Frailty among the non-survivors: Assessing the effects of urban living on infant and maternal health through a comparative study of dental stress markers between two Dutch post medieval populations.

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Frailty Among the Non-Survivors

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Oriana Chiappa Zugazagoitia

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Metsu, G. (c. 1664 - c. 1666). *The Sick Child* [Painting]. Rijksmuseum, Amsterdam, Netherlands. https://www.rijksmuseum.nl/nl/collectie/SK-A-3059.

Frailty Among the Non-Survivors:
Assessing the effects of urban living on infant and maternal health through a comparative study of dental stress markers between two Dutch post- medieval populations (Arnhem and Middenbeemster).

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Chapter 1: Introduction

Urbanization and urban living not only reshaped the social, cultural, political, and economical aspects of European societies, but also had a great impact on people’s health. These effects can be observed in the archeological record through the analysis of skeletal remains from urban sites and comparing them to their rural counterparts (DeWitte and Betsinger 2020). Many osteoarcheological studies have focused on the effects of urbanization in terms of adult health, stress, frailty, and survivorship (Betsinger et al., 2020; DeWitte, 2010; Gamble et al., 2017). Recently, more studies have focused on children, since they represent the most vulnerable members of society (Garcin et al., 2010). However, there is a lack of research done on the repercussions of urbanization and urban living on children and mother’s health, specifically in terms of survivorship and frailty. This research focuses on this issue, assessing the impact of urban living on non-adult and maternal health during post medieval times in the Netherlands (1500-1850 AD). To do so, dental pathological conditions from a rural (Middenbeemster) and an urban (Arnhem) collection of post-medieval Dutch non-adults and young adults were analyzed and compared. This chapter will serve as an introduction to this study, by setting the background and context, along with the essential definitions and concepts for this research; followed by the problem statement, the research question, the objectives, and finally the broad relevance of the study.

1.1 Problem statement

According to DeWitte and Betsinger (2020, p. 2), cities can be defined as “relatively large human settlements with systems of infrastructure, governance, and organization necessary to accommodate and support large numbers of people”. Although throughout the history of humankind cities have had different sizes, shapes, and administrations, nowadays, more than half of the people from all over the world live in cities (UNCTAD, 2021). Therefore, the study of the effects of urban centers is not only important to understand past societies, but to comprehend our current lifestyle.

There seems to be a dispute amongst scholars about the general outcome of urban living on humankind. On one hand, researchers argue that urbanization has had a general positive impact on people’s health; while on the other hand, other scholars suggest the opposite. As seen from several social indicators analyzed in current urban centers, (such as life expectancy, poverty index, and access to basic needs such as education, health services, sanitization, water, and food), living standards in cities are generally better in urban areas compared to rural environments (DeWitte & Betsinger, 2020; Hagerty et al., 2002).

Nonetheless, historians have pointed out that people living in cities have not always enjoyed a better quality of life, specifically in European urban centers during medieval and post-medieval times. During the medieval period (500-1550 CE), many European societies underwent a major structural transformation caused by the migration of people from rural areas toward urban centers (Schats, 2021). This process known as urbanization led to a rapid population increase in the cities, having an enormous impact on people's lifestyles. In other words, urbanization shifted how human beings interacted with each other as well as with their environment, transforming urban living into what we know today (Betsinger & DeWitte, 2021).

According to historic sources, during the 16th century, some of the largest European cities, such as Paris, reached up to 225,000 inhabitants, and a population density of 512 inhabitants per hectare (Bairoch, 1988, p. 278). Although Dutch towns could not compare in population size to these cities due to their small sizes, their population density must have been comparable or higher (Schats, 2016, p. 10). Due to their high number of residents, as well as the lack of sanitation systems, urban centers were often described as insalubrious places, ideal for the spread of plagues and infectious diseases (Lucassen & Willems, 2011, p. 16). Historians suggest that people were aware of the health and security issues of these conglomerate places, but the plenty of advantages that cities offered were enough to oversee the burdens of the urban lifestyle (Lucassen & Willems, 2011). For instance, in the Low Countries, the vast political, social, and economic opportunities attracted people from all over Europe, which resulted in culturally diverse urban centers with relatively well-paid occupations and the possibility for vertical status mobility (Lucassen & Willems, 2011; Price, 2014). Other authors claim that the reason behind the migration towards cities was not the attractive opportunities they presented, but the lack of them in rural settings due to the labor surplus generated by the transition from grain cultivation to cattle breeding (Hoppenbrouwers, 2001).

Recently, the analysis of human archeological skeletal remains has helped to fill in the missing gaps from the historical evidence, since bones and teeth show a permanent record of stress markers and diseases that past individuals suffered from (Buikstra et al., 2022). In addition, archeological research provides us with empirical data, which can help overcome the biases of historic sources. Therefore, studying health conditions from an osteoarcheological perspective can provide relevant information about the effects of urban living on people's health and lifestyle (Betsinger & DeWitte, 2021). Specifically for European medieval and post-medieval populations, archeological and historical data complement each other to depict a more complete representation of how people experienced the early urban phenomenon.

To assess the living conditions of European societies during the transition to urban living in terms of health, osteoarcheologists have looked for evidence of what are called *stress indicators*. These have been broadly defined as any type of marker in the body tissue which could indicate a physical disruption of the “normal” condition of the human body due to environmental or cultural constraints (Goodman & Armelagos, 1989; Larsen, 2002, p. 2; Marklein et al., 2016). Stress markers can be identified in the skeletal record as growth disruption, evidence of disease, and even death, and they have been used as a proxy to determine the *frailty* and *survivorship* of individuals; these terms refer to the biological state of a person resulting from an accumulative decline of one or more body functions, increasing an individual's vulnerability to stress factors, and decreasing its ability to overcome them (resiliency) (Marklein et al., 2016, p. 2010; Reitsema & McIlvaine, 2014). High levels of frailty in an individual can negatively affect their morbidity and mortality rates, and therefore also affect their ability to survive (survivorship) (Edinborough & Rando, 2020, p. 109).

There is a vast repertoire of studies focused on comparing skeletal stress markers to assess urbanization in terms of frailty, health, and living conditions of archeological populations from medieval and post-medieval Europe (e.g. Bennike et al., 2005; Betsinger et al., 2020; Crane-Kramer & Buckberry, 2020; Griffin, 2017; Miszkiewicz, 2015; Palubeckaitė et al., 2002; Yaussy et al., 2016). Specifically in the Netherlands, osteological studies of medieval and post-medieval collections have shown similar health conditions for urban and rural individuals (Casna et al., 2021; Schats, 2016).

However, these studies are often focused on adult skeletal remains, leaving us with a gap in knowledge on the effects of urban living on infants and children. Recent trends in osteoarcheology have stated the importance of including non-adults in osteoarcheological research as well. Because infants represent the most frail and vulnerable members of society, mortality patterns and health conditions of these individuals provide an overall image of the health conditions of the broad population (DeWitte & Betsinger, 2020, p. 4; Halcrow, 2020; Kamp, 2001). In addition, the nexus between maternal and infant health has started to be investigated from an osteoarcheological perspective, since the first few years of an individual’s life, including the fetal period, are majorly influenced by maternal health (Capra et al., 2013; Gowland & Halcrow, 2020). Thus, any sign of stress or disease in the youngest individuals (including death) could be representing the maternal-infant health relationship (Gowland & Halcrow, 2020, p. 6). In addition, infancy and childhood have been identified as highly vulnerable stages in an individual's life, and any disruption during this period could negatively impact health conditions even later in life (Gamble & Bentley, 2022; Gowland, 2015; Wadhwa et al., 2009).

One of the biggest limitations that comprises studying young individuals is the state of preservation of the bones; the level of organic material in dental and skeletal remains of children is higher than those of fully matured individuals since they have not reached full levels of mineralization (Bartelink, 2007; Halcrow & Ward, 2018). Therefore, the degradation of these bones is quicker than in those of fully developed adults (Guy et al., 1997). Although deciduous or "milk" teeth are less mineralized than permanent dentition, due to their material and composition they are still more resistant to degradation than the rest of their skeleton (Stodder, 2018). This characteristic makes teeth an extremely useful material to analyze when preservation is not guaranteed, as in the case of juvenile skeletal remains (Hillson, 1996).

Another key characteristic of dental tissue is that, unlike bone, teeth do not remodel throughout an individual’s life, and therefore, any pathological condition remains marked on the tooth as a permanent record of any stressful event (Fitzgerald & Rose, 2008; Lacruz et al., 2017). This research focuses on two of the most prevalent oral pathologies in infant and adult teeth: linear enamel hypoplasia and dental caries.

Linear enamel hypoplasia is a developmental defect in teeth linked to stress during development of the dentition (Bereczki et al., 2018; Goodman & Rose, 1991; Hillson, 1996). Due to its multifactorial etiology, this condition has been catalogued as a non-specific stress indicator widely used to assess episodes of systematic stress during an individual's early life-stages (Goodman et al., 1984; Goodman & Rose, 1991). In addition, dentistry and pediatric studies have provided researchers with a great understanding of teeth development timing for both deciduous and permanent dentitions, which can be used to determine the age of an individual at the time of a stress insult (Cares Henriquez & Oxenham, 2019; Goodman & Rose, 1991). Therefore, investigating linear enamel hypoplasia in permanent and deciduous teeth allows researchers to assess an individual's health during specific periods of time, even when the rest of the skeleton is not present or complete.

Multiple studies have focused on analyzing time at formation of dental stress markers in adult individuals to address issues of child frailty and survivorship, with the premise that although these individuals suffered from stressful events during childhood, they survived into adulthood (e.g Amoroso et al., 2014; Boldsen, 2007; Cucina, 2011; Goodman & Armelagos, 1989; Ham et al., 2021). However, these studies often exclude the analysis of enamel hypoplasia on deciduous teeth, leaving out important information about frail individuals who *did not survive*. Unlike permanent teeth which are developed mainly during the first years of childhood, deciduous teeth are mainly formed *in utero* providing information about the child's first months, as well as the mother's health by the time of gestation (Cook & Buikstra, 1979; López-Lázaro et al., 2022; Temple, 2020).

As for carious lesions, even though dental cavities have been thoroughly examined in archeology to determine dietary patterns and subsistence strategies, due to the infectious character of the disease, they are also considered an indicator of physiological stress, related to the nutritional and immune status of an individual (Caselitz, 1998; Crall & Forrest, 2018; Hillson, 1996). Very few osteoarcheological studies have investigated dental caries in deciduous teeth, and even less known studies have researched childhood caries as an indicator of stress (e.g Cook & Buikstra, 1979; Halcrow et al., 2013; Lingström & Borrman, 1999). Nonetheless, multiple pediatric studies have investigated childhood caries, and some studies have linked the presence of caries in children with maternal oral health (e.g Crall & Forrest, 2018; Davies, 1998; Seow, 2012).

