, 8.5 years. Whereas the adult females in the post-medieval period experienced an increase in the average age-at-death despite increased morbidity, the subadult individuals do not show a similar trend. As adult females aged and had increased exposure to pathogens and stress, individual resistance to these influences may have been acquired. As for the subadults, their foreshortened lives would have diminished their ability to acquire the same resistance as the adults. While the post-medieval adults would have also passed through the dangerous childhood years, the type of stress they were exposed to and individual variability in biological resistance would have allowed some individuals to reach adulthood and gain further resistance.

6.9 REVISITING THE FOUR HYPOTHESES

The four hypotheses and the results of this analysis are important as they contribute to a better understanding of the stress response and the interaction (if any) between these multiple indicators of poor health. Further, these hypotheses provide an opportunity to explore the benefit of combining traditional osteological methods with biochemical signatures to explore health. Based on the discussion above, each hypothesis is examined within the framework of the Black Friars population and the general trends that emerged from this research.

Hypothesis 1 stated that if morbidity (occurrence of illness) and mortality (occurrence of death) were elevated within this population, it is expected that skeletal evidence should indicate stress, or that stress indicators should be present. Within the Black Friars sample all individuals showed some indication of stress, whether lesion-based or growth-based. The biochemical data, while preliminary, also hinted at fluctuations associated with increased glucocorticoid release from stress response activation. How stress was differentially internalized across this population was likely the result of stress severity and duration and the various insulating aspects of human biology (i.e. differential frailty, tissue vulnerability). From a biochemical perspective, the release of glucocorticoids does not suppress all aspects of immunity equally during periods of stress, but can prompt a myriad of responses depending on the chronic or acute nature of the stressors (Elenkov et al. 1999). Therefore, while the statement can be made that all individuals in the Black Friars sample were exposed to periods of stress resulting in skeletal disruption, a closer examination of the biological stress pathways may better help extrapolate why these differences in skeletal manifestation occur.

Hypothesis 2 stated that the severity of that stress and how it was manifest in the skeleton would be reduced in successive age cohorts (i.e. after a certain age, those affected by the

increased stress would have likely succumbed to these insults). In observing the stress sum data and the relationship between each lesion-based indicator, there was no distinct relationship between these lesions where the presence of one may preclude the development of another. However, there was a clear decline in stress severity in the oldest adult age group, suggesting those individuals who survived into later adulthood were less stressed during their development period. While the biochemical data shows the expected decrease of osteocalcin levels into older adulthood, this trend is primarily dictated by underlying biological processes. It is likely that stress is still present in this older age group, but individuals may have developed coping mechanisms that reduce the level of circulating glucocorticoids in tandem with these already known biological reductions.

Armelagos et al. (2009) discuss how the relationship between increased stress and increased morality can be the result of three independent processes difficult to differentiate archaeologically: 1) individual differential frailty, 2) pre-exposure frailty and 3) confounding stressors. How an individual responds to stress is driven both by internal and external factors. Differential frailty may predispose certain individuals within the population to show differing levels of stress in their skeletal tissues. Pre-exposure frailty may also influence the pattern between increased stress severity and mortality as individuals become "biologically damaged" (Armelagos et al. 2009:268), and are less able to cope with later life stress events. As discussed in Chapter 3, Section 3.8.1, frailty is not static and can change over an individual's lifetime (Boldsen 2007). This fluctuation in frailty inevitably leads to fluctuations in individual mortality as exposure to new stress during the life course continually influences overall health status.

Hypothesis 3 states that the severity of stress would be higher in children who represent the non-survivors of their community (i.e. individuals affected early in life by high levels of

physiological stress would not survive to adulthood). As such, it is expected that adult survivors would not demonstrate the same severity of stress as observed in the subadults. As discussed above, differential frailty greatly impacts how stress is manifested within the skeletal tissues, making the study of severity difficult. For example when observing the lesion-based indicators, the subadult individuals in the Black Friars population showed increased mortality when more indicators were present. The inverse was true for the adults where mortality was increased when each specific stress indicator showed increased severity. This would suggest that the co-prevalence of multiple stress indicators is more detrimental to overall health than fluctuations in the manifestation of each specific indicator as the subadults represent the non-survivors of the community. Perhaps then, the differing levels of manifestation for each specific indicator are more reflective of differential frailty and the heterogeneous response to stress in multiple tissues based on underlying biological factors (i.e. age and sex), and the influence of previous stress exposure (Armelagos et al. 2009).

Hypothesis 4 states that a small proportion of individuals, as a result of differential frailty (Wood et al. 1992), might live beyond what was expected despite showing an elevated severity of stress. In the oldest adult age group, the prevalence of stress lesions was relatively low, with no individuals having more than two different lesions present. The most prevalent stress lesions showing the most severity in the oldest age group were EHL and HL, whereas CO and PH were almost non-existent. For the BSI data all of the oldest adult individuals, except for one, had less than 50 percent of their BSI variables falling below the confidence interval, with one medieval male having no variables below the confidence interval. Interestingly, while the prevalence of stress lesions was low in this group and growth disruption moderate, pathology was relatively high with some individuals having as many as seven pathological conditions present at the time

of death. Table 6.8 is a summary of three individuals chosen for their high number of stress lesions, BSI variables falling below the confidence interval and the most pathology present and how they fit within the caveats of the fourth hypothesis.

For individual SBT81 GR479 (medieval male), the pathological conditions that are present better reflect later life health rather than insults incurred during the subadult years. This individual likely benefited from a naturally stronger immune system throughout childhood allowing him to pass through the subadults years relatively unscathed and to survive into later adulthood even when exposed to various pathological conditions. Individuals SBT81 GR633 (medieval male) and SBT81 GR253 (post-medieval female) show a different pattern of subadult health where they did develop more than one type of stress lesion which manifested quite dramatically (levels 2 and 3). However, the number of BSI growth variables affected by stress is noticeably different between these two examples where the post-medieval female demonstrates a much higher level of disruption (62% of variables below the confidence interval). The discrepancy between these two individuals could of course be tied to biological differences and individual constitution. However, the discussed hardships of the post-medieval period and the impact on health likely contributed to this pattern as well. Further, in assessing the pathological data, the post-medieval female would be identified as having worse overall health than the medieval male example. Important to note here is that both individuals have the same mean age-at-death, suggesting that despite more growth disruption during the childhood years and later life pathological processes, the post-medieval female continued to survive. This demonstrates that at an individual level when considering differential frailty, there will always be some in the oldest age categories with visibly poorer health yet still survive over those who appear nearly unscathed.

Table 6.8 Stress Summary Examples from the Oldest Adult Age Group (45-60 years)

Individual	Stress Lesions (severity level)	BSIs (% of variables below CI)	Pathology (Present)
SBT81 GR633 45 years medieval male	EHL – level 3 HL – level 3 No CO No PH	36.0	Periostitis
SBT81 GR253 45 years post-medieval female	EHL – level 3 HL – level 2 No CO No PH	62.0	TB Schmorl's nodes DJC
SBT81 GR479 51 years medieval male	HL – level 1 No EHL No CO No PH	33.0	Periostitis TB Treponema Fractures DSC Schmorl's Nodes DJC

6.10 SUMMARY

Overall the results and discussion of this research demonstrate that the stress response is not a uniform process and that the manifestation of stress in the hard tissues of the body is constantly influenced by a multitude of intrinsic and extrinsic factors. Despite the difficulty in determining the relationship between these different skeletal changes there was a clear trend in the data where the post-medieval group, particular the females, were experiencing higher levels of stress in the Black Friars population. Interestingly, this increase in stress did not lead to increased mortality in the post-medieval sample, suggesting that early childhood exposure to various types of stress may have provided some insulation against later life stress events. Although the medieval period was host to the outbreak of the Black Death and the beginning of the Late Medieval Agrarian Crisis (Gissel 1981; Jäger 1981; Benedictow 2004), it was the post-medieval period that was left with the consequences of these events in addition to an expanding urban environment and increasing political and religious upheaval. These cultural disruptions along with the physical and psychological stressors associated with these hardships would have

increasingly affected the post-medieval Black Friars population and their daily lives in the city of Odense.

CHAPTER 7: CONCLUSIONS

The main objective of this research was to examine different indicators of skeletal stress and how the relationship between these stressors can better inform our understanding of health and well-being in the past. Situating each of these indicators within a larger clinical understanding of the stress response and the mechanisms that instigate skeletal disruption, this study provided a comprehensive look at stress. By expanding the analytical framework of well-established methods and integrating new biochemical analyses, this study demonstrated the importance of comparing and contrasting multiple data types in the study of stress.

This research also provided an opportunity to explore health in the Black Friars cemetery population from the medieval into the post-medieval period. Informed by historical texts of the cultural, social and environmental changes in Denmark between the medieval and post-medieval period, it was expected that these two time periods would display distinct health trends that could be captured within the caveats of the research objectives and four hypotheses.

7.1 REVISITING THE RESEARCH OBJECTIVES

Revisiting the research objectives outlined in Chapter 1 of this dissertation, the following conclusions were drawn. A clinical understanding of the stress response, specifically the influence of glucocorticoids, should be used to better assess the patterns of skeletal disruption, particularly when these disruptions are characterized differently (e.g. systemic vs. localized stress; complete growth halting vs. growth disruption). This clinical knowledge of glucocorticoid release timing, the distribution of their receptors, and the underlying biological factors that control their circulation patterns all contribute to the underlying stress response process that eventually results in hard tissue change. While a direct correlation between glucocorticoids and macroscopic skeletal changes to date has not been made in in archaeological remains, knowledge

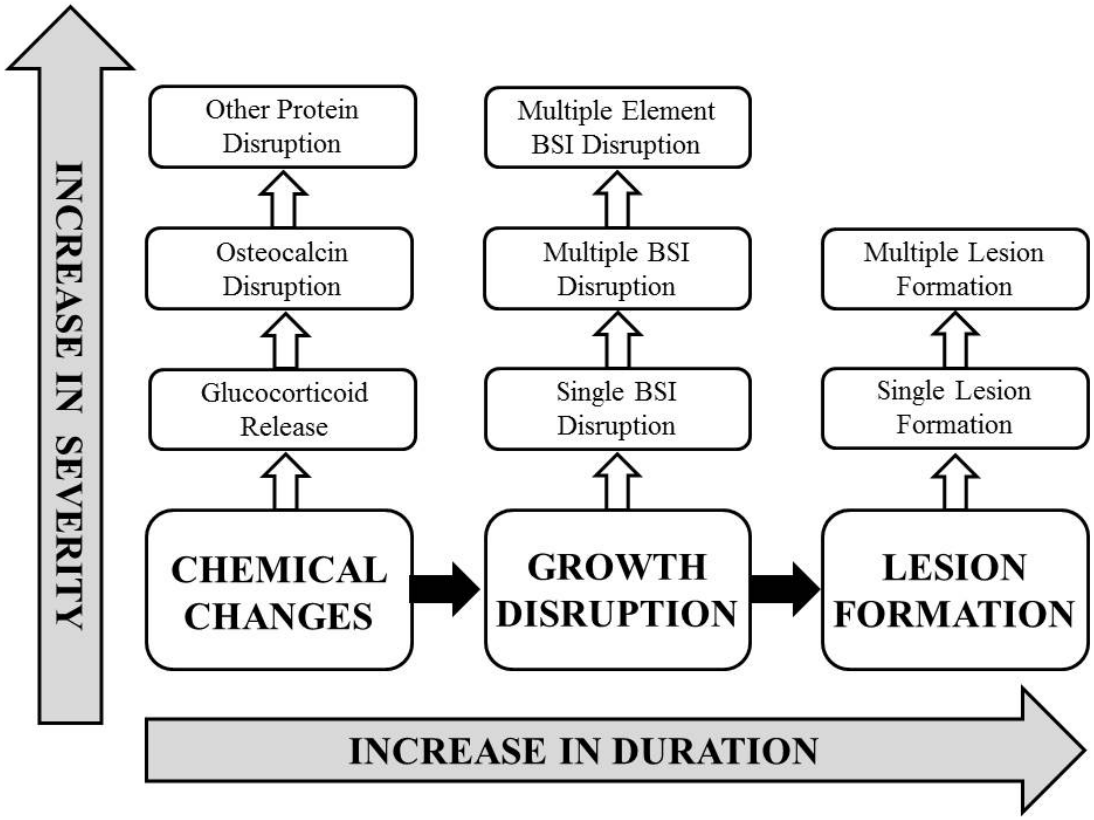
of these steroids and their release at the onset of stress provides the necessary foundation to better understand the biological processes that mediate the initial stress response and how over time this response translates into visible skeletal disruption. Therefore, through a better integration of this clinical knowledge osteologists can improve and advance their approach to the stress process and its study in ancient populations. While this relationship has been hinted at in the osteological literature in the past, this dissertation provides an in-depth analysis of how clinical knowledge of the stress response can better inform our osteological expectations. Further, the introduction of this discussion provides an opportunity to reframe our research questions and how we may interpret the presence or absence of specific stress indicators in archaeological remains.

The lesion- and growth-based data showed similar results within the Black Friars population, but of course represent different mechanisms of the stress response. In theory, when the stress response is activated, glucocorticoids lead to primary skeletal changes that then target the growth function of the body resulting in immediate growth disruption that over time eventually leads to lesion formation. Therefore, these indicators do not necessarily reflect differing severities of stress, but rather the *duration* of the stress. In contrast, severity seems to be best measured through increased skeletal involvement within each tissue type. Figure 7.1 presents a schematic summary of this based on clinical observations and our expectations for osteological remains. This schematic represents our current understanding of the stress mechanism and resultant hard tissue changes at an individual level and cannot necessarily be used to compare individuals when factors such as age-at-death, individual frailty and pre-exposure that can differentially affect osteological evidence of stress manifested over the life course. The stress response is not a simple step-wise matrix, with individual and population

factors influencing how these trends can be explored and interpreted archaeologically. In looking specifically at this progression, duration is best captured through the type of skeletal change(s) occurring, beginning first with chemical changes, then a disruption of growth and finally the formation of lesions. Based on the biological factors driving these specific processes, this progression is fairly straightforward. While it is likely that growth disruption occurs first before lesion changes due to the specific influence of glucocorticoids on this mechanism, this response to stress may not present as simplistically in an archaeological context as factors such as catch-up growth impede our ability to clarify this process. Severity and how it is captured in skeletal remains however, is much more difficult to identify and confidently assess as each of the three components (i.e. chemical changes, growth disruption, and lesion formation) are marked by different skeletal change and involvement. When assessing the initial phase of chemical change, glucocorticoid release identifies the onset of stress followed by disruption to secondary processes, particularly the production of osteocalcin. Similar to the disruption of osteocalcin, it is expected that similar proteins will also be affected during more severe periods of stress and warrant further investigation (see Section 7.2). In looking at growth disruption, specifically through BSI analysis, the initial stages of stress lead to disruption in single BSI variables. With increasing severity more BSI variables are affected, culminating in multiple BSIs being disrupted across multiple skeletal elements. Finally, the severity of stress lesions is perhaps best analyzed through an examination of multiple lesions. Whereas the varying degree of manifestation for one lesion type may be regulated by differential frailty, the involvement of multiple lesions across different tissue types suggests a more severe form of stress. While this explanation and schematic represent an idealized projection of the stress response in skeletal tissues, it does

contribute to a more refined understanding of duration and severity and how these elements are a necessary component of osteological analyses.