Therefore, by studying dental stress markers on both deciduous and permanent teeth, we will not only have a better understanding of early childhood and maternal stress, but we will be able to compare the differences between survivors and non-survivors. In sum, this research will focus on analyzing the prevalence and time-at-formation of linear enamel hypoplasia and the prevalence of carious lesions on deciduous and permanent teeth from a rural and an urban population, to tackle the lack of information about the impact of urban living on non-adults and maternal health in the Netherlands during post-medieval times.

1.2 Research questions and objectives

Main research question:

How did urban-living impact non-adults and maternal health in the Netherlands?

Through the comparison of chronic and acute stress markers (caries and LEH) between skeletal samples from an urban and a rural site, I will research how urban living impacted non-adults and maternal health in the Netherlands during the post-medieval period.

Specific questions:

It is important to state that for every question, the comparison between urban and rural sites will be considered, as well as the age at death of the individual.

Questions for linear enamel hypoplasia.

1. What is the prevalence of linear enamel hypoplastic events in deciduous teeth?
2. What is the prevalence of linear enamel hypoplastic events in permanent teeth?
3. At what age are non-adults more likely to acquire LEH based on the age of formation?

Questions for carious lesions

1. What is the prevalence of carious lesions in deciduous teeth?
2. What is the prevalence of carious lesions in permanent teeth?
3. Is there a relationship between the prevalence of carious lesions and LEH in deciduous and/or permanent teeth?

Answering these specific questions about enamel hypoplasia and dental caries in an age-specific manner will allow us to compare data between different age groups at the time of death, revealing information about markers of oral stress in those individuals who survived to later stages of life compared to those who died earlier (survivors vs. non-survivors). In addition, we will be able to compare these patterns between rural and urban settings.

To address these questions, the sample to be analyzed had to meet certain criteria. First, the sample had to be composed of Dutch post-medieval individuals from a rural and an urban site to be able to make comparisons between these two contexts. Second, to investigate maternal and non-adult health, the sample had to be composed of non-adult and adult individuals of multiple age categories. Finally, to avoid biases related to sex, gender, or social status, the sample required individuals of both sexes and from similar social strata. These characteristics were met by the Arnhem and Middenbeemster skeletal collections, which together with other characteristics explained in later chapters, made them the perfect assemblages for this research.

1.3 Thesis outline

The next chapter of this thesis, chapter 2, contains the theoretical background in which this research is embedded, including a critical review of the most relevant frameworks of the osteoarcheological discipline.

Chapter 3 is devoted to the methodological background, covering the definitions and concepts necessary to understand the methods used, as well as a literature review of previous work related to this subject.

In chapter 4, I present a comprehensive description of the materials selected for this research, providing important details on the archeological and historical, context of both contexts and samples; followed by a detailed explanation of the methods applied and their rationale, as well as the followed ethical guidelines.

Chapter 5 is devoted exclusively to presenting the results of the research according to the methods applied.

Finally, Chapter 6, dedicated to the discussion, provides a comprehensive interpretation of the results, critically discussing the outcomes of the research, as well as the limitations and potential topics and recommendations for future research. This will be followed by chapter 7, containing the conclusions and final remarks, where I will summarize the most interesting findings of the research.

Chapter 2: Theoretical background

In this chapter, I will define the theoretical framework in which this thesis is embedded. Since this research is based on osteological data, the first two sections of this chapter provide contextual information about the concepts of health, stress, frailty, and survivorship within an osteoarcheological framework[1], including the Osteological Paradox. The third section is dedicated to the most recent paradigms relevant to this research, which englobe childhood archeology, infant and maternal health, and the Developmental Origins of Health and Disease Hypothesis.

2.1 Determining health in osteoarcheology

One of the main goals of osteoarcheology as a discipline is to answer questions about the behavior and lifestyle of human past populations through their osteological remains; this includes the study of cultural and biological aspects of individuals or populations, as well as their interaction with the environment (Buikstra & Beck, 2009, p. 477; Larsen, 2002, p. 26). In osteoarcheology, important attention has been given to the study of health and disease since these play a crucial part in defining someone's life experience. This resulted in the emergence of paleopathology as a subdiscipline dedicated to study the abnormal variation of archeological human remains (Larsen, 1997; Ortner, 2003).

However, for our discipline, it has been challenging to define what is considered healthy and what is not. Generally, *health* is thought to be a holistic concept that captures the life quality of an individual, their daily functioning and social interactions which are reflected in a physical and mental state (Reitsema & McIlvaine, 2014, p. 181). In fact, the World Health Organization denominates health as "a state of complete physical, mental, and social well-being and not merely the absence of disease, or infirmity", meaning that health and disease are not mutually exclusive and can coexist and interact with each other as a continuous (Reitsema & McIlvaine, 2014, p. 181; World Health Organization, 2006, p. 1).

Although this definition of health may be considered vague, the concept of disease is just as difficult to define. In osteoarcheology, these two concepts are subject to constant debate, and

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[1] It is important to note that in European archeology, the term osteoarcheology is equivalent to the term bioarcheology as used in American literature.

new definitions are continuously being implemented; however, for this specific study, the definition of disease will be intrinsically related to the concept of stress, in the sense that *stress* is the bodily response to any disruption in the organism's biological homeostasis caused by genetic, nutritional, environmental, and, or cultural elements (Goodman & Armelagos, 1989; Grauer, 2018, p. 905; Larsen, 2002, p. 6). In other words, when one or more stress factors interact with our body, there is a disruption of the proper functioning of the organism, which can generate certain anomalies in the growth process of the individual, or result in what is referred to as a state of disease (Goodman & Armelagos, 1989; Grauer, 2018, p. 905; Larsen, 2002, p. 6).

To explain the development of stress, Goodman & Armelagos (1989, p. 226) developed a model in which environmental restrains, cultural systems, and the organism's resistance interact with each other continuously throughout an individual's life. This model can be better appreciated in the figure below (figure 2.1). According to these authors, the environmental and cultural systems provide and regulate the resources to survive, but also can generate stress factors that can cause physiological disruptions in the organism. However, some stressors do not signify a biological threat because of the organism's capacity to respond and/or the existence of a "cultural buffering system" that helps to counteract them (Goodman & Armelagos, 1989, p. 226). When the individual fails to resist these threats, then a physiological disruption occurs which can be reflected as "decreased health, decreased work capacity, decreased reproductive capacity, or a disruption in the sociocultural activities" (Goodman & Armelagos, 1989, p. 226). If an individual suffers from acute or prolonged periods of stress, they might develop marks in their body tissue, including bones and teeth, which are used in osteoarcheology as a proxy to determine health in individuals (Goodman & Armelagos, 1989, p. 227; Larsen, 2002, p. 8; Temple & Goodman, 2014, p. 188). This model can also translate into a population level, showing processes of adaptability to certain stressors (Grauer, 2018, p. 906).

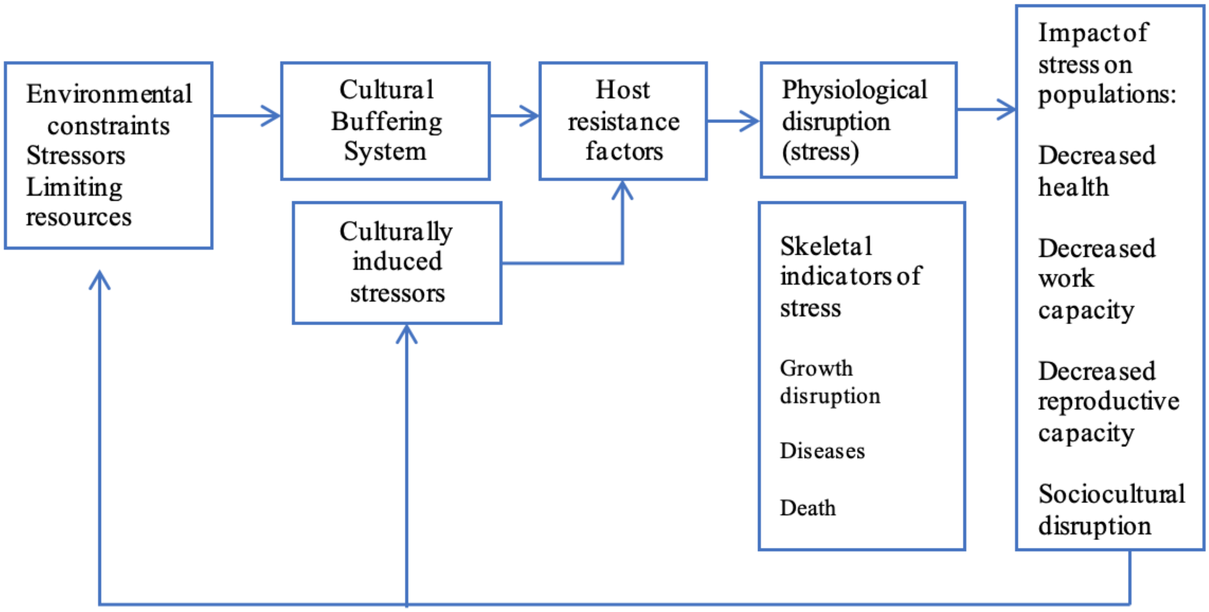

Figure 2. 1 Stress development model of past populations. Taken from Goodman and Armelagos (1989, p. 285)

Skeletal indicators of stress

As stated before, in our discipline we are restraint to analyze stress markers from osteological remains since soft tissue is rarely preserved in archeological contexts (Ortner, 2003, p. 23). Hence, assessing stress through the prevalence of skeletal markers related to pathological conditions is our only way to understand health, morbidity, and mortality of past populations (Marklein et al., 2016, p. 208).

There are multiple types of skeletal lesions and conditions with diverse etiologies, ranging from trauma (healed or unhealed fractures), lesions associated to congenital diseases, lesions related to ageing, skeletal lesions caused by infectious disease, metabolic and endocrine disease amongst many others[2]. Nonetheless, to assess health and stress in past populations researchers have focused on those skeletal and dental lesions related to disruptions of growth and development, as well as other signs of physiological stress (Gamble & Bentley, 2022, p. 18). Although some of these skeletal markers can be tracked down to a specific causal origin (*e.g.* infectious diseases such as tuberculosis or leprosy), most researchers focus on other indicators of stress with multiple or nonspecific etiologies, often called nonspecific stress indicators (DeWitte & Stojanowski, 2015, p. 402).

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[2] For an extensive reading on paleopathology and skeletal lesions see Ortner (2003) and Roberts & Manchester (2007).

Among the most frequently analyzed nonspecific stress indicators are porotic hyperostosis and *cribra orbitalia* as indicators of nutritional stress, related to vitamin deficiency, such as scurvy and anemia (DeWitte & Stojanowski, 2015, p. 402; Godde & Hens, 2021, p. 632). These are resorptive, porous lesions formed during childhood; the first one can be found on the cranial vault, while the latter one forms in the orbital roof of the skull (Gamble & Bentley, 2022, p. 18).