Figure 7.1 Summary of the Stress Response with Focus on Severity and Duration



Through this in-depth exploration and discussion this research contributes further insight and observations into the stress response and how different indicators of stress and their variability in manifestation may be better integrated into osteological interpretations. Further, this research has attempted to explore the relationship between lesion based changes and growth disruptions and how underlying biological mechanisms mediate these responses differently. While not the primary focus of this study, this dissertation provides the foundation on which to further explore how BSIs may be further integrated into stress research.

The introduction of biochemistry into studies of health and well-being can also inform the subtle changes associated with stress that until now have been invisible archaeologically.

While particular attention must be paid to the diagenetic influences of protein preservation, the use of osteocalcin to support the well-established methods of stress assessment is invaluable. One of the primary points made by Wood and colleges (1992) is our inability to account for those suffering from poor health without any physical evidence. Advancing biochemical techniques and methods however, can address this issue and as shown in this research can/should be used in tandem with macroscopic methods. Further, in instances where skeletal preservation may be poor, or remains badly damaged, bioarchaeological research is limited by what can be observed. However, with the integration of biochemical techniques that can be reliably used to assess health research, collections previously overlooked due to issues of preservation may be re-explored. This dissertation directly contributes to this growing area of research by providing a viable method of osteocalcin extraction for human remains and preliminary interpretations between this protein and other osteological indicators of stress. Additionally this dissertation provides the theoretical foundation linking the clinical literature with the current osteological understanding of osteocalcin production and preservation over time.

Overall the Black Friars population experienced more stress in the post-medieval period, with females in particular showing the greatest biological strain. Evidence of stress lesions, growth disruption and osteocalcin fluctuations in the post-medieval population suggests that a variety of hardships such as: poor nutrition, heavy manual labour, an introduction of new pathogens in a growing urban environment, psychological stressors and civil unrest impacted the overall well-being of these individuals. While the medieval period was also exposed to many stressors, the increasing urban environment of the post-medieval period exacerbated by diminishing social assistance with the shift to Protestantism created an increased strain on the lower classes of Danish society in the second half of the 16th century. At the onset of this

research, it was expected that the socioeconomic shift experienced in the late medieval and early post-medieval period in Denmark would have led to improved health in the post-medieval population due to improved dietary supplementation. Despite this new reliance on cattle and cattle products however, it appears that the majority of these new foodstuffs were traded in European markets and not necessarily researching the general population in Denmark. This is not to say that certain individuals did not benefit from this socioeconomic shift, but the majority of the population likely still subsisted off of foods similar to those from the medieval period. Yoder (2010) also found no significant dietary changes when comparing early and late medieval populations, reiterating that "there was a shift in the agricultural system, (but) there was not much of a shift in the actual diet" (2234). While the contents of the Danish diet may have remained fairly stable between the medieval and post-medieval periods, the amount of available foodstuffs possibly influenced the unexpected poorer health observed in the post-medieval population. While the medieval period experienced agricultural devastation during the Little Ice Age (Fagan 2000) and the Late Medieval Agrarian Crisis (Gissel 1981; Jäger 1981), agricultural production eventually rebounded creating a surplus after the population decline following the Black Death (Hybel and Poulsen 2007). This surplus however was short-lived as political unrest and social upheaval in the post-medieval period saw the loss of one of Denmark's main agricultural region to Sweden (i.e. the Halland, Skåne and Blekinge provinces). Since the introduction of agriculture in Denmark, the Skåne province in particular was an important agricultural region; therefore, its loss during a period of urban expansion and population growth no doubt contributed to some extent to declining volumes of food for those living in the post-medieval period. These constant food woes in addition to the multiple other confounding factors

discussed were likely responsible for the poorer overall health of the post-medieval Black Friars population despite the economic successes of the European cattle trade.

7.2 FUTURE RESEARCH

From a traditional standpoint, the methods and protocols for how stress indicators are captured and studied can be continually re-evaluated and improved upon. The benefit of this constant improvement and validation is consistency within this field of research which contributes to the reliability and repeatability of the results. Additionally, further exploration into the hierarchical nature of stress can also better inform the interpretations we make about particular populations. While the summary presented in Figure 7.1 may represent the visible patterns in the Black Friars population, it may not be as consistent in other contexts with different influences affecting the outcome of skeletal change associated with stress. As an area of study decidedly complex and difficult to ascertain concrete rules, the study of stress is best approached from a multi-method perspective where indicators that cover various time periods of maturation can be analyzed in tandem. This integration of multiple methods capturing various aspects of the stress response does more than simply complicate research goals; it provides an opportunity to explore the various biological responses that may occur from one particular stressor. In better understanding how these different indicators of stress piece together, bioarchaeologists will be in a better position to establish a more concrete understanding of severity and duration. The benefit of this is of course in the analytical and interpretative potential of this type of research, where degrees of severity and duration can enlighten the subtleties of stress which have been notoriously difficult to ascertain.

In order for biochemistry to become a cornerstone of stress research there is a need to truly understand the diagenetic processes that effect the preservation and extraction of proteins

such as osteocalcin. While this research used ELISA analysis to identify the amount of osteocalcin in each sample, more refined methods such as mass spectrometry would provide a better snapshot of the chemical composition of the bone and what diagenetic influences may be affecting the observed results. Similarly, the use of Fourier transform infrared spectroscopy (FT-IR) analysis to measure the amount of crystallinity within each sample would illustrate the rate of osteocalcin degradation compared to crystallinity (see Smith et al. 2005). FT-IR analysis could help establish the rate at which osteocalcin degrades when exposed to variables such as time depth, soil type, burial influences and underlying biological conditions. In addition to the further analysis of osteocalcin there is an opportunity to explore other similar proteins that reflect growth disruption during periods of stress such as bone-specific alkaline phosphatase (BSAP). Also produced by the osteoblast cells, BSAP is found in many biological tissues, including bone (van Straalen et al. 1991; Leung et al. 1993; Gomez et al. 1995; Christenson 1997; Rauch 2008). Similar to osteocalcin, it has been demonstrated that the quantitative measurement of BSAP can indicate specific bone diseases associated with bone loss or destruction such as Paget's and Cushing's disease (Gomez et al. 1995; Canalis and Delany 2002). More specifically, it has been clinically shown that decreases in BSAP reflect changes in the levels of circulating corticosteroids (van Straalen et al. 1991), similar to the glucocorticoids that are secreted during periods of prolonged stress. Recent archaeological studies have focused on the extraction of BSAP from ancient bone, demonstrating the longevity and viability of these biomarkers to explore paleopathological conditions in the past (Weser et al. 1995, Grupe and Turban-Just 1996; Weser et al. 1996; Schmidt-Schultz and Schultz 2004). In expanding the protocol for ancient protein extraction and establishing the diagenetic parameters of these particular proteins, there is an opportunity to guide stress research into the realm of biochemistry. This new methodology

arguably has the ability to clarify the visual and chemical expectations of stress from the onset of the biological response through to skeletal change, better informing how we explore health in the past and the types of questions that may be answered.

REFERENCES CITED

Aarden E, Burger E, Nijweide P. 1994. Function of osteocytes in bone. Journal of Cellular Biochemistry 55:287-299.

Abel W. 1976. Die wüstungen des ausgehenden mittelalters. Stuttgart: Quellen und Forschungen zur Agrargeschichte.

Abella Roth E. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):363.

Adamson M. 2004. Food in Medieval Times. Westport: Greenwood Press.

Agarwal S, Dumitriv M, Tomlinson C, Grynpas M. 2004. Medieval trabecular bone architecture: The influence of age, sex and lifestyle. American Journal of Physical Anthropology 124:33-44.

Aiello L, Wood B. 1994. Cranial variables as predictors of hominine body mass. American Journal of Physical Anthropology 95:409-426.

Ajie H, Hauschka P, Kaplan I, Sobel H. 1991. Comparison of bone-collagen and osteocalcin for determination of radiocarbon ages and paleodietary reconstruction. Earth and Planetary Sciences Letters 107:380-388.

Albala K. 2011. The ideology of fasting in the Reformation era. In: Albala K, Eden T, editors. Food and Faith in Christian Culture. New York: Columbia University Press, p 41-57.

Albert A, Greene D. 1999. Bilateral asymmetry in skeletal growth and maturation as an indicator of environmental stress. American Journal of Physical Anthropology 110:341-349.

Alfonso-Durruty M. 2011. Experimental assessment of nutrition and bone growth's velocity effects on Harris lines formation. American Journal of Physical Anthropology 145(2):169-180.

Alfonso M, Thompson J, Standen V. 2005. Re-evaluating Harris lines—a comparison between Harris lines and enamel hypoplasia. Collegium Antropologicum 29:393-408.

Aiello L, Wood B. 1994. Cranial variables as predictors of hominine body mass. American Journal of Physical Anthropology 95:409-426.

Amundsen D, Diers C. 1973. The age of menarche. Human Biology 45(3):363-369.

Anderson J. 2011. Daily Life during the Reformation. Santa Barbara: Greenwood Press.

Anderson D, Thompson G, Popovich F. 1976. Tooth, chin, bone and body size correlations. American Journal of Physical Anthropology 46:7-12.

Andrén A. 1989. State and towns in the Middle Ages: The Scandinavian experience. Theory and Society 18(5):585-609.

Angel J. 1966. Porotic hyperostosis, anemias, malarias, and marshes in the prehistoric eastern Mediterranean. Science 153(3737):360-363.

Armelagos G, VanGerven D. 2003. A century of skeletal biology and paleopathology: Contrasts, contradictions, and conflicts. American Anthropologist 105(1):53-64.

Armelagos G, Goodman A, Harper K, Blakey L. 2009. Enamel hypoplasia and early mortality: Bioarcheological support for the Barker Hypothesis. Evolutionary Anthropology 18:261-271.

Ashley B. 1990. The Dominicans. Collegeville: The Liturgical Press.

Aufderheide A. 2011. Soft tissue taphonomy: A paleopathology perspective. International Journal of Paleopathology 1:5-80.

Bamberger C, Schulte H, Chrousos G. 1996. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocrine Reviews 17(3):245-261.

Beaupré G, Orr T, Carter D. 1990. An approach for time-dependent bone modelling and remodelling. Theoretical development. Journal of Orthopaedic Research 8:651-661.

Becher E. 1999. Monastery of St. Peter in Odense. MA Thesis, University of Southern Denmark, Odense, Denmark.

Behrensmeyer A. 1978. Taphonomic and ecological information from bone weathering. Paleobiology 4:150-162.

Bell N, Epstein S, Greene A, Shary J, Oexmann J, Shaw S. 1985. Evidence for alteration of the vitamin D-endocrine system in obese subjects. Journal of Clinical Investigation 76:370-373.

Bell N, Godsen R, Henry D, Shary J, Epstein S. 1988. The effects of muscle-building exercise on vitamin D and mineral metabolism. Journal of Bone and Mineral Research 3:369-373.

Benedictow O. 1993. The Medieval Demographic System of the Nordic Countries. Oslo: Middelalderforlaget.

Benedictow O. 2003. Demographic conditions. In: Knut H, editor. The Cambridge History of Scandinavia Volume 1: Prehistory to 1520. Cambridge: Cambridge University Press, p 237-249.

Benedictow O. 2004. The Black Death 1346-1353: The Complete History. Rochester: Boydell Press.

Bennett R. 1937. The Early Dominicans: Studies in Thirteenth-Century Dominican History. New York: Russell and Russell.

Bennike P. 1985. Paleopathology of Danish Skeletons: A Comparative Study of Demography, Disease and Injury. Copenhaggen: Akademisk Forlag.

Bennike P, Lewis M, Schutkowski H, Valentin F. 2005. Comparison of child morbidity in two contrasting medieval cemeteries from Denmark. American Journal of Physical Anthropology 128:734-746.

Blair H, Kahn A, Crouch E, Jeffrey J, Teitelbaum S. 1986. Isolated osteoclasts resorb the organic and inorganic components of bone. Journal of Cell Biology 102:1164-1172.

Blanco R, Acheson R, Canosa C, Salomon J. 1974. Height, weight and lines of arrested growth in young Guatemalan children. American Journal of Physical Anthropology 40:39-48.

Bogin B. 1999. Patterns of Human Growth, Second Edition. Cambridge Studies in Biological Anthropology 3. Cambridge: Cambridge University Press.

Bogin B. 2001. The Growth of Humanity. New York: Wiely-Liss, Inc.

Boldsen J. 1984. Paleodemography of two southern Scandinavian Medieval communities. The Löddekkópinge Investigation. Papers of the Archaeological Institute, University of Lund 8:108.

Boldsen J. 1998a. Pathogenesis of dental abscesses in a medieval village community. Bullentins et Mémories de la Société d'anthropologie de Paris 10(3-4):345-356.

Boldsen J. 1998b. Body proportions in a medieval village population: Effects of early childhood episodes of ill health. Annals of Human Biology 24(4):309-317.

Boldsen J. 2005. Leprosy and mortality in the medieval Danish village of Tirup. American Journal of Physical Anthropology 126:159-168.

Boldsen J. 2007. Early childhood stress and adult age mortality – a study of dental enamel hypoplasia in the medieval Danish village of Tirup. American Journal of Physical Anthropology 132(1):59-66.

Boldsen J. 2008. Leprosy in medieval Denmark: Osteological and epidemiological analyses. Anthropologischer Anzeiger 67(4):407-425.

Boldsen J, Mollerup L. 2006. Outside St. Jørgen: Leprosy in the medieval Danish city of Odense. American Journal of Physical Anthropology. 130:344-351.

Boldsen J, Milner G, Konigsberg L, Wood J. 2002. Transition analysis: A new method for estimating age from skeletons. In: Hoppa R, Vaupel J, editors. Paleodemography: Age Distributions from Skeletal Samples. Cambridge: Cambridge University Press, p 73-106.

Boldsen J, Milner G, Weise S. 2015. Cranial vault trauma and selective mortality in medieval to early modern Denmark. Proceedings of the National Academy of Sciences of the United States of America 112(6):1721-1726.

Boldsen J, Søgaard J. 1997. A history of height in Denmark. In: Komlos J, Baten J, editors. The Biological Standard of Living in Comparative Perspective. Stuttgart: Franz Steiner Verlag, p 467-482.

Boucher B. 1957. Sex differences in the fetal pelvis. American Journal of Physical Anthropology 15:591-600.

Brainerd Arey L. 1965. The skeletal system. In: Brainerd Arey L, editor. Development Anatomy: A Textbook and Laboratory Manual of Embryology, Seventh Edition. Philadelphia: W.B. Saunders, p 396-425.

Brenner H, Kliebsch U. 1996. Dependence of weighted kappa coefficients on the number of categories. Epidemiology 7(2):199-202.

Brickley M, Howell P. 1999. Measurement of changes in trabecular bone structure with age in an archaeological population. Journal of Archaeological Science 26:151-157.

Bronner-Fraser M. 1994. Neural crest cell formation and migration in the developing embryo. The Journal of the Federation of American Societies for Experimental Biology 8:699-706.

Brooks S, Suchey J. 1990. Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. Human Evolution 5:227-238.

Brown T, Brown K. 2011. Biomolecular Archaeology: An Introduction. West Sussex: Wiley-Blackwell.

Bruzek J. 2002. A method for visual determination of sex, using the human hip bone. American Journal of Physical Anthropology 117:157-168.

Buchanan R. 1975. Effects of childbearing on maternal health. Journal of Family Planning Programs 8:125-1439.

Buckwalter J, Glimcher M, Cooper R, Recker R. 1995. Bone biology. The Journal of Bone and Joint Surgery 77A(8):1276-1289.

Buikstra J. 1977. Biocultural dimensions of archaeological study: A regional perspective. In: Blakey R, editor. Biocultural Adaptation in Prehistoric America. Athens: University of Georgia Press, p 67-84.

Buikstra J, Ubelaker D. 1994. Standards for Data Collection from Human Skeletal Remains. Arkansas: Arkansas Archeological Survey Research Series.

Caffey J. 1937. The skeletal changes in the chronic hemolytic anemias. American Journal of Roentgenology Radium Therapy and Nuclear Medicine 37:293-324.

Calvo M, Eyre D, Gundberg C. 1996. Molecular basis and clinical applications of biological markers of bone turnover. Endocrine Reviews 17(4):333-368.

Canalis E, Delany A. 2002. Mechanisms of glucocorticoid action in bone. Annals New York Academy of Sciences 966:73-81.

Cannon W. 1914. Emergency function of adrenal medulla in pain and major emotions. American Journal of Physiology 3:356-372.

Cardoso H. 2007. Environmental effects on skeletal versus dental development: Using a documented subadult skeletal sample to test a basic assumption in human osteological research. American Journal of Physical Anthropology 132:223-233.

Carter D, Beaupré G. 2001. Skeletal Function and Form: Mechanobiology and Skeletal Development, Aging and Regeneration. Cambridge: Cambridge University Press.

Cattaneo C, Gelsthorpe K, Philipps P, Waldron T, Booth J, Sokol R. 1994. Immunological diagnosis of multiple myeloma in a medieval bone. International Journal of Osteoarchaeology 4: 1-2.

Chamberlain A. 2000. Problems and prospects in palaeodemography. In: Cox M, Mays S, editors. Human Osteology in Archaeology and Forensic Science. London: Greenwich Medical Media, p 101-115.

Chambers T, Fuller K. 1985. Bone cells predispose surfaces to resorption by exposure of mineral to osteoclastic attack. Journal of Cell Science 76:155-165.

Charmandari E, Kino T, Chrousos G. 2004. Glucocorticoids and their actions: An introduction. Annals New York Academy of Sciences 1024:1-8.

Charmandari E, Tsigos C, Chrousos G. 2005. Endocrinology of the stress response. Annual Review of Physiology 67:259-284.

Chen L, Jacquet R, Lowder E, Landis W. 2015. Refinement of collagen-mineral interaction: A possible role for osteocalcin in apatite crystal nucleation, growth and development. Bone 71:7-16.

Christensen A. 1938. ‘Danmark’s Befolkning i Middelalderen’ in Schück 1938, 1ff.

Christensen A. 1988. Middelalderbyen Odense. Denmark: Centrum.

Christenson R. 1997. Biochemical markers of bone metabolism: An overview. Clinical Biochemistry 30(8):573-593.

Chrousos G. 2009. Stress and disorders of the stress system. Nature 5:374-381.

Chrousos G, Gold P. 1992. The concepts of stress and stress system disorders: Overview of physical and behavioural homeostasis. Journal of the American Medical Association 267(9):1244-1252.

Clark G. 1981 The paleopathology of tibial Harris lines and growth velocity in Dickson Mounds infant populations. American Journal of Physical Anthropology 54:209.

Clark G. 1988. New method for assessing changes in growth and sexual dimorphism in paleoepidemiology. American Journal of Physical Anthropology 77:105-116.

Clark G, Hall N, Armelagos G, Borkan G, Panjabi M, Wetzel T. 1986. Poor growth prior to early childhood: Decreased health and life span in the adult. American Journal of Physical Anthropology 70:145-160.

Clark J. 1972. Starr Carr: A Case Study in Bioarchaeology. Reading: Addison-Wesley.

Clarke S, Gindhart P. 1981. Commonality in peak age of early-childhood morbidity across cultures and over time. Current Anthropology. 22:574-575.

Cleland T, Voegele K, Schweitzer M. 2012. Empirical Evaluation of Bone Extraction Protocols. PLos ONE 7(2):1-9.

Cockburn A. 1963. The Evolution and Eradication of Infectious Diseases. Baltimore: Johns Hopkins Press.

Cohen J. 1960. A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20:37-46.

Cohen J. 1968. Weighted kappa: Nominal scale agreement with provision for scaled disagreement or partial credit. Physiological Bulletin 70(4):213-220.

Cohen M. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):358-359.

Cohen M. 1994. The osteological paradox reconsidered. Current Anthropology 35(5):629-637.

Cohen M, Tickle C. 1996. Limbs: A model for pattern formation within the vertebrate body plan. Trends in Genetics 12:253–57.

Cohn S. 2002. The Black Death Transformed: Disease and Culture in Early Renaissance Europe. London: Arnold.

Cole D, Gundberg C, Stirk L, Atkinson S, Hanley D, Ayer L, Baldwin L. 1987. Changing osteocalcin concentrations during pregnancy and lactation: Implications for maternal mineral metabolism. Journal of Clinical Endocrinology and Metabolism 65: 290-294.

Collins M, Child A, van Duin A, Vermeer C. 1998. Ancient osteocalcin: The most stable bone protein? Ancient Biomolecules 2:223-238.

Collins M, Gernaeu A, Nielsen-Marsh C, Vermeer C, Westbroek P. 2000. Slow rates of degradation of osteocalcin: Green light for fossil bone protein? Geology 28(12):1139-1142.

Collins M, Nielsen-Marsh C, Hiller J, Smith C, Roberts J, Prigodich R, Wess T, Csapo J, Millard A, Turner-Walker G. 2002. The survival of organic matter in bone: A review. Archaeometry 44(3):383-394.

Cook D. 1979. Subsistence base and health in prehistoric Illinois Valley: Evidence from the human skeleton. Medical Anthropology 4:109-124.

Cook D, Buikstra J. 1979. Health and differential survival in prehistoric populations: Prenatal dental defects. American Journal of Physical Anthropology 51:649-664.

Cooper M. 2004. Sensitivity of bone to glucocorticoids. Clinical Science 107:111-123.

Cooper M, Hewison M, Stewart P. 1999. Glucocorticoid activity, inactivity and the osteoblast. Journal of Endocrinology 163:159-164.

Cox M, Mays S. 2000. Human Osteology in Archaeology and Forensic Science. London: Greenwich Medical Media.

Cummings S, Kelsy J, Nevitt M, O'Dawd K. 1985. Epidemiology of osteoporosis and osteoporotic fractures. Epidemiological Reviews 17:178-208.

Cushing H. 1932. The basophil adenomas of the pituitary body and their clinical manifestations: Pituitary basophilism. Bulletin of the Johns Hopkins Hospital 50(4):137-195.

Cutress T, Suckling G. 1982. The assessment of noncarious defects of enamel. International Dental Journal 32:117-122.

Dahlbäck G. 2003. The towns. In Knut H, editor. The Cambridge History of Scandinavia Volume 1: Prehistory to 1520. Cambridge: Cambridge University Press, p 611-634.

Danforth M, Herndon K, Propst K. 1993. A preliminary study of patterns of replication in scoring linear enamel hypoplasias. International Journal of Osteoarchaeology 3:297-302.

DeLeon V. 2007. Fluctuating asymmetry and stress in a medieval Nubian population. American Journal of Physical Anthropology 132:520-534.

Delmas P. 1995. Biochemical markers of bone turnover. Acta Orthopaedica Scandinavica 66:176-182.

Demirjian A, Goldstein H, Tanner J. 1973. A new system of dental age assessment. Human Biology 45(2):211-227.

Demirjian A, Goldstein H. 1976. New systems for dental maturity based on seven and four teeth. Annals of Human Biology 3:411-427.

Dennison E, Hindmarsh P, Fall C, Kellingray S, Barker D, Phillips D, Cooper C. 1999. Profiles of endogenous circulating cortisol and bone mineral density in healthy elderly men. Journal of Clinical Endocrinology and Metabolism 84(9):3058-3063.

Derry T. 1979. A History of Scandinavia: Norway, Sweden, Denmark, Finland and Iceland. Minneapolis: University of Minnesota Press.

DeSilva J. 1999. Sex hormones and glucocorticoids: Interactions with the immune system. Annals of New York Academy of Sciences 876:102-117.DeWitte S. 2010. Sex differential in frailty in medieval England. American Journal of Physical Anthropology 143(2):285-297.

DeWitte S. 2010. Sex differentials in frailty in medieval England. American Journal of Physical Anthropology 143:285-297.

DeWitte S, Bekvalac J. 2011. The association between periodontal disease and periosteal lesions in the St. Mary Graces cemetery, London, England A.D. 1350-1538. American Journal of Physical Anthropology 146:609-618.

DeWitte S, Wood J. 2008. Selectivity of Black Death mortality with respect to pre-existing health. Proceedings of the National Academy of Sciences 105(5):1436-1441.

Dienstbier R. 1989. Arousal and physiological toughness: Implications for mental and physical health. Psychological Review 96:84-100.

Djurić-Srejić M, Roberts C. 2001. Paleopathological evidence of infectious disease in skeletal populations from later medieval Serbia. International Journal of Osteoarchaeology 11:311-320.

Donoghue H, Marcsik A, Matheson C, Vernon K, Nuorala E, Molto J, Greenblatt C, Spigelman M. 2005. Co-infection of Mycobacterium tuberculosis and Mycobacterium leprae in human archaeological samples: A possible explanation for the historical decline of leprosy. Proceedings of the Royal Society B 272:389-394.

Drancourt M, Raoult D. 2002. Molecular insights into the history of the plague. Microbes and Infection 4:105-109.

Dreizen S, Spirakis C, Stone R. 1964. The influence of age and nutritional status on 'bone scar' formation in the distal end of the growing radius. American Journal of Physical Anthropology 22:295-306.

Ducy P, Schinke T, Karsenty G. 2000. The osteoblast: A sophisticated fibroblast under central surveillance. Science 289:1501-1504.

Dyer C. 1989. Standards of Living in the Later Middle Ages: Social Change in England C.1200-1520. Cambridge: Cambridge University Press.

Eberhardt A, Yeager-Jones A, Blair H. 2001. Regional trabecular bone matrix degeneration and osteocyte death in femora of glucocorticoid-treatment rabbits. Endocrinology 142(3):1333-1340.

Eisenberg L. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):359-360.

Elenkov I, Webster E, Torpy D, Chrousos G. 1999. Stress, corticotropin-releasing hormone, glucocorticoids, and the immune/inflammatory response: Acute and chronic effects. Annals New York Academy of Sciences 22(876):1-11.

El-Najjar M, Desanti M, Ozebek L.1978. Prevalence and possible etiology of dental enamel hypoplasia. American Journal of Physical Anthropology 48:185-192.

El-Nofely A, İşcan M.1989. Assessment of age from the dentition in children. In: İşcan M, editor. Age Markers in the Human Skeleton. Springfield: Charles C. Thomas Publishing Ltd., p 237-253.

Elster H. 1991. Age determination of fossil bone by amino acid racemization. PhD Thesis, Weizmann Institute of Science, Rehovot, Israel.

Erslev K. 1889. Erik Plovpennings Strid med Abel. Historisk Tidsskrift 6(2):359-442.

Eveleth P. 2008. Timing of menarche: Secular trends and population differences. In: Lancaster J, Hamberg B, editors. School-age Pregnancy and Parenthood: Biosocial Dimensions. Piscataway: Transaction Publishers, p 39-52.

Eveleth P, Tanner J. 1990. Worldwide Variation in Human Growth, Second Edition. Cambridge: Cambridge University Press.

Fagan B. 2000. The Little Ice Age: How Climate Made History 1300-1850. New York: Basic Books.

Fan J. 2006. Schmorl's nodes: Implications for occupational stress and gendered division of labor in a late medieval population from York, England. Paper presented at the 33rd Annual North America Meeting of the Paleopathology Association, 7–8 March, Anchorage, Alaska.

FitzGerald C, Saunders S, Bondioli L, Macchiarelli R. 2006. Health of infants in an imperial Roman skeletal sample: Perspective from dental microstructure. American Journal of Physical Anthropology 130:179-189.