Another commonly analyzed bone anomalies as indicators of stress are periosteal new bone formation, a layer of woven bone produced by the periosteum as an inflammatory response to a variety of conditions, such as trauma, metabolic disease, and infectious diseases(Gamble & Bentley, 2022, p. 19; Roberts & Manchester, 2012, p. 385). When there is no causal information of the condition, its associated with overall health and mortality (DeWitte, 2014, p. 1354; Gamble & Bentley, 2022, p. 19).

Because an individual's height has been associated with nutritional and environmental factors, stature and growth patterns have been investigated in osteoarcheology as a predictor of stress (Marklein et al., 2016, p. 213). The assessment of body dimensions, including the estimation of stature, as well as other measurements such as diaphyseal length and vertebral neural canals are used to determine overall health during development periods (Gamble & Bentley, 2022, p. 19; Marklein et al., 2016, p. 213). Short stature has been catalogued as an indicator of stress with the premise that “adult stature, relative to other individuals within the population, likely indicates poor health and poor nutrition during the developmental years” (DeWitte & Hughes-Morey, 2012, p.1412).

Skeletal signs of growth disruptions include Harris lines, which are defined as a transverse increases in bone density on the long bones, created by changes in mineralization during long bone formation (Gamble & Bentley, 2022, p. 18; Roberts & Manchester, 2012, p. 362). These lines occur when there is a developmental disruption due to periods of stress during childhood; in adult individuals, Harris lines might not be visible due to natural bone remodeling processes (Gamble & Bentley, 2022, p. 18).

Similar to Harris lines, enamel hypoplasia has been researched as a developmental disruption related to early childhood stress (DeWitte & Stojanowski, 2015, p. 402; Gamble & Bentley, 2022). Other dental indicators of stress frequently analyzed in osteoarcheological studies include dental caries, periodontal disease, and abscesses as markers related to compromised immunity and nutritional deficiencies (DeWitte & Stojanowski, 2015, p. 402; Marklein et al., 2016, p. 213).

Many studies have opted for a multiple proxy approach to assess frailty and stress in past populations, demonstrating that this methodology can improve the results by providing a better understanding of the health conditions of the population (Betsinger et al., 2020; DeWitte & Hughes-Morey, 2012; Wissler, 2021). However, when it comes to juvenile skeletal remains, in archeological contexts it is unusual to come across complete skeletons. Due to the size and composition of juvenile bones, these are more susceptible than adult bones to experience preservation issues (Guy et al., 1997, p. 223; Stodder, 2018, p. 77). Particularly infants and children have shown very high levels of diagenetic damage attributed to the increased porosity of such remains (Manifold, 2015, p. 540). For newborns and fetuses, often only the teeth and very dense bones from the cranium, such as the mandible, are preserved (Manifold, 2015, p. 540) .

Therefore, oral pathologies are a special area of interest in the study of infant and child health. Enamel hypoplasia and dental caries are especially useful in complicated and comingled contexts, as they can be studied in individual teeth without the presence of the maxilla and mandible, as in the case of periodontitis and ante-mortem tooth loss (Hillson, 1996, p. 16, 2001, p. 365). More detailed information on these pathologies will be provided in the following chapters.

2.2 The Osteological Paradox

In 1992, Wood and colleagues published a controversial article questioning the traditional model of determining stress in past populations. “The Osteological Paradox: Problems of Inferring Prehistoric Health from Past Populations” became a staple in osteoarcheology, highlighting several points about the problematics of the discipline, specifically of interpreting health through the study of skeletal samples. The main topics discussed by the authors include three main issues: demographic non-stationarity, selective mortality, and hidden heterogeneity. In the next few paragraphs, a brief explanation of these terms will be provided, followed by information on how osteoarcheology as a discipline has dealt with these issues since the release of the influential article.

Demographic non-stationarity

The first problematic Wood and colleagues addressed in the Osteological Paradox is the issue of demographic non-stationarity, referring to this concept as "the departure of a population from the stationary state". This alludes to the conceptual error paleodemography often falls into by assuming a population is stationary, when most of them are not. In other words, paleodemographic studies focus on assessing mortality patterns by constructing life tables based on observable age-at-death distribution of skeletal samples (DeWitte & Stojanowski, 2015, p. 405; Wood et al., 1992, p. 343). However, some factors that can modify the demographics of a population are often disregarded in these tables, including migrations, shifts in mortality and fertility rates, constant growth rate, and a continuous variation of age distribution in a population (Wood et al., 1992, p. 344). By overseeing these factors, studies based solely on age-at-death distribution of skeletal samples might under or overestimate the number of individuals of the living population, directly affecting the accuracy of life expectancies estimations (DeWitte & Stojanowski, 2015, p. 405; Wood et al., 1992, p. 343). For instance, an increase of the number of death non-adults in a skeletal collection of a stationary sample might be an indicator of a higher mortality rate; nonetheless, for non-stationary populations, this could be related to an increase in fertility rate (DeWitte & Stojanowski, 2015, p. 405; Milner et al., 2008, p. 563; Wood et al., 1992, p. 343). However, this issue does not directly intervene with the assessment of a population's health *per se*, and therefore, remains as a secondary matter of concern for paleopathologists, being selective mortality and hidden heterogeneity the main limitations.

Selective mortality and hidden heterogeneity

Goodman and Armelagos (1989, p.226) mention that due to cultural, environmental, and biological differences, some individuals might be at a higher risk than others to be affected by stress factors. This increase of an individual's vulnerability to stress factors, and their decreased ability to overcome them compared to other individuals from their same birth cohort has also been determined as *frailty* (Marklein et al., 2016, p. 2010; Vaupel et al., 1979, p. 151; Wood et al., 2002, p. 344).

High levels of frailty in an individual can have a negative effect in its morbidity and mortality rates, and therefore also affect their capacity to survive (survivorship) (Edinborough &

Rando, 2020). That is to say that every individual from a population has a different chance of dying at a certain age, and therefore populations are "heterogenous for frailty" (DeWitte & Stojanowski, 2015, p. 407; Milner et al., 2008, p. 586). Likewise, frailty is not constant; its distribution changes according to age as frail individuals are discarded from the population (Milner et al., 2008, p. 586). Thus, a skeletal sample is composed of the frailest individuals of each age group, in other words, the ones who did not survive.

Wood and colleagues (1992) argue that the fundamental paradox in osteoarcheology relies on the attempt to determine a population's health by assessing skeletal lesions from an "inherently biased sample" (DeWitte & Stojanowski, 2015, p. 407). They define this issue as *selective mortality* since skeletal samples are "...highly selective for lesions that increase the risk of death at a certain age..." and "...overestimate the true prevalence of the conditions in the general population" (Wood et al., 1992, p. 348). Consequently, the skeletal lesions observed in a collection of human remains might not be a true representation of the living population, but of the frailest individuals.

Similarly, the concept of hidden heterogeneity refers to the fact that as archaeologists, we are unable to observe the variations in frailty, and we ignore the number of factors and conditions that could directly affect an individual's frailty, complicating the assessment of health for the entirety of the population. As mentioned before, the variation in the frailty of the individuals can be caused by countless sources such as immunological differences associated with genetic, cultural, nutritional, or biological compounds; differences in exposure to stress factors induced by behavioral, environmental or cultural reasons (e.g risk- taking behavior, exposure to occupational hazards, exposure to environmental pollutants); among many others (Buikstra & DeWitte, 2019, p. 17; DeWitte & Stojanowski, 2015, p. 406).

Consequently, when we are unable to assess the sources that could have caused variations in frailty of the individuals we cannot assure that the observed patterns (such as stature, age-at-death distribution, non-specific stress markers, and other skeletal and dental lesions), are representative of the living population (Buikstra & DeWitte, 2019, p. 21). This is why selective mortality and hidden heterogeneity are two key concepts to take into account when assessing health of past populations.

Resiliency and Survivorship

Wood and colleagues (1992), based on reflections by Ortner (1991), stated another paradoxical issue faced by osteoarcheologists, related to the concepts of *resiliency* and *survivorship*. As stated previously, in osteoarcheology, skeletal stress markers are used as proxies of health and disease in the sense that individuals with lower rates of skeletal lesions were in better health conditions than the ones who present higher rates (DeWitte & Stojanowski, 2015, p. 407; Temple & Goodman, 2014, p. 188). However, based on the fact that lesions in bone and teeth take longer to develop than lesions in soft tissues, the authors argue that in some cases, individuals that show higher rates of skeletal lesions could be "healthier" than those who show no lesions at all (Buikstra & DeWitte, 2019, p. 17; DeWitte & Stojanowski, 2015, p. 409; Ortner, 2003, p. 15; Wood et al., 1992, p. 351). In other words, individuals with visible skeletal manifestations of stress were able to survive these episodes for longer periods of time, showing signs of resiliency, compared to those individuals who might have died before skeletal lesions could even develop. It is important to note that this scenario must not be taken always as a true statement, since it represents only one of the many plausible interpretations that the skeletal data could provide, and thus should be equally considered as well as the alternative options (DeWitte & Stojanowski, 2015, p. 408).

Engaging with the Osteological Paradox

Since the Osteological Paradox was published, it received many reactions from scholars around the field, creating space for dialogue and debate. This forced researchers to rethink the traditional methods for determining health in osteoarcheology, and urged them to seek for new approaches to overcome the limitations presented by the osteological paradox (Wright & Yoder, 2003, p. 45). However, this was easier in theory than in practice since few publications in the following decade managed to engage with Wood and colleagues' (1992) article in other ways that were not restrained to acknowledging the osteological paradox as a research limitation or as a great theoretical contribution (DeWitte & Stojanowski, 2015; Wissler, 2021, p. 6; Wright & Yoder, 2003). Nowadays, more authors are responding to the Osteological Paradox in their papers, as multiple efforts are being made to explore the possible solutions to the conceptual obstacles in our discipline (Buikstra et al., 2022; Wissler, 2021, p. 15).

Regarding hidden heterogeneity a and selective mortality, DeWitte and Stojanowski (2015) identified four areas of interest in which archaeologists could focus to approach the osteological paradox. These areas include paying crucial attention to the archeology context, understanding lesion-formation processes, investigating frailty and demography, and researching non-adults as non-survivors (DeWitte & Stojanowski, 2015, p. 418). In addition to these areas of focus, the recent advances in biomolecular sciences, epidemiology, and genetics have worked complementary to investigate selective mortality and hidden heterogeneity from a different approach, adding information that would be impossible to retrieve from the typical skeletal analysis.

To minimize the effects of heterogeneous frailty, Wood et al. (1992) had already suggested gathering as much information as possible from the archeology context, including data about the social status of the individuals, ethnic background, gender, and any other details that could provide information about possible sources (Buikstra et al., 2022, p. 17). For this, some researchers have focused on short term use graveyards, such as the black death cemeteries in London, in which the cause of death is known, together with other background information such as sex, age at death, temporality, and social status (DeWitte, 2014). In these rare scenarios, selective mortality can be addressed, allowing more accurate interpretations (DeWitte & Stojanowski, 2015, p. 415). Nonetheless, sites in where the cultural context is well identified are certainly not common, and researchers have had to implement a wide array of techniques to determine factors that could cause hidden heterogeneity. For instance, ancient DNA has been analyzed to detect presence of pathogens in ancient populations (Fonzo et al., 2020; Immel et al., 2021; Spyrou et al., 2018); and the use of GIS has been applied to identify construction patterns linked to social status (DeWitte & Stojanowski, 2015, p. 415; Sosna et al., 2013).

DeWitte and Stojanowski (2015) mention that apart from familiarizing ourselves with the context, investigating frailty from a demographic perspective is essential for understanding morbidity and mortality patterns. For this, epidemiology and epigenetics play an important role, by studying the association between the exposure to stress sources during key development periods and health conditions on living populations (Buikstra et al., 2022, p. 78). Yaussy and collegues (2016) researched the effects of selective mortality of famine victims in medieval London by analyzing the association between stress indicators, sex, and age of the individuals; revealing an increased frailty in those individuals who were affected by stressors earlier in life. Similarly, understanding lesion-formation processes is crucial for tackling the osteological paradox. This includes recording healed vs. non-healed lesions, as well as examining them from an etiological and physiological perspective (Buikstra et al., 2022; Buikstra & DeWitte, 2019).

Finally, Wood and colleagues (1992) as well as (Buikstra & DeWitte, 2019) recommend using age-structured data to overcome the osteological paradox, since the thorough examination of age-related skeletal lesions can provide information about hidden heterogeneity. For instance. DeWitte, (2014) investigated age patterns related to skeletal lesions to assess the overall health of the population pre-Black Death and post-Black Death, showing a correlation between age and rates of skeletal lesions, which suggests higher resiliency and health improvement in the post-Black Death sample. This demonstrates the importance of using age-structured data when assessing heterogeneous frailty. Likewise, Godde & Hens, (2021) analyzed the relation between cribra orbitalia as a stress indicator and mortality patterns in medieval and post-medieval London, considering sex, status, and age-at-death of the individuals. This study showed higher mortality rates for individuals with cribra orbitalia at younger ages than older ones, meaning that cribra orbitalia could be a potential indicator of selective mortality.

Because age-at-death of juvenile skeletons can be more accurately determined, many authors have focused on non-adults as a proxy to determine frailty by comparing the individuals who died at early stages in life against those who survived into adulthood (e.g Amoroso et al., 2014; Amoroso & Garcia, 2018; Bennike et al., 2005; Stodder, 1997; Usher, 2016).

2.3 The study of children in osteoarcheology

As mentioned before, analyzing skeletal stress indicators in non-adults[3] can be used as a proxy to assess frailty. However, due to the misrepresentation of juvenile skeletons in the archeological record, it was not until recently when children started to be investigated in

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[3] In the literature, non-adults are often referred to as *subadults*. However, in accordance to multiple studies, I have opted for the term non-adult to encompass all children from the fetal stages up to late adolescence as an attempt to raise awareness of the importance of studying children in osteoarcheological contexts (see Lewis, 2006, p. 2).

terms of frailty in osteoarcheology (Halcrow, 2020, p. 19; Lewis, 2006, p. 12). Some authors attribute this to the preservation issues related to non-adults' bones size and composition, nonetheless, other factors that affect the preservation of juvenile remains in archeological collections include excavation damage, deficient documentation strategies, conservation issues, and poor storage conditions (Stodder, 2018, p. 76). Because of this lack of representation in archeological collections, non-adults were often disregarded from osteoarcheological studies.

Other academics argue that rather than a practical issue, it was a theoretical one, as children were seen as a "peripheral topic to the main issues of interest" (Kamp, 2001, p. 2). However, since the emergence of gender archeology and childhood archeology as disciplinary branches, more attention has been given to the study of children in the past, including their relationship with their sociocultural and physical environment (Halcrow, 2020, p. 22; Lewis, 2006, p. 12; Lillehammer, 1989, p. 90). It is important to mention that "childhood" refers to a social state, rather than a biological one. According to Gowland (2006, p. 145) a difference must be established between the biological or physiological age and the social or cultural age; the first one being associated to the natural ageing of the body and related to the amount of time passed since the moment of birth (chronological age); while the second one relates to the social role, attitudes, and behaviors attributed to individuals of a certain age group within a specific society, and can vary between populations (*ibidem*). In osteoarcheology, the age-at-death assigned to an individual corresponds to the biological age, rather than the social one. Although there can be discrepancies between social and biological ages, there is an undeniable relation between them in the sense that the biological age determines the physiological abilities of an individual, influencing how society interacts with a child depending on their life-stage (Lewis, 2006, p. 5). Therefore, to answer questions about the health and lifestyle of children in archeological populations, we must not only take into account the difference between social and biological ages, but we must also distinguish between the different life stages of individuals.

Recent trends in osteoarcheology and paleopathology push towards an integrative understanding of an individual's life course, with the idea that "individuals are products of their previous social and biological experiences" (Cheverko, 2020, p. 62). In other words, a holistic approach of life stages, or *life history* is needed to better understand health and disease of past populations. This can be done by distinguishing between development stages of non-adults, including the fetal stages (Halcrow, 2020, p. 21).

Development and frailty: Infant and maternal stress.

Life history theory, or life course approach is enrooted in an evolutionary and biological context in which plasticity plays an important role in the development and success of an individual (Cheverko, 2020, p. 64). According to Hill & Kaplan (1999, p. 399), when exposed to an external stressor during development, individuals will unconsciously prioritize systems that are crucial for survival, "trading off" the amount of energy needed for current growth and development, jeopardizing future health and reproduction. In other words, these processes of developmental plasticity and "trade-offs" during early life-stages can have long lasting effect later in life, determining an individual's frailty (Gamble & Bentley, 2022, p. 18; Suzuki, 2018, p. 266).

In evolutionary biology and medical fields, this is referred to as the Developmental Origins of Health and Disease (DOHaD) hypothesis (or Barkers hypothesis), based on Barker's findings of the correlation between fetal undernutrition during gestation and coronary heart disease in adults (Barker, 1995; Barker, 2012; Buikstra et al., 2022; Edwards, 2017; Gowland, 2015; Lewis, 2018). Similarly, many studies have found correlations between maternal caloric deficiency during pregnancy and health conditions in their offspring during adulthood, such as obesity, coronary heart disease, and insulin resistance (Barker, 2012, p. 187; Wadhwa et al., 2009, p. 4). This hypothesis shows that the fetal and infant stages (1000 days after conception) are critical for the health outcome of an individual (Barker, 2012, p. 186).

Throughout the first years of life, individuals go through several critical periods of vulnerability. The first one occurs in utero where most body structures start to develop. At this stage of development, organs and systems have the capacity to transform phenotypically depending on the external circumstances to which the organism is exposed (developmental plasticity) (Barker, 2012, p. 187; Cheverko, 2020, p. 62; Edwards, 2017, p. 21). Therefore, individuals in the intrauterine period are highly sensitive to environmental stressors (Cheverko, 2020, p. 65). Since, fetal nutritional and health status are directly dependent on the mother, any disruption on maternal health and diet before and during pregnancy might affect the development of the bodily structures of an individual, repercussing on their future health status (Barker, 2012, p. 185; McDade, 2003, p. 111).

After birth, another time of vulnerability occurs, as the immature state of the immune system, coupled with constant exposure to new pathogens, leaves infants especially susceptible to suffer or perish from disease (Goodman & Armelagos, 1989; Lewis, 2006, p. 15, 2018, p. 5). During this time, breast milk provides infants with antibodies that protect them against infectious disease. Thus, maternal health also plays an important role in this stage, as the mother's health condition influences the quality of breastmilk (McDade, 2003, p. 111). When the breastfeeding period ceases and the infant starts weaning, the passive immunity acquired through breastmilk is no longer effective, leaving children once again, highly susceptible to diseases (Lewis, 2018, p. 6). Because of these multiple moments of vulnerability, infancy and childhood are considered a decisive period in an individual's life; suffering from stress during this time could be negatively reflected in their future health (Gowland, 2015; Halcrow, 2020).

After this, the individual achieves stability for a few years until the onset of puberty and adolescence (Lewis, 2018, p. 6). Here, the individual is once again in a state of vulnerability, as hormonal development triggers the "trade-off" mechanisms, prioritizing pubertal growth and compromising immune system (Cheverko, 2018, p. 36; McDade, 2003, p. 115).

DOHaD in osteoarcheology

Recently, the DOHaD hypothesis was adopted in archeological sciences and osteoarcheology with the intention of explaining morbidity and mortality patterns as well as infant and maternal stress from archeological populations (Buikstra et al., 2022, p. 78). Although several health conditions cannot be observed in skeletal remains, through different methods, osteoarcheologists have been able to detect correlations between early life stress and increased adult morbidity and mortality (Temple, 2019, p. 36). These methods include the analysis of a variety of stress indicators, such as assessing the risk of death associated with short stature and body measurements (Amoroso & Garcia, 2018; DeWitte & Yaussy, 2017; Usher, 2016); comparing the presence of dental and skeletal indicators of early life stress between adult age cohorts (Amoroso et al., 2014; Betsinger et al., 2020; DeWitte & Bekvalac, 2010; Marklein et al., 2016; Wissler, 2021); and comparing surviving and non-surviving elements of a sample that includes pre-adults and adults (Bennike et al., 2005; Cucina, 2011; Yaussy & DeWitte, 2018).

Many of these studies have found positive outcome in osteoarcheological data to support the DOHaD hypothesis. For instance, in terms of body size, DeWitte and Yaussy (2017) measured and compared femur lengths as a proxy for stature between famine burials and contemporaneous attritional, finding that people with shorter statures were more likely to die during conditions of famine. Similarly, Usher (2016) estimated the adult stature of non-surviving non-adults from a Tirup cemetery and compared it to the stature of the adults, finding short stature to be a significant predictor of mortality. However, other studies focusing on different body measures have found contrasting results. Amoroso and García (2018) examined the vertebral neural canal size of individuals from different age cohorts from a Portuguese skeletal collection from the 19th–20th century, to test the hypothesis that small vertebral neural canal size is associated with a decreased lifespan, finding no significant relation between these variables.

This example shows how the application of the DOHaD in our discipline must be critically examined. As Cheverko (2018, p. 46) mentions, one of the biggest limitations of testing the DOHaD from an osteoarcheological perspective is the lack of a consistent methodology. Researchers have focused on a wide array of techniques to score skeletal indicators of childhood stress; however, a consistent methodology is needed to better understand the physiological constraints that occur after surviving early episodes of stress.