Fleiss J, Cohen J. 1973. The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. Educational and Psychological Measurement 33:613-619.

Follis R, Park E. 1952. Some observations on bone growth, with particular respect to zones and transverse lines of increased density in the metaphysis. The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine 68:709-724.

Fonseca V, D'souza V, Houlder S, Thomas M, Wakeling A, Dandona P. 1987. Vitamin D deficiency and low osteocalcin concentrations in anorexia nervosa. Journal of Clinical Pathology 41:195-197.

Frisancho A. 1979. Human Adaptation. St. Louis: C.V. Mosby Co.
Gallagher S. 2005. Physical limitations and musculoskeletal complaints associated with work in unusual or restricted postures: A literature review. Journal of Safety Research 36(1):51-62.

Garn S, Lewis A, Kerewsky R. 1965. Genetic, nutritional and maturational correlates of dental development. Journal of Dental Research 44:228-242.

Garn S, Silverman F, Hertzog K, Rohmann C.1968. Lines and bands of increased density. Their implications to growth and development. Medical Radiography and Photography 44(3):58-89.

Genovés S. 1959. L'estimation des différences sexuelles dans l'os coxal; différences métriques et différences morphologiques. Bulletins et mémoires de la Société d'Anthropologie de Paris 10:3-95.

Gilchrist R. 2013. Medieval Life: Archaeology and the Life Course. Woodbridge: The Boydell Press.

Gill-King H. 1996. Chemical and ultrastructural aspects of decomposition. In: Haglund W, Sorg M, editors. Forensic Taphonomy: The Postmortem Fate of Human Remains. Boca Raton: CRC Press, p 93-108.

Gissel S. 1981. The late medieval agrarian crisis in Denmark. In: Skyum-Nielsen N, Lund N, editors. Danish Medieval History, New Currents. Copenhagen: Museum Tusculanum Press, p 238-250.

Giustina A, Mazziotti G, Canalis E. 2008. Growth hormone, insulin-like growth factors, and the skeleton. Endocrine Reviews 29(5):535-559.

Gleiser I, Hunt Jr. E. 1955. The permanent mandibular first molar: Its calcification, eruption and decay. American Journal of Physical Anthropology 13:253-284.

Gomez B, Ardakani S, Ju J, Jenkins D, Cerelli M, Daniloff G, Kung V. 1995. Monoclonal antibody assay for measuring bone-specific alkaline phosphatase activity in serum. Clinical Chemistry 41(11):1560-1566.

Goodman A. 1993. On the interpretation of health from skeletal remains. Current Anthropology 34(3):281-288.

Goodman A, Armelagos G. 1985. Chronological distribution of enamel hypoplasia in human permanent incisor and canine teeth. Archives of Oral Biology 30(6):503-507.

Goodman A, Armelagos G. 1988. Childhood stress and decreased longevity in a prehistoric population. American Anthropologist 90:936-944.

Goodman A, Armelagos G. 1989. Infant and childhood morbidity and mortality risks in archaeological populations. World Archaeology 21(2):225-243.

Goodman A, Rose J. 1990. Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures. Yearbook of Physical Anthropology 33:59-110.

Goodman A, Song R. 1999. Sources of variation in estimated ages at formation of linear enamel hypoplasias. In: Hoppa R, FitzGerald C, editors. Human Growth in the Past: Studies from Bones and Teeth. Cambridge: Cambridge University Press, p 210-240.

Goodman A, Armelagos G, Rose J. 1980. Enamel hypoplasias as indicators of stress in 3 prehistoric populations from Illinois. Human Biology 52(3):515-528.

Goodman A, Brooke Thomas R, Swedlund A, Armelagos G. 1988. Biocultural perspectives on stress in prehistoric, historical, and contemporary population research. Yearbook of Physical Anthropology 31:169-202.

Goodman A, Martin D. 2002. Reconstructing health profiles from skeletal remains. In: Steckel R, Rose J, editors. The Backbone of History: Health and Nutrition in the Western Hemisphere. Cambridge: Cambridge University Press, p 11-60.

Goran M, Carpenter W, Poehlman E. 1993. Total energy expenditure for children between 4-6 years. American Journal of Physiology 264(5)706-711.

Gordon C, Buikstra J. 1981. Archaeology soil pH, bone preservation, and sampling bias at mortuary sites. American Antiquity 46(3):566-571.

Grauer A. 1993. Patterns of anemia and infection from medieval York, England. American Journal of Physical Anthropology 91(2):203-213.

Grauer A, Roberts C. 1996. Paleoepidemiology, healing and possible treatment of trauma in the medieval cemetery population of St. Helen-on-the-walls, York, England. American Journal of Physical Anthropology 100:531-544.

Grine F. 2005. Enamel thickness of deciduous and permanent molars in modern Homo sapiens. American Journal of Physical Anthropology 165:14-31.

Grupe G, Turban-Just S. 1996. Serum proteins in archaeological human bone. International Journal of Osteoarchaeology 6:300-308.

Gundberg C, Looker A, Nieman S, Calvo M. 2002. Patterns of osteocalcin and bone specific alkaline phosphatase by age, gender, and race or ethnicity. Bone 31(6):703-708.

Gunnar M, Vazquez D. 2001. Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. Development and Psychopathology 13:515-538.

Habib K, Gold P, Chrousos G. 2001. Neuroendocrinology of stress. Neuroendocrinology 30(3):695-728.

Hall B, Miyake T. 2000. All for one and one for all: Condensations and the initiation of skeletal development. Bioessays 22:138-147.

Hallberg L. 2001. Perspectives on nutritional iron deficiency. Annual Review of Nutrition 21:1-21.

Hanawalt B. 1998. 'Of Good and Ill Repute': Gender and Social Control in Medieval England. Oxford: Oxford University Press.

Hanawalt B. 2002. Medievalists and the study of children. Speculum 77:440-460.

Harper K, Zuckerman M, Harper M, Kingston J, Armelagos G. 2011. The origin and antiquity of Old World pre-Columbian evidence for treponemal infection. Yearbook of Physical Anthropology 54:99-133.

Hassett B. 2014. Missing defects? A comparison of microscopic and macroscopic approaches to identifying linear enamel hypoplasia. American Journal of Physical Anthropology 153:463-472.

Hauschka P, Lian J, Cole D, Gundberg C. 1989. Osteocalcin and matrix Gla protein: Vitamin K-dependent proteins in bone. Physiological Reviews 69(3):990-1047.

Hedges R, Millard A, Pike A. 1995. Measurements and relationships of diagenetic alteration of bone from three archaeological sites. Journal of Archaeological Science 22:201-209.

Henneicke H, Herrmann M, Kalak R, Brennan-Speranza T, Heinevetter U, Bertollo N, Day R, Huscher D, Buttgereit F, Dunstan C, Seibel M, Zhou H. 2011. Corticosterone selectively targets endo-cortical surfaces by an osteoblast-dependent mechanism. Bone 49:733-742.

Hens S, Rastelli E, Belcastro G. 2008. Age estimation from the human os coxa: A test on a documented Italian collection. Journal of Forensic Science 53(5):1040-1043.

Herman J, Cullinan W. 1997. Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. Trends in Neuroscience (20):78-84.

Himes J. 1978. Bone growth and development in protein-calorie malnutrition. World Review of Nutrition and Dietetics 28:13-187.

Hinnebusch W. 1973. The History of the Dominican Order: Intellectual and Cultural Life to 1500, Volume 2. New York: Alba House.

Hoffman R. 2001. Frontier foods for late medieval consumers. Culture, economy, ecology. Environment and History 7:131-167.

Högberg U, Iregren E, Siven CH, Diener L. 1987. Maternal deaths in medieval Sweden: An osteological and life table analysis. Journal of Biosocial Science 19:495-503.

Holden L. 1882. Human Osteology, Sixth Edition. London: Churchill.

Holland A. 1995. Estimation of stature from the adult calcaneus and talus. American Journal of Physical Anthropology 96:315-320.

Holt P, Jones C. 2000. Development of the immune system during pregnancy and early life. Allergy Review Series VI: The Immunology of Fetuses and Infants 55:688-697.

Hoppa R, FitzGerald C (editors). 1999. Human Growth in the Past: Studies from Bones and Teeth. Cambridge: Cambridge University Press.

Hughes C, Heylings D, Power C. 1996. Transverse (Harris) lines in Irish archaeological remains. American Journal of Physical Anthropology 101:115-131.

Hummert J, Van Gerven D. 1983. Skeletal growth in a medieval population from Sudanese, Nubia. American Journal of Physical Anthropology 60:471-478.

Humphrey L. 1998. Growth patterns in the modern human skeleton. American Journal of Physical Anthropology 105:57-72.

Humphrey L. 2003. Linear growth variation in the archaeological record. In: Thompson J, Krovitz G, Nelson A, editors. Patterns of Growth and Development in the Genus Homo. Cambridge: Cambridge University Press, p 144-169.

Hunt Jr. E, Gleiser I. 1955. The estimation of age and sex of preadolescent children from bones and teeth. American Journal of Physical Anthropology 13:479-487.

Hunziker E. 1994. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. Microscopy Research and Technique 28:505-519.

Huq N, Tseng A, Chapman G. 1990. Partial amino acid sequence of osteocalcin from an extinct species of ratite bird. Biochemistry International 21:491-496.

Huss-Ashmore R, Goodman A, Armelagos G. 1982. Nutritional inference from paleopathology. Advances in Archaeological Method and Theory 5:395-474.

Hutchinson D. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):360.

Hybel N, Poulsen B. 2007. The Danish Resources c.1000 – 1550: Growth and Recession. Leiden: Brill.

Ingram R, Park Y, Clarke B, Fitzpatrick L. 1994. Age- and gender-related changes in the distribution of osteocalcin in the extracellular matrix of normal male and female bone. Journal of Clinical Investigation 93:989-997.

Isaksson O. 1982. Growth hormone stimulates longitudinal bone growth directly. Science 26:1237-1239.

Jackes M. 2011. Representativeness and bias in archaeological skeletal samples. In: Agarwal S, Glencross B, editors. Social Bioarchaeology. Chichester: Blackwell Publishers Ltd., p 107-146.

Jacobi K, Danforth M. 2002. Analysis of interobserver scoring patterns in porotic hyperostosis and cribra orbitalia. International Journal of Osteoarchaeology 12:248-258.

Jacobsen G. 1983. Women's work and women's role: Ideology and reality in Danish urban society. The Scandinavian Economic History Review and Economy and History 31(1):3-20.

Jåger H. 1981. The International background- late medieval agrarian crisis and deserted settlements in Central Europe. In: Skyum-Nielsen N, Lund N, editors. Danish Medieval History, New Currents. Copenhagen: Museum Tusculanum Press, p 223-237.

Jakobsen J. 2008. The Role of Friars Preachers in Medieval Danish Society. PhD Thesis, University of Southern Denmark, Odense, Denmark.

Jankauskas R, Česnys G. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):360-361.

Jantz L, Jantz R. 1999. Secular change in long bone length and proportion in the United States 1800-1970. American Journal of Physical Anthropology 110:57-67.

Jantz R, Owsley D. 1984. Long bone growth variation among Arikara skeletal populations. American Journal of Physical Anthropology 63:13-20.

Jantzen C, Kieffer-Olsen J, Madsen P. 1994. De små brødres hus I Ribe. Ribe: Mark og Montre, p 26-37.

Johnston F. 1962. Growth of the long bones of the infants and young children at Indian Knoll. American Journal of Physical Anthropology 20:249-254.

Johnston F. 1969. Approaches to the study of developmental variability in human skeletal populations. American Journal of Physical Anthropology 31:335-342.

Johnston F, Zimmer L. 1989. Assessment of growth and age in the immature skeleton. In: İşcan M, Kennedy K, editors. Reconstruction of Life from the Skeleton. New York: Wiley-Liss, p 11-21.

Jones A, Godfrey K, Wood P, Osmond C, Goulden P, Phillips D. 2006. Fetal growth and andrenocortical response to psychological stress. The Journal of Clinical Endocrinology and Metabolism 91(5):1866-1871.

Jordan W. 1996. The Great Famine: Northern Europe in the Early Fourteenth Century. Princeton: Princeton University Press.

Judd M, Roberts C. 1998. Fracture patterns at the medieval leper hospital in Chichester. American Journal of Physical Anthropology 105:43-55.

Katzenberg A. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):361.

Kaufmann J, Kaufmann H. 2004. The Medieval Fortress: Castles, Forts and Walled Cities of the Middle Ages. Cambridge: De Capo Press.

Kieffer-Olsen J. 1993. Grav og gravskik I det middelalderlige Danmark. Afdelingen for Middelalderarkæologi og Middelalderarkæologisk Nyhedsbrev.

Kemeny M. 2003. The psychobiology of stress. Current Directions in Psychological Science 12:124-129.

Khan A, Sanchez S, Pflieger A. 1998. Flioviruses haemorrhagic fevers. British Mediecal Bulletin 54:675-692.

King T, Humphrey L, Hillson S. 2005. Linear enamel hypoplasias as indicators of systemic physiological stress: Evidence from two known age-at-death and sex populations from postmedieval London. American Journal of Physical Anthropology 128(3):547-559.

King A, Ulijaszek S. 1999. Invisible insults during growth and development: Contemporary theories and past populations. In: Hoppa R, FitzGerald C, editors. Human Growth in the Past: Studies from Bones and Teeth. Cambridge: Cambridge University Press, p 161-182.

Kirschbaum C, Wust S, Hellhammer D. 1992. Consistent sex differences in cortisol responses to psychological stress. Psychosomatic Medicine S4:648-657.

Klaus H, Tam M. 2009. Contact in the Andes: Bioarchaeology of systemic stress in colonial Mórrope, Peru. American Journal of Physical Anthropology 138:356-368.

Klein S. 2000. The effects of hormones on sex differences in infection from genes to behavior. Neuroscience and Biobehavioral Review 24:627-638.

Kronenberg H. 2003. Developmental regulation of the growth plate. Nature 423:332-336.

Kular J, Tickner J, Chim S, Xu J. 2012. An overview of the regulation of bone remodelling at the cellular level. Clinical Biochemistry 45:863-873.

LaCourte R, Trotta F, Adami S. 2010. Glucocorticoid receptors and bone. Current Pharmaceutical Design 16(32):3586-3592.