Although without specifically mentioning the DOHaD hypothesis, dental stress markers have been extensively analyzed in osteoarcheology in terms of survival, since teeth do not remodel throughout an individual's life (e.g Armelagos et al., 2009; Goodman & Armelagos, 1989; Goodman & Rose, 1991). Therefore, there is already a relatively consistent methodology to study them. The following chapter includes a bibliographic review of previous work carried out within this framework.

Chapter 3 Methodological background

To be able to study dental pathologies such as enamel hypoplasia and caries, we must first have a broad understanding of the tooth's anatomy, as well as the tooth's formation process. In the first few paragraphs of this chapter, I will provide a brief explanation of dental anatomy and development, focusing specifically on the enamel formation process. Posteriorly, the dental stress markers used for this thesis will be defined, followed by a literature review of the most recent research in this area.

3.1Teeth anatomy

During our lifespan as human beings we possess two sets of dentitions: the deciduous one, colloquially known as "milk teeth", and the permanent dentition. The first one starts developing during the prenatal months, around 15 weeks after fertilization, and continues until late childhood; it consists of 20 teeth: 8 incisors, 4 canines, and 8 molars that later get replaced by the permanent dentition (figure 3.1). This latter one develops during the first years of an individual's life, and it consists of 32 teeth: 8 incisors, 4 canines, 8 premolars, and 12 molars (Hillson, 1996, p. 18; White & Folkens, 2005, p. 107).

Anatomically speaking, teeth are divided into three major parts according to their composition and function: the crown, the neck, and the root (figure 3.2). The crown is the part of the tooth that emerges above the gingival line, varying in their morphology depending on the function of each tooth. For example, teeth used for cutting and tearing food (such as incisors and canines) have a more elongated shape with a sharp edge, while teeth used for grinding (molars and premolars) have a more robust and flatter surface (Hillson, 1996, p. 13). The neck is the section of the tooth that connects the crown and the root, and it is also referred to as *cervix* or cement-enamel junction (CEJ), since in this part of the tooth, these structures merge, forming a visible border line (Hillson, 1996, p. 13).

The root is the part that remains inside the socket, embedded in the bony process of the jaw (alveolar process); its function is to fixate the tooth into the periodontal ligaments and collagen fibers, forming the dental arch (Hillson, 1996, p. 14; Nelson, 2014, p. 23). Depending on the type of tooth and its morphology, they may have one or more root.

Teeth are composed of four types of tissues: dentine, enamel, cement, and pulp. Dentine composes most of the tooth, and it is composed by a material formed by a mineralized inorganic tissue called hydroxyapatite (70%), and collagen protein (30%). In a tooth's crown, dentine is recovered by a thick layer of enamel, a highly resistant white material formed 96% by hydroxyapatite crystals, and 4% by organic matter and water. Due to these high levels of mineralization and low levels of collage, enamel cannot be remodeled, serving as a protective layer for the inner structures of the tooth (Lacruz et al., 2017, p. 942).

Meanwhile, the root is coated by an external layer of hard tissue called *cementum* or cement. The inner section of the tooth (pulp cavity) withholds an organic soft tissue called pulp; this section starts inside the crown (pulp chamber) and continues until the root canal, innervating the tooth and connecting it to the bloodstream (Hillson, 1996, p. 16; Nelson, 2014, p. 25). Because of its organic composition, the pulp is no longer found in archeological skeletal remains (Fitzgerald & Rose, 2008).

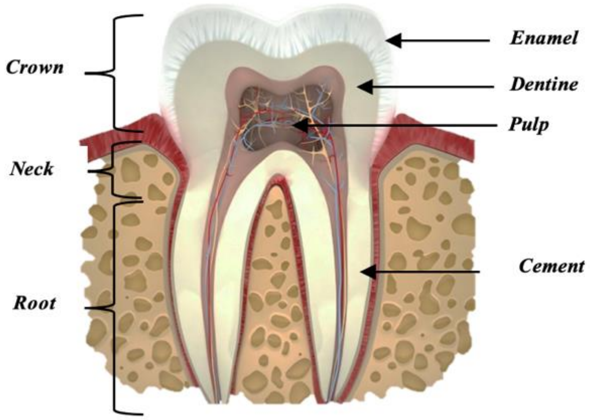

Figure 3. 1 Diagram of a human molar showing the anatomical structures and segments of teeth. Image retrieved from 21stcenturydental.com

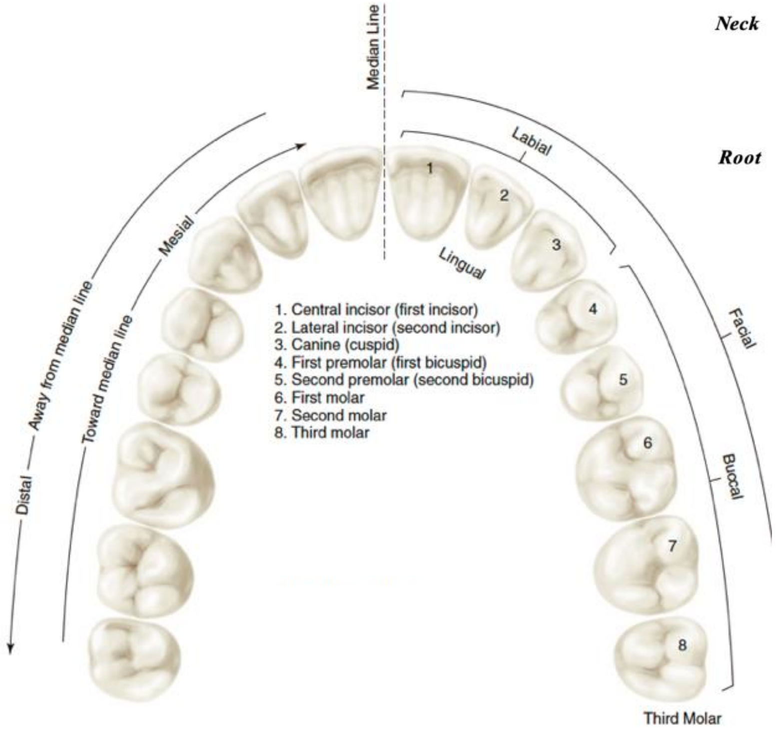

Figure 3. 2 Diagram of the upper dental arcade showing the different anatomical planes, and surfaces of the teeth with respect to their location in the mouth. Tooth numbers 1 to 8 represent each type of teeth and correspond to the left maxillary teeth. Image retrieved from Nelson (2014, p. 7).

As seen in the figure above (figure 3.2), teeth can be divided into four anatomic planes according to their location in the mouth: buccal or labial, lingual, mesial, distal, and occlusal. The buccal or labial plane is the one in contact with the internal surface of the lips and cheeks respectively; and the lingual plane is the one in contact with the tongue. The mesial plane is the one located towards the medial line, while the distal plane goes away from the medial line. Finally, the occlusal plane corresponds to the masticatory area of teeth (Hillson, 1996, p. 22).

Teeth can also be identified according to their position in the dental arcade. If they are located in the maxilla, they are referred to as superior teeth, whereas teeth in the mandible are called inferior teeth. In addition, anterior teeth are those located towards the labial section (incisors and canines), and posterior teeth are those in the buccal section (premolars and molars) (Lacruz et al., 2017, p. 942).

3.2 Teeth development

Odontogenesis

It should be taken into consideration that the complete development and eruption of teeth is a lengthy complex process that begins in utero with the formation of the primary dentition, and finishes during early adulthood, with the eruption of the third molars (Lacruz et al., 2017, p. 940).

The first stage of the process (bud stage) starts with the formation of the dental lamina composed of a layer of epithelial cells. Here, the position of the future deciduous and permanent teeth is determined by a focal thickening of the dental lamina in bud-like shapes and setting the basis for what will, later, be called the enamel organ. With the formation of the enamel organ, the bell stage happens, dividing the future teeth from the forming jaw (Fitzgerald & Rose, 2008, p. 242; Nelson, 2014, p. 14).

Subsequently, in the enamel epithelium, a histodifferentiation process begins, resulting in the development of two hard-tissue-forming cells: ameloblasts (enamel-forming cells) and odontoblasts (dentine-forming cells). Odontoblasts start with the process of dentine formation, inducing the activation of ameloblasts, which begin to secrete the enamel matrix. Enamel production consists of a deposition of a series of dome-like layers. The first layers to form are the more distal ones, that will build the future cusps. After these layers have formed, further layers, with gradually increasing dimensions, are deposited on top of each other down the walls of the crown, until enamel formation ceases when they reach the tooth surface. These stages shape the crown, which is then followed by the development of the roots in a similar course of action; internal and external enamel epithelial cells multiply in a descending way to create the dimensions of the root of the teeth (Fitzgerald & Rose, 2008, p. 242; Nelson, 2014, p. 14).

Mineralization

Right after the first layers of enamel have been positioned, the mineralization process of the enamel begins (Lacruz et al., 2017). For deciduous teeth, this process beginning in utero at around 15 weeks after fertilization, and concretes postnatally with the completion of the root. On the other hand, the mineralization process of the permanent dentition occurs entirely after birth. It is important to note that the timing of calcification and eruption varies with each tooth, allowing researchers to link the different stages of development to a specific age range of the individual, making this extremely useful for estimating age-at-death of non-adults (see figure 3.3).

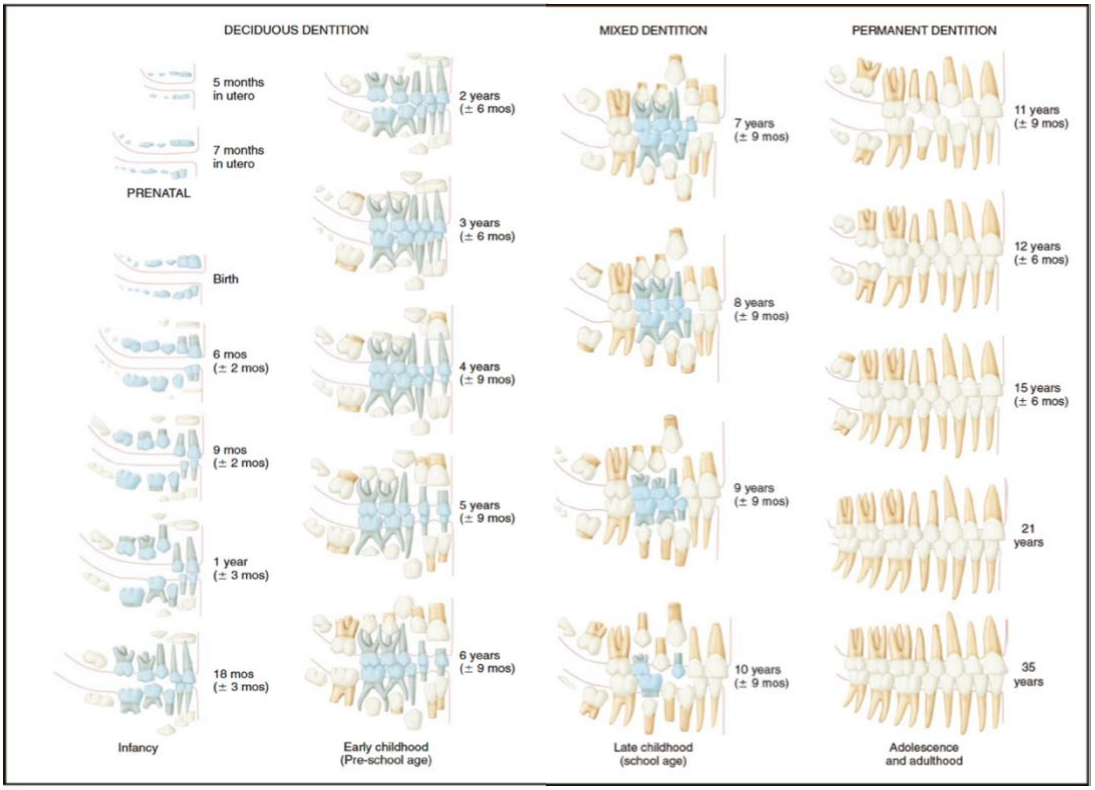

Figure 3.3 Images showing the development of the human dentition from 5 months in utero (left) to maturity (right). Teeth shadowed correspond to deciduous teeth, while the brighter teeth correspond to permanent dentition. Retrieved from Nelson (2014, pp.14-16)

Enamel developmental disruptions

During the process of enamel formation, the methodical deposition of ameloblasts results in the formation of growth markers that can be observed microscopically (Lacruz et al., 2017, p. 942). These microscopic structures are the result of different metabolic processes linked to daily and weekly body rhythms which alter the production of enamel (Hillson, 1996). Although some of these markers are a natural phenomenon of amelogenesis (such as cross striations, striae of Retzius, and perikymata), others have been identified as pathological marks since they do not correspond to the typical behavior of ameloblasts.

These abnormal types of physical disruption during amelogenesis can lead to anomalies in the quantity and quality of enamel (Goodman & Rose, 1991; Salanitri & Seow, 2013). Qualitative defects, also known as hypocalcifications, are characterized by alterations in the process of mineralization of the enamel, causing changes in the coloration and opacity of the tooth. Meanwhile, quantitative defects, also known as *enamel hypoplasias*, are associated with a deficiency in the secretion of enamel, resulting in thinner enamel layers (Blakey & Armelagos, 1985; Goodman & Rose, 1991; Hillson, 1996; Ritzman et al., 2008).

According to Hillson (1996, pp. 166-167), enamel hypoplasias can also be grouped by their form into three basic categories:

1. Furrow-type defects: The most common type, characterized by an exaggeration of the perikymata[4] (a greater than the normal spacing of perikymata grooves) This type of defects are also known as linear enamel hypoplasia.
2. Pit-type defects: These usually appear as small, dense, bounding on the cusps of posterior teeth or the edges of anterior teeth.
3. Plane-type defects: These produce large facets of brown striae planes, usually found on the cusp of posterior teeth.

In the next figure (figure 3.4), a classification of main pathological and non-pathological enamel markers is presented, showing pathological enamel defects as irregular phenomena that can occur at any time during amelogenesis due to an external trigger.

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[4] Perikymata: minute transverse ridges on the surface of the enamel of a tooth which correspond to the incremental lines in the enamel of a tooth (lines of Retzius) (Merriam-Webster Dictionary)

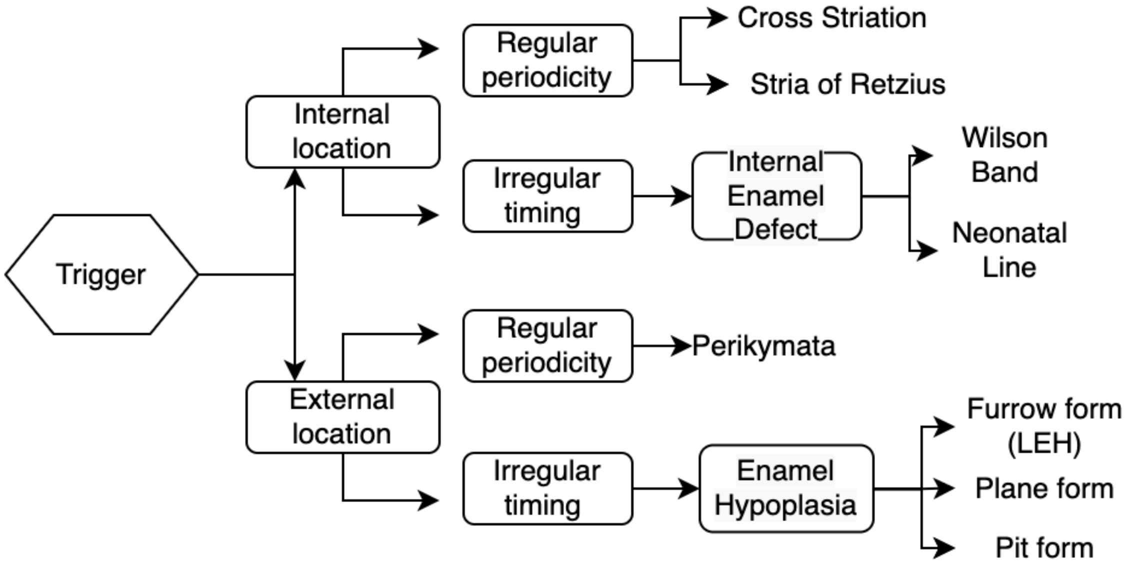

Figure 3.4 Pathological and non-pathological enamel markers. Adapted from Fitzgerald and Rose (2008, p. 289)

3.3Linear Enamel hypoplasia

Etiology of LEH

Enamel defects can be attributed to several number of factors, which can be classified into three major groups: hereditary, localized, and systemic defects.

Enamel hypoplasia has been identified as the most common type of enamel defect, and although several hereditary conditions have been related to enamel defects in both permanent and deciduous dentition, these represent the minority of the cases (Hillson, 1996; Nikiforuk & Fraser, 1981; Salanitri & Seow, 2013). Hereditary enamel hypoplasia are characteristically severe defects affecting most teeth in permanent and deciduous dentition.

In the same way, localized hypoplasias in specific tooth are more likely to occur in permanent dentition, linked to injuries, inflammation or direct trauma, however the incidence of these cases is low (Goodman & Rose, 1990, 1991; Kanchan et al., 2015; Salanitri & Seow, 2013). Localized hypoplasias generally appear in a specific area of the dental arcade, affecting one tooth, or several consecutive teeth (Goodman & Rose, 1990; Suckling, 1989).

The most frequent type of enamel hypoplasias are furrow-type-defects that occur horizontally on the buccal or labial surface of teeth, because of this, they are also known as linear enamel hypoplasia (LEH from now onwards) (Goodman & Rose, 1991; Nikiforuk & Fraser, 1981). These types of defects are distributed in more than one non-consecutive tooth throughout the dental arcade and can be paired between teeth (Hillson, 1996). They are considered a sign of systemic period of physiological stress during an individual's developmental stage, and they are considered a sign of a *non-specific* stress disorder, since multiple conditions have been associated with this systemic disruption of the enamel (Bereczki et al., 2018; Goodman & Rose, 1990; Hillson, 1996).

During the second half of the last century, multiple efforts were made to establish the main causes of enamel hypoplasia. In permanent dentition, Nikiforuk & Fraser (1981) found a relation between calcium insufficiency and enamel hypoplasia. In the same way, Klein (1945) observed a link between enamel defects and vitamin A or D deficiency. Sweeney and coworkers (1971) found a higher prevalence of LEH in malnourished children from Guatemala, while Goodman and Rose (1991, p. 184) reported a lower prevalence of LEH in adolescents who received dietary supplements during childhood than their peers who did not, concluding that "a mild to moderate undernutrition during enamel formation is causally linked to the formation of LEHs". Furthermore, in both permanent and deciduous teeth, LEH has been associated with severe viral, parasitic, and bacterial infectious diseases characterized by symptoms that include high fevers, diarrhea, and or vomits (Bereczki et al., 2018; Ford et al., 2009; Goodman & Rose, 1990; Suckling, 1989).

The causes for LEH formation in the deciduous dentition imply both pre-natal and post-natal periods, considering the formation timing for these teeth. Therefore, maternal health plays a significant role in the development of enamel hypoplasias during the intra-uterine period (Temple, 2020, p. 68). Fetuses are more susceptible to develop LEH if the mother is suffering from congenital or acquired pathological conditions such as diabetes, kidney disease, heart disease or any type of infectious disease (Cook & Buikstra, 1979). Moreover, Purvis et al. (1973) found a correlation between maternal vitamin D deficiency, neonatal tetany, and linear enamel hypoplasia in newborns. Likewise, congenital syphilis acquired from the mother, and hereditary vitamin D dependency rickets have also been linked to enamel hypoplastic defects (Nikiforuk & Fraser, 1981; Salanitri & Seow, 2013). In addition, during the post-natal period, studies have shown that enamel hypoplasia is more frequently present in prematurely born children with lower birth weight than full term newborns with average birth weights (Clarke, 1980; Cook & Buikstra, 1979; Lukacs et al., 2001; Salanitri & Seow, 2013).

Although most of the causes listed above are related to nutritional deficiencies, dietary restrictions, and infectious diseases, LEH's etiology has proven to be multifactorial, and thus considered a non-specific stress marker of physiological stress in osteoarcheological studies. The relation between timing of teeth development and age of individuals, plus enamel's characteristic of not remodeling, provides a permanent record of physiological stress during an individual's lifespan (Hillson, 1996, p. 206; Temple, 2020, p. 68). These characteristics have allowed researchers to estimate the age at formation of LEH marks on teeth.

Calculating chronology of LEH

The most used methods for estimating the age of an individual at the time of LEH formation are the ones proposed by Goodman & Rose (1990), and Reid and Dean (2000; 2006). The first one, consists of a linear regression equation based on a study by Swärdstedt (1966) who calculated the growth rate of each tooth and divided their crown into six-month linear segments. To develop this equation, Goodman and Rose (1990, p. 97) used Swärdstedt's (1966) crown growth rates, the total crown height, and the distance from the CEJ to the LEH defect.

Some of the critiques for this methodology include the assumption that enamel growth rates are constant, as well as the exclusion of cuspal enamel formation, which tends to underrepresent the early formation of enamel defects (Ritzman et al., 2008).