Landers J. 1993. Death and the Metropolis: Studies in the Demographic History of London 1670–1830. Cambridge: Cambridge University Press.

Landis R, Koch G. 1977. The measurement of observer agreement from categorical data. Biometrics 33(1):159-174.

Larsen C. 1997. Bioarchaeology: Interpreting Behaviour from the Human Skeleton. Cambridge: Cambridge University Press.

Lauring P. 1976. A History of the Kingdom of Denmark. Copenhagen: Høst and Søn.

Lefort M, Bennike P. 2007. A case study of possible differential diagnoses of a medieval skeleton from Denmark: Leprosy, ergotism, treponematosis, sarcoidosis or smallpox? International Journal of Osteoarchaeology 17:337-349.

Leonard M, Zemel B. 2004. Assessment of bone mineralization in children and adolescents. Clinical Reviews in Bone and Mineral Metabolism 2(1):3-18.

Leung K, Fung K, Sher A, Li C, Lee K. 1993. Plasma bone-specific alkaline phosphatase as an indicator of osteoblastic activity. Journal of Bone Joint Surgery 75-B:288-292.

Leung B, Forbes M, Houle D. 2000. Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits. The American Naturalist 155(1):101-115.

Lewis M. 2000. Non-adult pathology: Current stature and future potential. In: Cox M, Mays S, editors. Archaeology and Forensic Science. London: Greenwich Medical Media, p 39-58.

Lewis M. 2002. The impact of industrialisation: Comparative study of child health in four sites from medieval and post-medieval England (850–1859 AD). American Journal of Physical Anthropology 119:211-223.

Lewis M. 2007. The Bioarchaeology of Children: Perspectives from Biological and Forensic Anthropology. Cambridge: Cambridge University Press.

Lewis, A. and S. Garn (1960) The relationship between tooth formation and other maturational factors. Angle Orthodontist 30:70-77.

Lewis M, Roberts C. 1997. Growing pains: The interpretation of stress indicators. International Journal of Osteoarchaeology 7(6):581-586.

Lietman T, Porco T, Blower S. 1997. Leprosy and tuberculosis: The epidemiological consequences of cross-immunity. American Journal of Public Health 87(12):1923-1927.

Livi-Bacci M. 1991. Population and Nutrition: An Essay on European Demographic History. Cambridge: Cambridge University Press.

Lockhart P. 2004. Frederik II and the Protestant Cause: Denmark's Role in the Wars of Religion, 1559-1596. Leiden: Brill.

Lovejoy C, Meindl R, Pryzbeck T, Mensforth R. 1985. Chronological metamorphosis of the auricular surface of the ilium: A new method for the determination of adult age at death. American Journal of Physical Anthropology 68:15-28.

Lukacs J. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):361-362.

Maat G. 1984. Dating and rating of Harris lines. American Journal of Physical Anthropology. 63:291-299.

Macchiarelli R, Bondioli L, Censi L, Kristoff Hernaez M, Salvadei L, Sperduti A. 1994. Intra- and interobserver concordance in scoring Harris lines: A test on bone sections and radiographs. American Journal of Physical Anthropology 95:77-83.

Magnusson P, Hager A, Larsson L. 1995. Serum osteocalcin and bone and liver alkaline phosphatase isoforms in healthy children and adolescents. Pediatric Research 38(6):955-961.

Mahoney P. 2012. Incremental enamel development in modern human deciduous anterior teeth. American Journal of Physical Anthropology 147:637-651.

Manchester K. 1984. Tuberculosis and leprosy in antiquity: An interpretation. Medical History 28:162-173.

Manelli F, Giustina A. 2000. Glucocorticoid-induced osteoporosis. Trends in Endocrinology Metabolism 11(3):79-85.

Manolagas S. 2000. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine Reviews 21(2):115-137.

Maresh M. 1943. Growth of major long bones in healthy children. American Journal of Diseases of Children 66(3):227-257.

Maresh M. 1955. Linear growth of long bones of extremities from infancy through adolescence. American Journal of Diseases of Children 89(6):725-742.

Maresh M. 1970. Measurements from roentgenograms. In: McCannon R, editor. Human Growth and Development. Springfield: Charles C. Thomas, p 157-188.

Marieb E, Hoehn K. 2013. Human Anatomy and Physiology, Ninth Edition. Boston: Pearson.

Marshall W. 1968. Problems in relating the presence of transverse lines in the radius to the occurrence of disease. In: Brothwell D, editor. Skeletal Biology of Earlier Human Populations. London: Academic Press, p 245–261.

Marshall L. 1989. Bone modification and "the laws of burial." In: Bonnichsen R, Sorg M, editors. Bone Modification. Orono: Center for the Study of the First Americans, p 7-24.

Martin D, Armelagos G. 1979. Morphometrics of compact bone: An example from Sudanese Nubia. American Journal of Physical Anthropology 51:571-578.

Martin D, Armelagos G. 1985. Skeletal remodeling and mineralization as indicators of health: An example from prehistoric Sudanese Nubia. Journal of Human Evolution 14:527-537.

Martinez M, De Pedro C, Catalan P, Salinas M, Balaguer G, Ordas J. 1985. Levels of osteocalcin in normal pregnancy. American Journal of Obstetrics and Gynecology 153:708-709.

Mason J. 1968a. A review of psychoendocrine research on the pituitary-adrenal cortical system. Psychosomatic Medicine 30:576-629.

Mason J. 1968b. A review of psychoendocrine research on the sympathetic-adrenal medullary system. Psychosomatic Medicine 30:631-653.

Mason J. 1971. A re-evaluation of the concept of "non-specificity" in stress theory. Journal of Psychosomatic Research 8:323-334.

Mason J, Maher J, Hartley L, Mougey E, Perlow M, Jones L. 1976. Selectivity of corticosteroid and catecholamine response to various natural stimuli. In: Serban G, editor. Psychopathology of Human Adaptation. New York: Plenum, p 147-171.

Mays S. 1985. The relationship between Harris line formation and bone growth and development. Journal of Archaeological Science 12:207-220.

Mays S. 1995. The relationship between Harris lines and other aspects of skeletal development in adults and juveniles. Journal of Archaeological Science 22:511-520.

Mays S. 2007. The human remains. In: Mays S, Harding C, Heighway C, editors. Wharram: A Study of Settlement on Yorkshire Wolds, Vol. 11, The Churchyard. York: York University Archaeology Publications 13, p 77-192.

Mays S, Brickley M, Ives R. 2008. Growth in an English population from the Industrial Revolution. American Journal of Physical Anthropology 136:85-92.

McCance R. 1960. Severe undernutrition in growing and adult animals. l. Production and general effects. British Journal of Nutrition 14:59-73.

McCance R, Dickerson J, Bell G, Dunbar O. 1962. Severe undernutrition in growing and adult animals. 9. The effect of undernutrition and its relief on the mechanical properties of bone. British Journal of Nutrition 16:1-12.

McCance R, Ford E, Brown W. 1961. Severe undernutrition in growing and adult animals. 7. Development of the skull, jaws, and teeth in pigs. British Journal of Nutrition 15:213-224.

McEwan J, Mays S, Blake G. 2005. The relationship of bone mineral density and other growth parameters to stress indicators in a medieval juvenile population. International Journal of Osteoarchaeology 15:155-163.

McGrath J. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):362-363.

McHenry H. 1992. Body size and proportions in early hominids. American Journal of Physical Anthropology 87:407-431.

McHenry H, Schultz P. 1976. The association between Harris lines and enamel hypoplasias. American Journal of Physical Anthropology 44:507-512.

Meindl R, Lovejoy C. 1989. Age changes in the pelvis: Implications for paleodemography. In İşcan M, editor. Age Markers in the Human Skeleton. Springfield: Charles C. Thomas, p 137-167.

Meindl R, Swedlund A. 1977. Secular trends in mortality in the Connecticut Valley, 1700-1850. Human Biology 49(3):389-414.

Meinlschmidt G, Heim C. 2005. Decreased cortisol awakening response after early loss experience. Psychoneuroendocrinology 30:568-576.

Mensforth R. 1985. Relative tibia long bone growth in the Libben and Bt-5 prehistoric skeletal populations. American Journal of Physical Anthropology 68:247-262.

Merchant V, Ubelaker D. 1977. Skeletal growth of the protohistoric Arikara. American Journal of Physical Anthropology 46:61-72.

Micozzi M. 1996. Frozen environments and soft tissue preservation. In: Haglund W, Sorg M, editors. Forensic Taphonomy: The Postmortem Fate of Human Remains. Boca Raton: CRC Press, p 171-199.

Miles A, Bulman J. 1994. Growth curves of immature bones from a Scottish island population of sixteenth to mid-nineteenth centuries: Limb-bone diaphyses and some bones of the hand and foot. International Journal of Osteoarchaeology 4:121-136.

Miller G, Cohen S, Ritchey A. 2002. Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid resistance model. Health Psychology 21:531-541.

Miller G, Chen E, Zhou E. 2007. If it goes up, must it come down? Chronic stress and the HPA axis in humans. Psychological Bulletin 133(1):25-45.

Milner G. 1992. Determination of Age and Sex: A Manual Prepared for the Dickson Mounds Reburial Team. Lewiston: Dickson Mounds Museum.

Milner G, Boldsen J. 2012. Transition analysis: A validation study with known-age modern American skeletons. American Journal of Physical Anthropology 148:98-110.

Milner G, Boldsen J, Weise S, Lauritsen L, Freund U. 2015. Sex related risk of trauma in medieval to early modern Denmark, and its relationship to change in interpersonal violence overtime. International Journal of Paleopathology 9:59-68.

Mollat M. 1986. The Poor in the Middle Ages: An Essay in Social History. New Haven: Yale University Press.

Mollerup L, Boldsen J. 2010. The temporal distribution of syphilis and syphilis like lesions in the Black Friars in Odense, Denmark: A likelihood based approach to cemetery analysis. ADBOU 1992-2009: Forskningsresultater, University of Southern Denmark, Odense, Denmark.

Møller-Christensen V, 1953. Ten lepers from Næstved in Denmark, a study of skeletons from a medieval Danish leper hospital. Copenhagen: Danish Science Press.

Møller-Christensen V. 1958. Bogen om Æbelholt Kloster. Copenhagen: Dansk Videnskabs Selskab.

Møller-Christensen V. 1961. Bone Changes in Leprosy. Copenhagen: Munksgaard.

Møller-Christensen V. 1967. Evidence of leprosy in earlier peoples. In: Browthwell D, Sandison A, editors. Diseases in Antiquity. Springfield: Charles C. Thomas, p 295-307.

Møller-Christensen V. 1978. Leprosy Changes in the Skull. Odense: University Press.

Møntegården Odense bys Museer (2010) Public Information Plaque. Sorte Brøde Torv, Odense, Denmark.

Moore-Jansen P, Ousley S, Jantz R 1994. Data Collection Procedures for Skeletal Material. Report of Investigations No. 48. Department of Anthropology, University of Tennessee, Knoxville.

Moorrees C, Fanning E, Hunt Jr. E. 1963a. Formation and resorption of three deciduous teeth in children. American Journal of Physical Anthropology 21:205-213.

Moorrees C, Fanning E, Hunt Jr. E. 1963b. Age variation of formation stages for ten permanent teeth. Journal of Dental Research 42:1490-1502.

Mora S, Prinster C, Bellini A, Weber G, Proverbio MC, Puzzovio M, Bianchi C, Chiumello G. 1997. Bone turnover in neonates: Changes of urinary excretion rate of collagen type I cross-linked peptides during the first days of life and influence of gestational age. Bone 20:563-566.

Morrison N, Eisman J. 1993. Role of the negative glucocorticoid regulatory element in glucocorticoid repression of the human osteocalcin promoter. Journal of Bone and Mineral Research 8(8):969-975.

Muldrew C. 2011. Food, Energy and Creation of Industriousness. Oxford: Oxford University Press.

Muyzer G, Sandberg P, Knapen M, Vermeer C, Collins M, Westbroek P. 1992. Preservation of the bone protein osteocalcin in dinosaurs. Geology 20:871–874.

Naveed H, Abed S, Davagnanam I, Uddin J, Adds P. 2012. Lessons from the past: Cribra orbitalia, an orbital roof pathology. Orbit 31(6):394-399.

Neve A, Corrado A. 2011. Osteoblast physiology in normal and pathological condition. Cell and Tissue Research 343:289-302.

Neve A, Corrado A, Cantatore F. 2013. Osteocalcin: Skeletal and extra-skeletal effects. Journal of Cellular Physiology 228(6):1149-1153.

Nicolson N. 2004. Childhood parental loss and cortisol levels in adult men. Psychoneuroendocrinology 29:1012-1018.

Nielsen E. 1982a. Udgravningsberetning Sortebrødre Torv 1979. Møntergården, Odense, Denmark.

Nielsen E. 1982b. Udgravningsberetning Sortebrødre Torv 1981. Møntergården, Odense, Denmark.

Nikiforuk G, Fraser D. 1981. The etiology of enamel hypoplasia: A unifying concept. Journal of Pediatrics 98(6):888-893.

Ninomiya J, Tracy R, Calore J, Gendreau M, Keim R, Mann K. 1990. Heterogeneity of bone. Journal of Bone and Mineral Research 5(9):933-938.

Nilsson O, Marino R, DeLuca F, Phillip M, Baron J. 2005. Endocrine regulation of the growth plate. Hormone Research 64:157-165.

Noble B, Stevens H, Loveridge N, Reeve J. 1997. Identification of apoptotic changes in osteocytes in normal and pathological human bone. Bone 20(3):273-282.

North M. 2012. The Expansion of Europe, 1250 – 1500. Manchester: Manchester University Press.

Nowak O, Piontek J. 2002. The frequency of appearance of transverse (Harris) lines in the tibia in relationship to age at death. Annals of Human Biology 29:314-325.

Oakley S. 1972. The Story of Denmark. London: Faber and Faber.

O'Brian C, Jia D, Plotkin L, Bellido T, Powers C, Stewart S, Manolagas S, Weinstein R. 2004. Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocrinology 145(4):1835-1841.

Ohlsson C, Begtsson B, Isaksson O, Andreassen T, Slootweg M. 1998. Growth hormone and bone. Endocrine Reviews 19(1):55-79.

Olsen B, Reginato A, Wang W. 2000. Bone development. Annual Review of Cell and Developmental Biology 16:191-220.

Orrman E. 2003a. Rural conditions. In: Knut H, editor. The Cambridge History of Scandinavia Volume 1: Prehistory to 1520. Cambridge: Cambridge University Press, p 250-311.

Orrman E. 2003b. Church and society. In: Knut H, editor. The Cambridge History of Scandinavia Volume 1: Prehistory to 1520. Cambridge: Cambridge University Press, p 421-462.

Ortner D. 2003. Identification of Pathological Conditions in the Human Skeleton. San Diego: Academic Press.

Oxenham M, Cavil I. 2010. Porotic hyperostosis and cribra orbitalia: The erythropoietic response to iron-deficiency anaemia. Anthropological Science 118(3):199-200.

Paine R. 2000. If a population crashes in prehistory, and there is no paleodemographer there to hear it, does it make a sound? American Journal of Physical Anthropology 112(2):181-190.

Paine R, Boldsen J. 2002. Linking age-at-death distributions and ancient population dynamics: A case study. In: Hoppa R, Vaupel J, editors. Paleodemography: Age Distributions in Skeletal Samples. Cambridge: Cambridge University Press, p 169-180.

Palkovich A. 1996. Historic depopulation in the American southwest: Issues of interpretation and context-embedded analyses. In: Baker B, Kealhofer L, editors. Bioarchaeology of Native American Adaptation in the Spanish Borderlands. Gainesville: University Press of Florida, p 179-197.

Palmer A, Strobeck C. 1986. Fluctuating asymmetry: Measurement, analysis and patterns. Annual Review of Ecology and Systematics 17:391-421.

Palubeckaitë Z, Jankauskas R, Boldsen J. 2002. Enamel hypoplasia in Danish and Lithuanian late medieval/early modern samples: A possible reflection of child morbidity and mortality patterns. International Journal of Osteoarchaeology 12:189-201.

Palumbo C, Palazzini S, Marotti G. 1990. Morphological study of intercellular junctions during osteocyte differentiation. Bone 11:401-406.

Papageorgopoulou C, Suter S, Rühli F, Siegmund F. 2011. Harris lines revisited: Prevalence, comorbidities, and possible etiologies. American Journal of Human Biology 23:381-391.

Parfitt A. 1977. The cellular basis of bone turnover and bone loss. Clinical Orthopaedics and Related Research 127:236-247.

Parfitt A. 1983. The physiological and clinical significance of bone histomorphometric data. In: Recker R, editor. Bone Histomorphometry, Techniques and Interpretations. Boca Raton: CRC Press, p 142-223.

Parfitt A. 1984. Age related structural changes in trabecular and cortical bone: Cellular mechanisms and biomechanical consequences. Calcified Tissue International 36:S37-S45.

Parfitt A. 1994. Osteonal and hemi-osteonal remodelling: The spatial and temporal framework for signal traffic in adult human bone. Journal of Cellular Biochemistry 55:273-286.

Parfitt A. 2002. Misconceptions (2): Turnover is always higher in cancellous than in cortical bone. Bone 30(6):807-809.

Parfitt A. 2010. Skeletal heterogeneity and the purpose of bone remodelling: Implications for the understanding of osteoporosis. In: Marcus R, Feldman D, Nelson D, Rosen C, editors. Fundamentals of Osteoporosis. Burlington: Academic Press, p 35-54.

Park E. 1964. Imprinting of nutritional disturbances on the growing bone. Paediatrics 29 (Supplement):815-862.

Park E, Richter C. 1953. Transverse lines in the bone: The mechanism of their development. Bulletin of the Johns Hopkins Hospital 93:234-248.

Petersen H, Boldsen J, Paine R. 2006. Population relationships in and around medieval Danish towns. In: Storey G, editor. Urbanism in the Preindustrial World: Cross-Cultural Perspectives. Tuscaloosa: University of Alabama Press, p 110-120.

Phenice T. 1969. A newly development visual method of sexing in the *os pubis*. American Journal of Physical Anthropology 30:297-301.

Pindborg J. 1970. Pathology of the Dental Hard Tissues. Philadelphia: W. B. Saunders.

Porter A. 1999. The prediction of physique from the skeleton. International Journal of Osteoarchaeology 9:102-115.

Porter R, Pavitt D. 1987. The vertebral canal: 1. nutrition and development, an archaeological study. Spine 12(9):901-906.

Pósa A, Maixner F, Sola C, Bereczki Z, Molnár E, Masson M, Lovász G, Wicker E, Perrin P, Dutour O, Zink A, Pálfi G. 2015. Tuberculosis infection in a late-medieval Hungarian population. Tuberculosis (DOI: http://dx.doi.org/10.1016/j.tube.2015.02.010).

Prader A, Tanner J, von Harnack G. 1963. Catch-up growth following illness or starvation: An example of developmental canalization in man. The Journal of Pediatrics 62(5):646-659.

Prigodich R, Uesely M. 1997. Characterization of the complex between bovine osteocalcin and type 1 collagen. Archives of Biochemistry and Biophysics 345(2):339-341.

Randsborg K. 2009. The Anatomy of Denmark: Archaeology and History from the Ice Age to the Present. London: Duckworth.

Raoult D, Aboudharam G, Crubezy E, Larrouy G, Ludes B, Drancourt M. 2000. Molecular identification by "suicide PCR" of *Yersinia pestis* as the agent of medieval black death. Proceedings of the National Academy of Sciences USA 97:12800-12803.

Rauch F. 2008. The growing skeleton is a busy place – can biochemical bone markers keep track of the action? The Journal of Pediatrics 153:454-455.

Raxter M, Auerbach B, Ruff C. 2006. Revision of the Fully technique for estimating statures. American Journal of Physical Anthropology 130:374-384.

Rehman Q, Lane N. 2003. Effect of glucocorticoids on bone density. Medical and pediatric Oncology 41:212-216.

Reid D, Ferrell R, Walton P. 2002. Histology derived canine crown formation times from a medieval Danish sample. American Journal of Physical Anthropology 34:129.

Reid D, Dean M. 2006. Variation in modern human enamel formation times. Journal of Human Evolution 50:329-346.

Reid D, Ferrell R. 2006. The relationship between number of striae of Retzius and their periodicity in imbricational enamel formation. Journal of Human Evolution 50:195-202.

Ribot I, Roberts C. 1996. A study of non-specific stress indicators and skeletal growth in two medieval subadult populations. Journal of Archaeological Science 23:67-79.

Rifken B, Gay C. 1992. Biology and Physiology of the Osteoclast. Boca Raton: CRC Press.

Roberts C, Manchester K. 2007. The Archaeology of Disease. Ithaca: Cornell University Press.

Roberts S, Smith C, Millard A, Collins M. 2002. The taphonomy of cooked bone: Characterizing boiling and its physico-chemical effects. Archaeometry 44:485-494.

Robins S. 1994. Biochemical markers for assessing skeletal growth. European Journal of Clinical Nutrition 48 (Supplement 1):S199-S209.

Roffey S. 2012. Medieval leper hospitals in England: An Archaeological perspective. Medieval Archaeology 56:203-233.

Rose J, Armelagos G, Lallo J. 1978. Histological enamel indicators of childhood stress in prehistoric skeletal samples. American Journal of Physical Anthropology 49:511-516.

Rosenfield R. 1996. Essentials of growth diagnosis. Endocrinology and Metabolism Clinics of North America 25(3):743-758.

Ruff C. 2002. Variation in human body size and shape. Annual Review of Anthropology 31:211-232.

Saluja G, Fitzpatrick K, Bruce M, Cross T. 1986. Schmorl's nodes (intravertebral herniations of intervertebral disc tissue) in two historic British populations. Journal of Anatomy 145:87-96.

Saunders S. 2008. Juvenile skeletons and growth related studies. In: Katzenberg A, Saunders S, editors. Biological Anthropology of the Human Skeleton, Second Edition. Hoboken: Wiley-Liss, p 117-147.

Saunders S, Hoppa R. 1993. Growth deficit in survivors and non-survivors: Biological mortality bias in subadult skeletal samples. Yearbook of Physical Anthropology 36:126-151.

Sawyer B, Sawyer P. 1993. Medieval Scandinavia: From Conversion to Reformation, circa 800-1500. Minneapolis: University of Minnesota Press.

Schafer M, Black S, Scheuer L. 2009. Juvenile Osteology: A Laboratory and Field Manual. Burlington: Elsevier.

Scheuer L, Black S. 2000. Developmental Juvenile Osteology. Oxford: Elsevier Academic Press.

Schuster C. 2004. A note on the interpretation of weighted kappa and its relations to other rater agreement statistics for metric scales. Educational and Psychological Measurement 64(2):243-253.

Schilling A, Kummer T, Marshall R, Bauerochse A, Jopp E, Pueschel K, Amling M. 2008. Brief communication: Two and three-dimensional analysis of bone mass and microstructure in a Bog Body from the Iron Age. American Journal of Physical Anthropology 135:479-483.

Schmidt-Schultz T, Schultz M. 2004. Bone protects proteins over thousands of years: Extraction, analysis and interpretation of extracellular matrix proteins in archaeological skeletal remains. American Journal of Physical Anthropology 123:30-39.

Schmorl G, Junghanns H. 1971. The Human Spine in Health and Disease. Grune & Stratton: New York.

Schour I, Massler M. 1944. Chart – Development of the Human Dentition, Second Edition. Chicago: American Dental Association.

Schwarz S. 2009. Syphilis in Medieval and Early Post- Medieval Denmark - An Osteological Analysis. MSc Thesis, University of Southern Denmark, Odense, Denmark.

Schwarz S, Skytte L, Rasmussen K. 2013. Pre-Columbian treponemal infection in Denmark? – A paleopathological and archaeometric approach. Heritage Science 1(19):1-12.

Schwartz G, Reid D, Dean C. 2001. Developmental aspects of sexual dimorphism in hominoid canines. International Journal of Primatology 22:837-860.

Segerstrom S, Miller G. 2004. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. Psychology Bulletin 130(4):601-630.

Scott A. 2009. Body Size Indicators and the Examination of Stress from a Growth and Development Perspective: A New Method of Bioarchaeological Assessment. MA Thesis, Department of Anthropology, The University of Western Ontario, London.

Scott A, Hoppa R (2015) A re-evaluation of the impact of radiographic orientation on the identification and interpretation of Harris lines. American Journal of Physical Anthropology 156(1):141-147.

Scott S, Duncan C. 2001. Biology of Plagues: Evidence from Historical Populations. Cambridge: Cambridge University Press.

Sellevold B. 1989a. Fødselogdød: kvinners dødelighet i forbindelse med svangerskap og fødsel I forhistorik tid og middelalder, belyst ut fra studier av skjelettmaterialer. In: Gunneng H, editor. Kvinnors Rosengård. Stockholm: Centrum, p 79-96.

Sellevold B. 1989b. Fokus på kvinner. Kvinner helse I middelalderen belyst gjennem skjelett studier. In: Gunneng H, editor. Kvinnors Rosengård. Stockholm: Centrum, p 59-78.

Selye H. 1936. A syndrome produced by diverse nocuous agents. Nature 138:32.

Selye H. 1973. The evolution of the stress concept: The originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones. American Scientist 61(6):692-699.

Sim J, Wright C. 2005. The kappa statistic in reliability studies: Use, interpretation, and sample size requirements. Physical Therapy 85(3):257-268.

Simpson S. 1999. Reconstructing patterns of growth disruption from enamel microstructure. In: Hoppa R, FitzGerald C, editors. Human Growth in the Past: Studies from Bones and Teeth. Cambridge: Cambridge University Press, p 241-263.

Šlaus M. 2000. Biocultural analysis of sex differences in mortality profiles and stress levels in the late medieval population from Nova Rača, Croatia. American Journal of Physical Anthropology 111:193-209.

Šlaus M. 2002. Demography and pathology of the medieval population from Stenjevec. Opvscvla Archæologica 26:257-273.

Slovik D, Gundberg C, Neer R, Lian J. 1984. Clinical evaluation of bone turnover by serum osteocalcin measurements in a hospital setting. Journal of Clinical Endocrinology and Metabolism 59(2):228-230.

Smith B. 1991. Standards of human tooth formation and dental age assessment. In: Kelley M, Larsen C, editors. Advances in Dental Anthropology. New York: Alan R. Liss, p 143-168.

Smith C, Craig O, Prigodich R, Nielsen-Marsh C, Jans M, Vermeer C, Collins M. 2005. Diagenesis and survival of osteocalcin in archaeological bone. Journal of Archaeological Science 32:105-113.

Sommer C. 1996. Ecotoxicology and developmental stability as an in-situ monitor of adaptation Ambio 25:374-376.

Sontag L, Comstock G. 1938. Striae in the bones of a set of monozygotic triplets. American Journal of Diseases of Children 56:301-308.

Spocter M, Manger P. 2007. The use of cranial variables for the estimation of body mass in fossil hominids. American Journal of Physical Anthropology 134:92-105.

Steckel R. 2005. Young adult mortality following severe physiological stress in childhood: Skeletal evidence. Economics and Human Biology 3:314-328.

Steckel R, Rose J (editors). 2002. The Backbone of History: Health and Nutrition in the Western Hemisphere. Cambridge: Cambridge University Press.

Steele J. 2000. Skeletal indicators of handedness. In: Cox M, Mays S, editors. Archaeology and Forensic Science. London: Greenwich Medical Media, p 307-323.

Steinbock R. 1976. Paleopathological Diagnosis and Interpretation. Springfiled: Charles Thomas.

St. Hoyme L, İşcan M. 1989. Determination of sex and race: Accuracy and assumptions. In: İşcan M, Kennedy K, editors. Reconstructions of Life from the Skeleton. New York: Liss, p 53-93.

Stojanowski C, Seidemann R, Doran G. 2002. Differential skeletal preservation at Windover Pond: Causes and consequences. American Journal of Physical Anthropology 119:15-26.

Stromstedt P, Poellinger L, Gustafsson J, Carlstedt-Duke J. 1991. The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: A potential mechanism for negative regulation. Molecular and Cellular Biology 11(6):3379-3383.

Stuart-Macadam P. 1982. A Correlative Study of a Palaeopathology of the Skull. PhD Thesis, Department of Physical Anthropology, Cambridge University. Cambridge.

Stuart-Macadam P. 1985. Porotic hyperostosis: Representative of a childhood condition. American Journal of Physical Anthropology 66:391-398.