The second method, developed by Reid and Dean (2000) solved this issue by providing a methodology based on a decile chart, in which the crown of a tooth was divided into ten segments as seen in the figures below (figure 3.5) ; then, they calculated the average number of days it takes for each segment to develop through histological research, considering factors such as cuspal enamel formation and the average number of days between birth and crown development in permanent teeth (Cares Henriquez & Oxenham, 2019; Ritzman et al., 2008).

Therefore, this method allows for the macroscopical analysis of LEH, by measuring the distance from the CEJ to the hypoplastic lesion, and matched with the correspondent age interval previously established (Hassett, 2014; Reid & Dean, 2006; Ritzman et al., 2008).

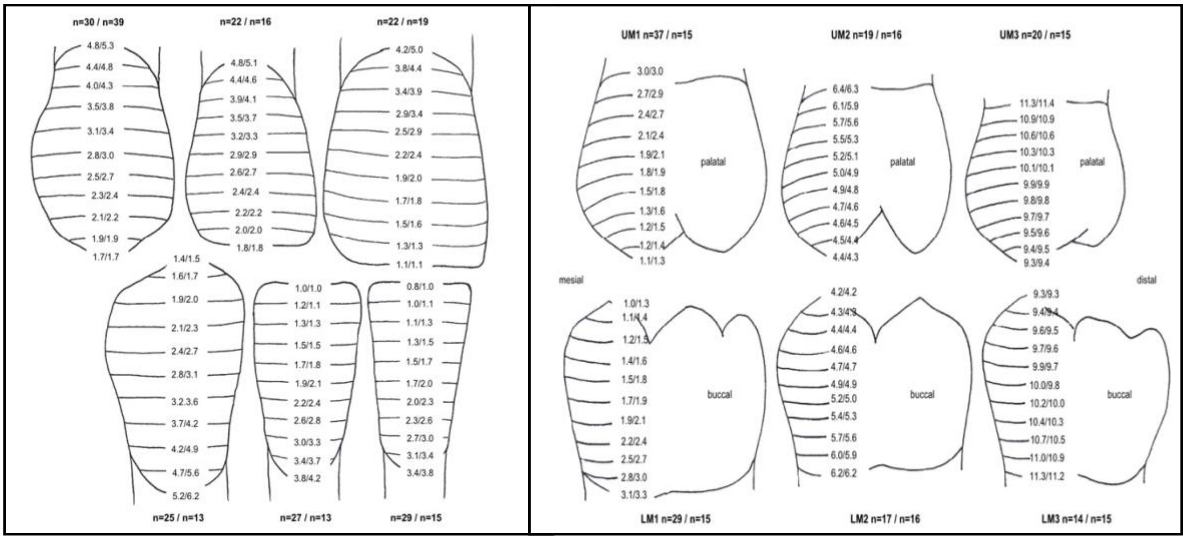

Figure 3.5 Chronological ages associated to each decile per tooth of the African sample/ and European sample (incisors and canines left, and molars right). Retrieved from Reid and Dean (2006, pp. 343-344)

LEH research in archeology

Because enamel hypoplasia has been considered a non-specific stress disorder linked to growth disruptions during childhood, there is a vast number of archeological studies focusing on investigating the relationship between possible sources of stress and LEH to determine children's health (e.g Blakey et al., 1994; Goodman & Armelagos, 1989; Goodman & Rose, 1990, 1991 and many others).

Some studies of LEH have served to identify the differences between socio-economic status and living conditions. For instance, Nakayama (2016) analyzed individuals from the 18th to 19th century in Japan, finding a higher prevalence of LEH in lower status individuals than those of higher social status, which indicates better living conditions during childhood for the more privileged individuals.

Moreover, LEH has been used to assess the effects of urban living in the past by comparing prevalence of LEH between urban and rural environments. Several studies have been made specifically for medieval and post-medieval European populations. Miszkiewicz (2015) compared prevalence of enamel hypoplasia of a rural and urban British medieval cemetery, revealing higher percentages of defects for rural inhabitants compared to the city dwellers. However, this trend has shown to differ depending on the context. Specifically for the Netherlands, Schats (2016) found no significant difference between LEH patterns of a rural medieval population compared to its urban counterpart. Among the most relevant ones in terms of frailty and urbanization, is the one by Palubeckaitė et al. (2002), who investigated differences in sex, status, and contexts from urban and rural post-medieval Danish and Lithuanian settings, revealing higher stress markers in rural populations, as well as higher child mortality compared to the urban contexts.

LEH in terms of frailty

LEH has also been useful to study patterns of frailty, morbidity, and mortality, with the premise that the presence of enamel hypoplasia is related to decreased life expectancy. For instance, Stodder (1997) analyzed the frequency and age at formation of LEH in the dentition of Latte Period sites in Guam, Mariana Islands; she found that individuals dying before age 16 and between 16 and 21 had a higher prevalence of LEH than those surviving into later life stages.

Multiple studies performed in a wide array of populations from different temporalities yield similar results, pointing towards an association between higher rates of early life stressors and decreased life expectancy (Armelagos et al., 2009; Boldsen, 2007; Goodman & Armelagos, 1985; Miszkiewicz, 2015; O'Donnell & Moes, 2021).

However, other studies have found opposite results when analyzing non-adults, in which individuals without LEH had longer average life spans than individuals with LEH. For example, Cucina (2011), analyzed stress during the early years of life of the Classic Mayan population of Xcambó; although he found higher frequencies of LEH in non-adults than adults; within age categories of non-adults, he found no association between presence of hypoplasia and age at death. In addition, Bennike et al. (2005) compared LEH rates between individuals from a lepra cemetery vs. individuals from a regular cemetery, finding that older individuals presented more lesions than younger individuals, which would go in accordance with the Osteological Paradox.

These example shows the importance of using both samples of non-adults and adults, since they can be reflecting different frailty patterns. Although these studies tend to vary, most of them points towards a tendency of higher prevalence of LEH in younger individuals compared to older ones, which has been proven useful to address frailty in non-survivors. However, as stated by Bennike et al. (2005, p.743), studies investigating associations between LEH and mortality should carefully consider the different external and internal circumstances that could influence the observed patterns.

LEH in relation to weaning and breastfeeding practices

Other factor that has been thought to influence the development of LEH in permanent teeth, is the process of weaning. This assumption was made based on different studies that found an association between high percentages of LEH during historically documented weaning periods (e.g Blakey et al., 1994; Maclellan, 2005).

According to these studies, the change of feeding strategies during weaning can represent high levels of physiological stress in children; the cease of breast milk, along with the introduction to new pathogens in the environment can lead to a compromised immunity system (Blakey et al., 1992; Goodman et al., 1984; Moggi-Cecchi et al., 1994, p. 300). An example of a study that agrees with the weaning hypothesis is the research by Moggi-Cecchi and colleagues (1994), who analyzed the age-at-formation of LEH from 38 individuals dated to the 19th century in Florence, Italy. Their research revealed a stress peak between 1.5 years and 3.5, which suggests a relationship between stress post-weaning and the gradual introduction to diet, since historical sources revealed a weaning period of 12 and 18 months (*idem*, p. 316). Other recent study that agree with the weaning hypothesis is the one reported by Temple (2020), who found that the majority of LEH lesions in a population of hunter gatherers occurred between 2.0 and 4.0 years of age, which could go in accordance to weaning ages.

Nonetheless, as Katzenberg and colleagues (1996) indicate, the relation between age at formation of LEH and weaning might be coincidental and influenced by methodological and theoretical biases. Some researchers have tested the weaning hypothesis using isotopic analysis on the basis that, during lactation, infants appear higher in the food web than their mothers and, therefore, their nitrogen values will be higher than those of their mothers; the weaning age corresponds to the time when nitrogen values decrease, and values of other isotopes related to diet appear (Sandberg et al., 2014, p. 281). The study carried out by Sandberg et al., (2014), have yielded results that are consistent with the weaning hypothesis, showing that LEH occurs during the process of weaning according to nitrogen and carbon values. However, in some populations this has not been the case, for example, Dąbrowski et al. (2020) did not find any association between the presence of LEH and isotopes related to breastfeeding and weaning (δ^{18}O and δ^{13}C), in early modern cemeteries in Wroclaw, Poland. This shows that the correlation between breastfeeding, weaning and the appearance of LEH lesions is complicated, and several factors must be taken into consideration before inferring direct correlations between these two phenomena.

LEH in Deciduous teeth

As seen before, there is a large body of research conducted on permanent teeth to assess early childhood stress, nonetheless fewer studies have investigated LEH in deciduous teeth. Some studies have been conducted in living populations; Sweeney and collegues (1971) examined children from Guatemala City who were recovering from malnutrition. According to the authors, 73% of children who presented third degree malnutrition had LEH, while only 42% of children with second degree malnutrition presented LEH.

One of the first archeological studies on this subject was conducted by Cook & Buikstra (1979), who investigated pre-natal enamel defects such as deciduous enamel hypoplasia, hypoplasia related caries, and hypocalcification in Middle and Late Woodland individuals. These authors found an association between deciduous enamel defects and other signs of stress, such as evidence of anemia and infectious disease. Later, Blakey et al. (1994) explored the chronological distribution of LEH in non-adults from the Dickson Mounds (A.D. 950-1300) to assess differences in survivorship, and frailty. With this pioneer study, the authors determined most affections during the intra-uterine period, which indicates evidence for maternal stress. More recently, López-Lázaro and collegues (2022) investigated enamel hypoplasia in deciduous teeth to determine socioeconomic status and health conditions during economic and social crisis during the 19th and 20th century in Granada, revealing that "23.8% of children presented enamel hypoplasia during the prenatal period, 19.1% during the perinatal period, and 57.1% in the postnatal period" (*idem*, p.22).

3.4 Caries lesions

Besides enamel hypoplasia, other common pathology affecting dental tissue is caries. Dental caries is nowadays considered an issue of public health, remaining the most prevalent chronic disease in children, and the main cause of permanent and deciduous tooth loss (Bagramian et al., 2009, p. 3). In its most severe states, caries can cause discomfort, inflammation, and tooth loss which has shown to negatively affect an individual's overall health, repercussing in their nutritional, social and physiological well-being (Crall & Forrest, 2018, p. 304).

This common pathology in human kind has been catalogued as an infectious disease characterized by the irreversible and progressive destruction of dental tissue (Caselitz, 1998; Hillson, 2001; Ortner, 2003). The infection starts by affecting the external layers of the tooth, and without the proper care, it can propagate onto the internal layers which can lead to the total destruction of the crown (Hillson, 2001). The affection is usually local, however, it can be transmitted to adjacent teeth or even to the bone, causing inflammation, abscess and tooth loss (Caselitz, 1998).