Stuart-Macadam P. 1989. Porotic hyperostosis: Relationship between orbital and vault lesions. American Journal of Physical Anthropology 80:187-193.

Stuart-Macadam P. 1992. Porotic Hyperostosis: A New Perspective. American Journal of Physical Anthropology 87:39-47.

Suter S, Harders M, Papageorgopoulou C, Kuhn G, Székely G, Rühli F. 2008. Technical note: Standardized and semiautomated Harris lines detection. American Journal of Physical Anthropology 137:362-366.

Sweeney E, Cabrera J, Urrutia J, Mata L. 1969. Factors associated with linear enamel hypoplasia of human deciduous incisors. Journal of Dental Research 48:1275-1279.

Szulc P, Seeman E, Delmas P. 2000. Biochemical measurement of bone turnover in children and adolescents. Osteoporosis International 11:281-294.

Tam P, Trainor P. 1994. Segmentation and specification of the paraxial mesoderm. Anatomy and Embryology 189:275-305.

Tanner J. 1978. Foetus Into Man: Physical Growth from Conception to Maturity. London: Open Books.

Tanner J. 1986. Growth as a target-seeking function: Catch-up and catch-down growth in man. In: Falkner F, Tanner J, editors. Human Growth a Comprehensive Treatise, Second Edition. Volume 1 Developmental Biology Prenatal Birth. New York: Plenum Press, p 167-179.

Teitelbaum S. 2000. Bone resorption by osteoclasts. Science 289:1504-1508.

Temple D, Nakatsukasa M, McGroarty J. 2012. Reconstructing patterns of systemic stress in a Jomon period subadult using incremental microstructures of enamel. Journal of Archaeological Science 39:1634-1641.

Thomas M, Gilbert P, Cuccui J, White W, Lynnerup N, Tiball R, Cooper A, Prentice M. 2004. Absence of *Yersinia pestis*-specific DNA in human teeth from five European excavations of putative plague victims. Microbiology 150:341-354.

Todd T. 1921. Age changes in the pubic bone. American Journal of Physical Anthropology 4(1):1-70.

Trotter M, Gleser G. 1958. A re-evaluation of estimation of stature based on measurements of stature taken during life and of long bone after death. American Journal of Physical Anthropology 16:79-123.

Trueman C, Behrensmeyer A, Tuross N, Weiner S. 2004. Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: Diagenetic mechanisms and the role of sediment pore fluids. Journal of Archaeological Science 31:721-739.

Tsigos C, Chrousos G. 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. Journal of Psychosomatic Research 53:865-871.

Tuross N. 1991. Recovery of bone and serum proteins from human skeletal tissue: IgG, osteonectin, and albumin. In: Ortner D, Aufderheide A, editors. Human Paleopathology. Current Syntheses and Future Options. Washington: Smithsonian Institution Press, p 51-54.

Ubelaker D. 1989. Human Skeletal Remains: Excavation, Analysis, Interpretation, Second Edition. Washington: Taraxacum.

Ubelaker D. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):363-364.

Üstündağ H. 2009. Schmorl's nodes in a post-medieval skeletal sample from Klostermarienberg, Austria. International journal of Osteoarchaeology 19:695-710.

Väänänen H, Horton M. 1995. The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure. Journal of Cell Science 108:2729-2732.

Van Gerven D, Hummert J, Burr D. 1985. Cortical bone maintenance and geometry of the tibia in prehistoric children from Nubia's Batn el Hajar. American Journal of Physical Anthropology 66:275-280.

van der Eerden B, Karperien M, Wit J. 2003. Systematic and local regulation of the growth plate. Endocrine Review 24(6):782-801.

Vanderschueren D, Gevers G, Raymaekers G, Devos P, Dequeker J. 1990. Sex- and age-related changes in bone and serum osteocalcin. Calcified Tissue International 46:179-182.

van Straalen J, Sanders E, Prummel M, Sanders G. 1991. Bone-alkaline phosphatase as indicator of bone formation. International Journal of Clinical Chemistry 201:27-34.

Vaughan J. 1975. The Physiology of Bone, Second Edition. Oxford: Clarendon Press.

Vercellotti G, Stout S, Boano R, Sciulli P. 2011. Intrapopulation variation in stature and body proportions: Social status and sex differences in an Italian medieval population (Trino Vercellese, VC). American Journal of Physical Anthropology 145:203-214.

Viera A, Garrett J. 2005. Understanding interobserver agreement: The kappa statistic. Family Medicine 37(5):360-363.

Wagner E, Karsenty G. 2001. Genetic control of skeletal development. Current Opinion in Genetics and Development 11(5):527-532.

Waldron I. 1983. Sex differences in human mortality the role of genetic factors. Social Science and Medicine 17(6):321-333.

Waldron T. 1994. Counting the Dead: The Epidemiology of Skeletal Populations. Wiley: Chichester.

Walker P. 2005. Greater sciatic notch morphology: Sex, age, and population differences. American Journal of Physical Anthropology 127(4):385-391.

Walker P, Bathurst R, Richman R, Ojerdrum T, Andrushko V. 2009. The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis. American Journal of Physical Anthropology 139:109-125.

Walker D, Powers N, Connell B, Redfern R. 2015. Evidence of skeletal treponematosis from medieval burial ground of St-Mary Spital, London and implications for the origins of the disease in Europe. American Journal of Physical Anthropology 156:90-101.

Weaver D. 1980. Sex differences in the ilia of a known sex and age sample of fetal and infant skeleton. American Journal of Physical Anthropology 52:191-195.

Webb E, Thomson S, Nelson A, White C, Koren G, Rieder M, Van Uum S. 2010. Assessing individual systematic stress through cortisol analysis of archaeological hair. Journal of Archaeological Science 37:807-812.

Webb E, White C, Van Uum S, Longstaffe F. 2015. Integrating cortisol and isotopic analyses of archeological hair: Reconstructing individual experiences of health and stress. American Journal of Physical Anthropology 156:577-594.

Weinberg J. 1993. Den gotiske labyrinth. Middelalderen og kirkerne i Danmark. Stockholm: Almqvist and Wiksell.

Weiner H. 1992. Perturbing the Organism: The Biology of Stressful Experience. Chicago: University of Chicago Press.

Weinstein R. 2001. Glucocorticoid-induced osteoporosis. Reviews in Endocrine and Metabolic Disorders 2:65-73.

Weinstein R, Jilka R, Parfitt M, Manolagas S. 1998. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids: Potential mechanisms of their deleterious effects on bone. Journal of Clinical Investigation 102(20):274-282.

Weiss E. 2005. Schmorl's nodes: A preliminary investigation. Paleopathology Association Newsletter 132:6-10.

Wells C. 1975. Ancient hazards and female mortality. Bulletin of the New York Academy of Medicine 51(11):1235-1249.

Weser U, Etspüler H, Kaup Y. 1995. Enzymatic and immunological activity of 4000 year aged bone alkaline phosphatase. FEBS Letter 375:280-282.

Weser U, Kaup Y, Etspüler H, Kenward N, Hedges R. 1996. Biochemically and immunologically active alkaline phosphatase in archaeologically important bone samples. The Journal of Archaeological Science 23:723-730.

West L. 2002. Defining critical windows in the development of the human immune system. Human and Experimental Toxicology 21:494-505.

White T. 1978. Early hominid enamel hypoplasia. American Journal of Physical Anthropology 49:79-83.

White T, Folkens P. 2005. The Human Bone Manual. Burlington: Elsevier Academic Press.

Wiggins R. 1991. Porotic Hyperostosis, Cribra Orbitalia, Enamel Hypoplasia, Periosteal Reaction and Metopism: A Correlation of their Prevalence and an Assessment of the Nature of Porotic Hyperostosis in Three British Archaeological Populations. MSc Thesis, University of Bradford, Bradford.

Wilkinson R. 1992. Comments - The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):364-365.

Wood J, Milner G, Harpending H, Weiss K. 1992. The osteological paradox: Problems of inferring prehistoric health from skeletal samples. Current Anthropology 33(4):343-370.

Wright L, Yoder C. 2003. Recent progression in bioarchaeology: Approaches to the Osteological Paradox. Journal of Archaeological Research 11(1):43-70.

Yehuda R. 2000. Biology of post-traumatic stress disorder. Journal of Clinical Psychiatry 61:14-21.

Yoder C. 2006. The Late Medieval Agrarian Crisis and Black Death Plague Epidemic in Medieval Denmark: A Paleopathological and Paleodietary Perspective. PhD Thesis, Department of Anthropology, Texas A&M University, College Station, USA.

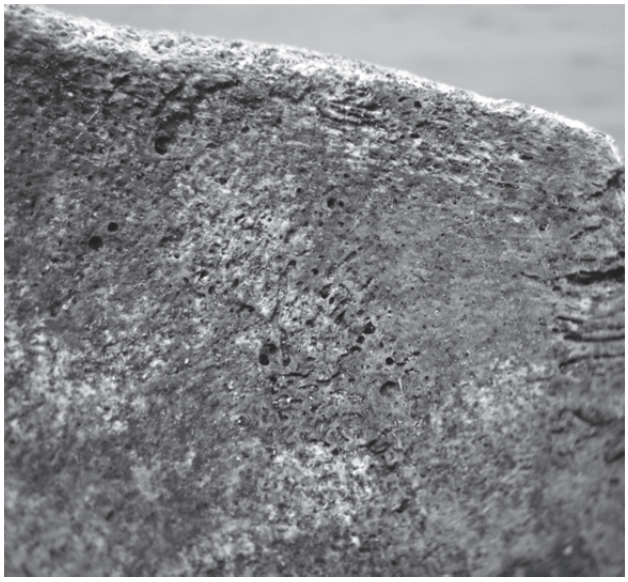
Yoder C. 2010. Diet in medieval Denmark: A regional and temporal comparison. Journal of Archaeological Science 37(9):2224-2236.

Youngs D. 2006. The Life Cycle in Western Europe, c.1300 – c.1500. Manchester: Manchester University Press.

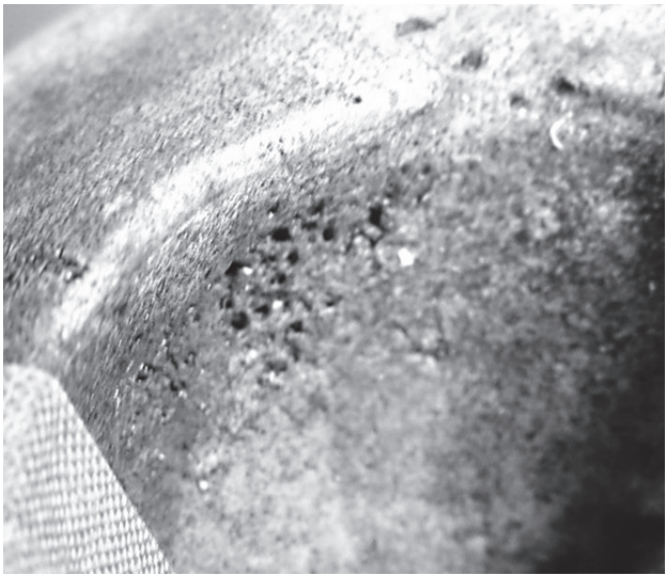
http://museum.odense.dk/viden-om/odense-historie/odense-bys-historie

APPENDIX A: STRESS LESION SEVERITY CATEGORIES

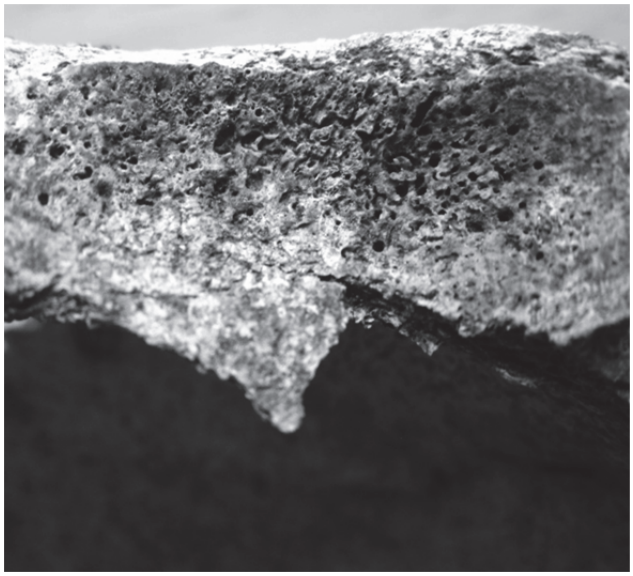
A.1 Cribra Orbitalia Severity Categories



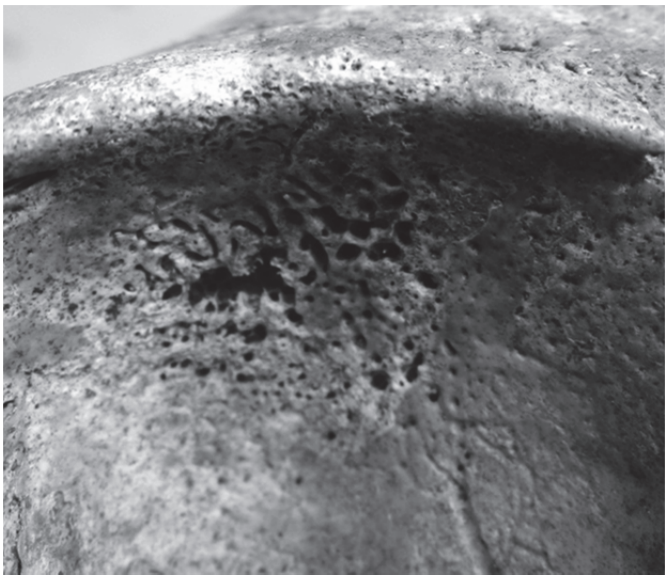
Category 1 (mild) (SBT81 GR174)



Category 2 (moderate) (SBT81 GR195)

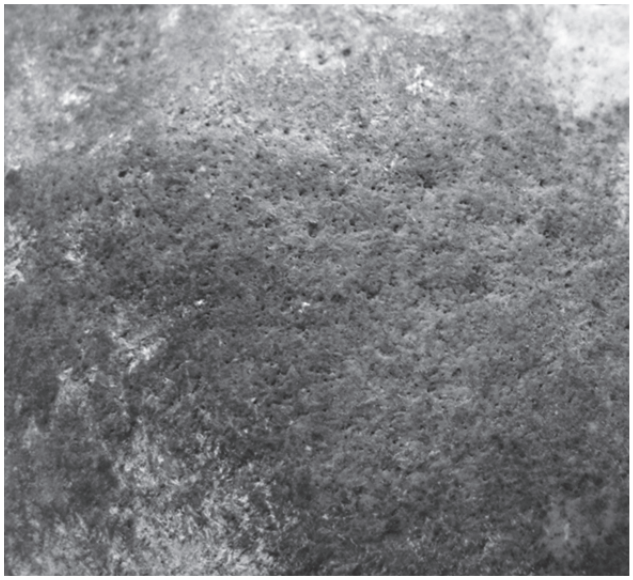


Category 3 (advanced) (SBT81 GR222)

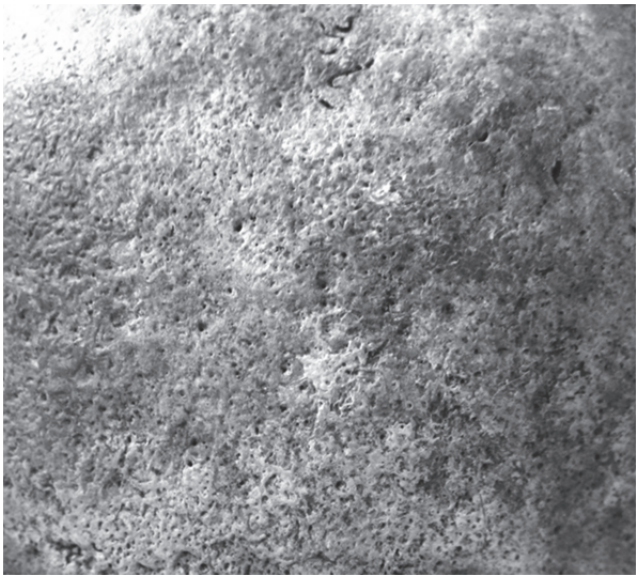


Category 4 (severe) (SBT81 GR586)

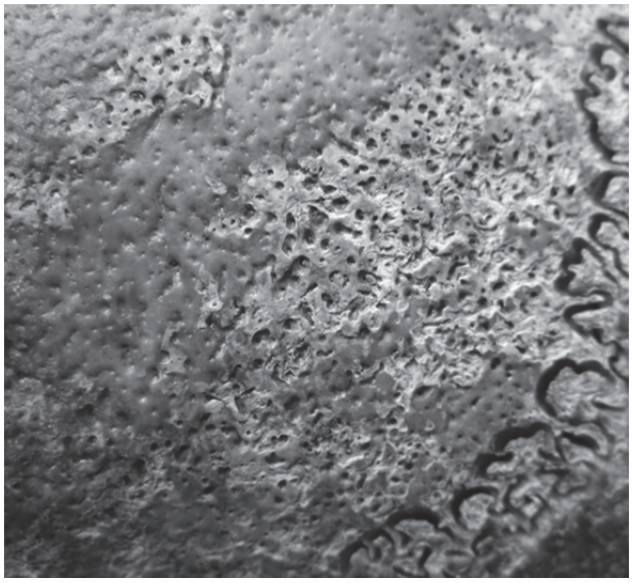
A.2 Porotic Hyperostosis Severity Categories



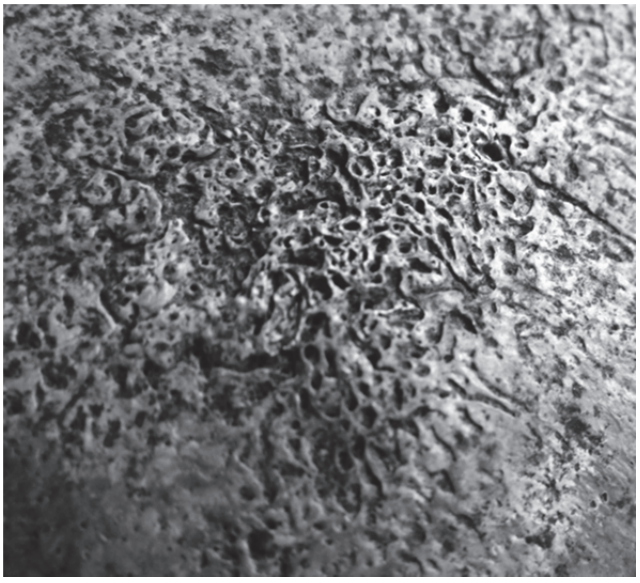
Category 1 (mild) (SBT81GR101)



Category 2 (moderate) (SBT81 GR128)



Category 3 (advanced) (SBT81 GR35)

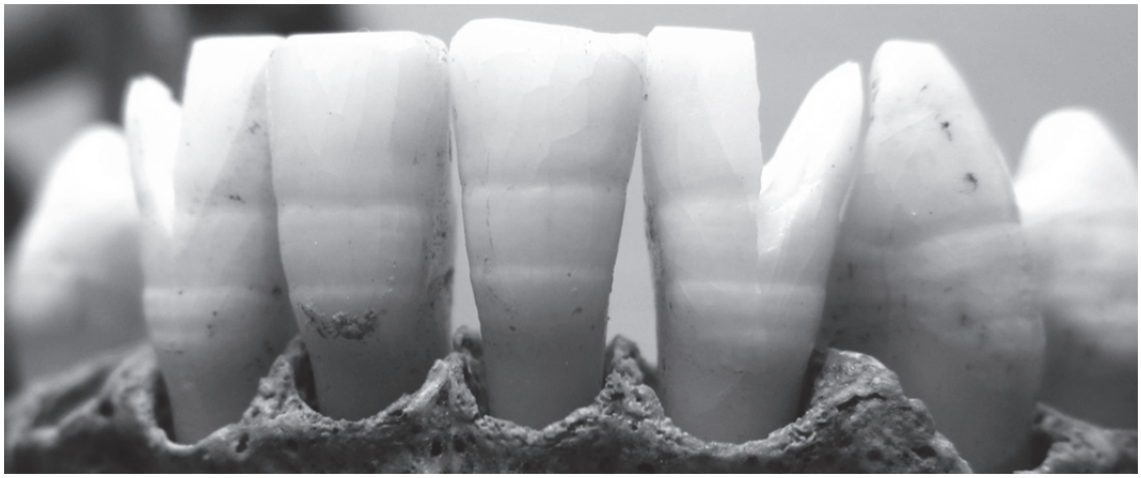


Category 4 (severe) (SBT81 GR266)

A.3 Enamel Hypoplastic Lesion Severity Categories

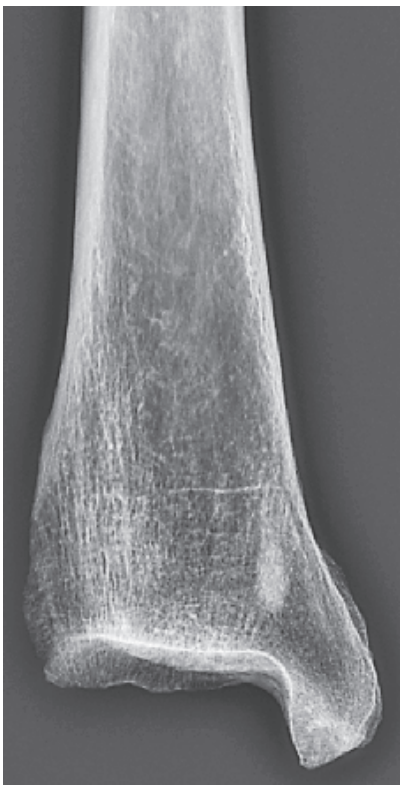
Category 1 (mild) (SBT81 GR483)

Category 2 (moderate) (SBT81 GR491)

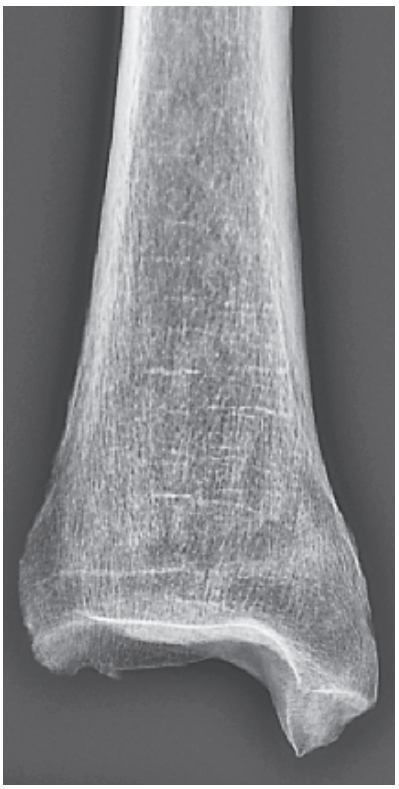


Category 3 (severe) (SBT81 GR15)

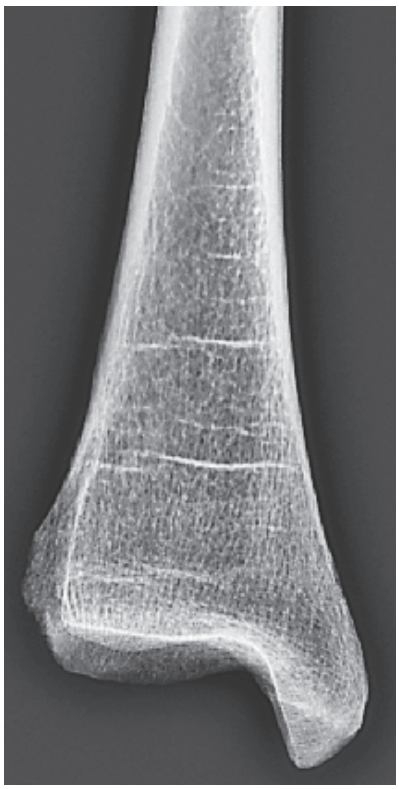
A.4 Harris Line Severity Categories



Category 1 (low)
(SBT81 GR142)



Category 2 (moderate)
(SBT79 GR22)



Category 3 (high)
(SBT81 GR464)

APPENDIX B: BIOCHEMICAL DATA

B.1 Adult Individuals Selected for Osteocalcin Extraction

Individual	Time Period	Sex	Age (years)
SBT81 GR378	Medieval	Male	20-25
SBT81 GR447	Medieval	Female	18-20
SBT81 GR457	Medieval	Male	20-25
SBT81 GR513	Medieval	Male	35-45
SBT81 GR550	Medieval	Male	32-39
SBT81 GR562	Medieval	Female	35-39
SBT81 GR661	Medieval	Male	30-34
SBT81 GR10	Post-medieval	Female	30-35
SBT81 GR131	Post-medieval	Female	45-55
SBT81 GR193	Post-medieval	Male	19-21
SBT81 GR208	Post-medieval	Female	25-29
SBT81 GR241	Post-medieval	Male	40-49
SBT81 GR279	Post-medieval	Female	35-40
SBT81 GR297	Post-medieval	Male	30-34
SBT81 GR306	Post-medieval	Male	20-24
SBT81 GR307	Post-medieval	Male	35-40
SBT81 GR313	Post-medieval	Female	27-32
SBT81 GR316	Post-medieval	Female	37-42
SBT81 GR344	Post-medieval	Male	27-32
SBT81 GR9	Post-medieval	Female	22-29

B.2 Total Protein Results from MicroBCA Analysis and PBS Amounts added for ELISA Analysis

Individual	4ug starting bone material	µl of PBS added to protein extract
SBT81 GR9 Femur	5.1	44.9
SBT81 GR9 Clavicle	4.0	46.0
SBT81 GR307 Femur	2.5	47.5
SBT81 GR307 Clavicle	3.1	46.9
SBT81 GR513 Femur	11.4	38.6
SBT81 GR513 Clavicle	19.0	31.0
SBT81 GR313 Femur	3.5	46.5
SBT81 GR313 Clavicle	3.0	47.0
SBT81 GR378 Femur	3.5	46.5
SBT81 GR378 Clavicle	4.9	45.1
SBT81 GR241 Femur	4.7	45.3
SBT81 GR241 Clavicle	8.4	41.6
SBT81 GR344 Femur	2.8	47.2
SBT81 GR344 Clavicle	3.5	46.5
SBT81 GR550 Femur	8.7	41.3
SBT81 GR550 Clavicle	9.2	40.8
SBT81 GR316 Femur	3.4	46.6
SBT81 GR316 Clavicle	3.3	46.7
SBT81 GR131 Femur	5.6	44.4
SBT81 GR131 Clavicle	5.2	44.8
SBT81 GR279 Femur	7.1	42.9
SBT81 GR279 Clavicle	5.0	45.0
SBT81 GR661 Femur	7.6	42.4
SBT81 GR661 Clavicle	5.3	44.7
SBT81 GR447 Femur	3.4	46.6
SBT81 GR447 Clavicle	3.7	46.3
SBT81 GR208 Femur	4.9	45.1
SBT81 GR208 Clavicle	4.3	45.7
SBT81 GR457 Femur	6.2	43.8
SBT81 GR457 Clavicle	6.2	43.8
SBT81 GR193 Femur	5.0	45.0
SBT81 GR193 Clavicle	3.8	46.2
SBT81 GR562 Femur	4.9	45.1
SBT81 GR562 Clavicle	5.5	44.5
SBT81 GR306 Femur	3.0	47.0
SBT81 GR306 Clavicle	2.8	47.2
SBT81 GR297 Femur	3.5	46.5
SBT81 GR297 Clavicle	3.2	46.8
SBT81 GR10 Femur	5.6	44.4
SBT81 GR10 Clavicle	8.4	41.6

B.3 Serial Dilution Calculation for ELISA Analysis

Well Position	ng/ml of Human Osteocalcin Standard*
Rows A, Columns 1 and 2	128
Rows B, Columns 1 and 2	64
Rows C, Columns 1 and 2	32
Rows D, Columns 1 and 2	16
Rows E, Columns 1 and 2	8
Rows F, Columns 1 and 2	4
Rows G, Columns 1 and 2	2
Rows H, Columns 1 and 2	0

* As per instructions provided with R&D Systems Quantikine ELISA – Human Osteocalcin kit (DSTCN0)