Etiology of caries

Due to the complexity of this condition, many efforts have been made over the years to understand the epidemiology and etiology of dental caries. Back in 1890, Miller proposed the Chemo-Parasitic Theory explaining the bodily mechanisms that contribute to the formation of caries. This theory states that saliva along with the acids produced by microorganisms during food degradation, specifically of sugars, are the main causes of dental decay (Mandel, 1983, p. 927). It was not only until 1960 that Keyes identified the three main biological factors for the emergence of caries: dental plaque, the tooth and the diet (also known as the Keyes Triad); setting the bases for the Ecological Hypothesis (Eriksen & Bjertness, 1991, p.477). This hypothesis explains the demineralization of the enamel as a consequence of changes in the pH of the mouth, caused by the fermentation of sugars by acidogenic microorganisms found in the dental flora (Takahashi & Nyvad, 2016). These sugars can be found in sweet foods with high content of natural sugars such as glucose, fructose, and any other type of processed sugar, as well as in starchy foods.

Once the superficial layer gets affected, colonies of microorganisms such as streptococci, lactobacilli and actinomycetes, settle on the tooth causing irreversible damage (Caselitz, 1998; Eriksen & Bjertness, 1991; Hillson, 2001).

Although the process of carious lesion formation has been related to dietary patterns and food consumption, because of the infectious character of the disease, host biologic factors such as immunological resistance and genetic predisposition to caries have shown to play an important role in the formation of dental caries (Scully, 1981, p. 110). Likewise, quality and quantity of saliva is highly important for preventing dental decay since it creates a "buffering effect" on acids and a rinsing effect, reducing the risk of coronal caries (Reich et al., 1999, p. 18).

Nowadays, the most accepted theory explains that the etiology of this infectious disease is multifactorial, involving a combination of intrinsic and extrinsic sociocultural and biological variables. This theory, known as the Caries Balance Theory, describes dental caries as a "dynamic disease" that depends on the "equilibrium of a myriad of risk factors" as well as "protective factors" (Crall & Forrest, 2018, p. 308). This theory recognizes the complex interaction between biological factors (e.g., dental microbiota, intraoral environment, tooth structure abnormalities, genetic predisposition to caries, biological sex, etc.) and behavioral and sociocultural factors related to individual and population dynamics (dietary practices, lifestyle of the individual, general oral health, socioeconomic status, access to cariogenic substances, etc.).

Research of caries in archeology

Since teeth are often preserved in the archeological record, many of the sociocultural and biological factors of dental caries have been studied from an osteoarcheological perspective. Regarding sociocultural factors, caries lesions disparities have been well documented between distinctive populations. For example, in terms of socioeconomic status, multiple studies indicate higher frequencies presented in people from lower strata (Caselitz, 1998, p. 216; Hillson, 2001; Larsen, 1997). This relation has been associated to dietary differences between lower and higher strata, as well as access to dental healthcare and an overall higher quality of life for privileged sectors of the population (Crall & Forrest, 2018) . Therefore, dental caries has served as an indicator of socioeconomic status in archeological studies.

Dental caries has also served as an indicator of the lifestyle of a population in relation to their diet as well as to detect shifts in their dietary patterns or subsistence strategies. This has been the case for researching the transition to agriculture, where a higher prevalence of caries has been recorded for agrarian societies compared to hunter-gatherers (e.g. Larsen, 1991; Temple & Larsen, 2007; Turner, 1979).

Likewise, the transition from rural to urban environments has also been researched in terms of oral health. For European populations, Pitts and Griffin (2012) explored oral health in individuals from late Roman Britain (250–410 C.E.), detecting lower caries lesions and signs of periodontal disease in urban settlements compared to rural contexts. Similarly, Griffin (2017) performed a diachronically study of the effects of urbanization on oral health from Roman to late medieval Britain, finding that in general, urban populations had less caries rates than rural populations; during the late medieval period oral health decreases significantly, with caries rates increasing for both contexts (*idem*, p. 354). For the Lower Countries, Schats et al. (2021) investigated the relationship between diet and urbanization during late medieval times in Holland by analyzing caries rates and Carbon and Nitrogen isotope levels ($\delta^{15}N$ and $\delta^{13}C$). For this time period and region, caries rates appear higher in urban contexts than in rural settlements, pointing towards a diet rich in cariogenic foods for urban citizens (*idem*, p. 151).

Regarding biological factors, the chronic and cumulative character of dental decay has been well established. According to Hillson (2001, p. 253), the incidence of caries increases with age due to the progressive and constant exposure to the oral environment. Therefore, in populations without access to dental healthcare, older individuals present higher rates of caries than the young. This has been the case for postmedieval European populations. The study conducted by (Lingström & Borrman, 1999) confirms an age-related increase in dental decay in a 17th century Swedish population. In their research, caries percentages go from 8.4% in young adults to 22% in middle adults (*idem*, p. 399).

Other biological factors include different rates of caries between biological sexes. Dental studies in archeological populations have detected this sex-related pattern regarding caries lesions, showing higher frequencies of caries in adult female individuals than adult males (Larsen, 1997). Although this pattern was first attributed to differences in gender specific dietary and cultural practices, clinical studies have demonstrated a physiological reason behind high rates of caries in females (Lukacs, 2008, p. 901, 2011a, p. 646; Lukacs & Largaespada, 2006, p. 549). According to Lukacs (2008, p. 905) females are predisposed to the formation of dental caries due to genetic and physiological factors related to the fluctuation of hormones during the menstrual cycle and pregnancy. These factors include saliva quantity and quality, which has shown to be generally lower in females than in males. Moreover, the peak of estrogen hormones (specifically during pregnancy) negatively affects the saliva flow and composition, altering the oral microbiome and enhancing caries formation (Kumar et al., 2022, p. 477; Lukacs, 2011, p. 658). Due to the hormonal fluctuations experienced in between menstrual cycles and in pregnancy, immune system is often compromised leading to a general dental health decline in female individuals throughout their life (Boggess & Committee, 2008).

In addition to these biological factors, behavioral and dietary patterns seem to exacerbate the cariogenic female pattern01/12/2023 12:59:00. For instance, labor division in many cultures associates women to food preparation tasks, which propitiates the constant interaction with carbohydrates and other cariogenic foods (Lukacs, 2011, p. 654). Likewise, high-sugary food cravings during pregnancy and menstrual cycle are also considered a factor for dental decay in females (Lukacs, 2008, p. 911).

Caries in deciduous teeth

Caries on deciduous teeth have been bidirectionally associated to health and nutritional deficiencies (Tanner et al., 2022, p. 105). On one hand, undernutrition affects the development of the teeth and alters the pH of saliva, which leads to the demineralization of the teeth resulting in a higher risk for dental caries (Tanner et al., 2022, p. 105). On the other hand, the presence of severe caries lesions can have an impact on a child's nutrition status, in the sense that pain and discomfort produced by the cavity can affect the ability of the infant to chew and process food (Crall & Forrest, 2018, p. 305). In fact, clinical studies have found a relation between low body weight and infants with nursing caries (Nicolau et al., 2007, p. 243). Moreover, severe caries during childhood have been linked to oral health issues in adulthood, as many studies confirm that children with early childhood caries present a higher risk of experiencing dental decay during adulthood (Crall & Forrest, 2018, p. 314).

An association between mother’s oral health and early childhood caries has also been documented. Because of the infectious character of the disease and the dependence of children to their caregivers (usually mothers) in early life stages, high levels of caries in the mother can be an indicator of caries risk among her offspring (Iida, 2017, p. 468). Cariogenic bacteria are often transmitted through behaviors such as food sharing and food preparation, in which saliva acts as method of transportation for these pathogens; this can occur before the eruption of teeth (Crall & Forrest, 2018, p. 317; Iida, 2017, p. 468). Caregivers relationship with cariogenic foods, as well as their cleaning habits, health beliefs, and behaviors have also reported to be an influential factor in development of caries in children (Seow, 2012, p. 160).

From a life-course perspective, recent studies have shown that prenatal maternal health may have an impact on the formation of early childhood caries. According to a research conducted by Schroth and collegues (2014), severe low levels of vitamin D in mothers result in significantly higher rates of caries in the primary dentition of their children. This is thought to be the result of a lack of mineralization of the teeth, leading to high risk of enamel defects and caries (Suárez-Calleja et al., 2021, p. 8).

Several clinical and osteoarcheological studies have identified a relation between enamel hypoplasia and caries. According to Cook & Buikstra (1979, p. 650) the so called “circular caries” are a distinctive lesion of the deciduous dentition resulting from severe enamel defects. Therefore, as Targino et al. (2011, p. 424) mention, enamel hypoplasia seems to be the most frequent enamel defect associated with dental caries. The presence of enamel hypoplasia increases the probability of dental decay specifically in deciduous teeth because of their higher vulnerability to bacteria colonization; likewise, the shape of the defect might propitiate dental plaque to accumulate in the area which might lead to dental decay (Infante & Gillespie, 1977, p. 495; Vargas-Ferreira et al., 2015, p. 543). This goes in accordance to the findings by Halcrow and Tayles (2008), who analyzed deciduous teeth in a late prehistoric Southeast Asian population, finding high frequencies of caries in the bucco-labial surface of teeth, which could be linked to enamel hypoplasias.

Chapter 4: Materials and Methods

In this chapter, I will first provide a thorough description of the assemblages selected for this research, including archeological and osteological information about them. Subsequently, the methods applied for this research will be explained, along with the different steps followed to complete this research.

Materials

As stated in previous chapters, the materials comprised for this study come from two different post-medieval Dutch contexts spanning from the 17th to the 19th centuries. The first collection of individuals originates from the city of Arnhem (1650 to 1829 A.D.), while the second collection comes from the Middenbeemster countryside (1623 to 1867 A.D). Currently, both collections are stored at the facilities of the Laboratory for Human Osteoarcheology at the Faculty of Archeology of Leiden University, where ongoing osteoarcheological research is being carried out. The following paragraphs describe more thoroughly the characteristics of these two assemblages of human skeletal remains and their historic and temporal context. The image below (figure 4.1) indicates the location of these sites in relation to the Netherlands; for more detailed maps of the regions, see Appendix A.

Figure 4. 1: Map indicating the location of Arnhem and Middenbeemster in relation to the Netherlands.

4.1 Arnhem

Historical context

The first sample selected for this study consists of 64 individuals retrieved from the St. Eusebius Church's cemetery of Arnhem and dated from post-medieval 17th and 19th century (1650- 1829). Arnhem is a city in the eastern part of the Netherlands, and capital to the province of Gelderland since 1817. The city is located between the Nederrijn and Sint-Jansbeek rivers, both highly important, since they remain the main sources of water for domestic and industrial purposes (Baetsen & Baetsen, 2020, p. 34). According to historical sources, before the 17th century, the city's main economic activities were based on small leather production companies and breweries; however, from the second half of the 17th century, the city became more industrialized, and many textile and shoe factories emerged (Klep, 2009, p. 117). Similarly, with the popularity of tobacco, much of the industry at the time centered around this activity (Roessingh, 1979, p. 48). These job opportunities attracted many people from all over the region, resulting in a major population expansion. Between 17th and 19th century, Arnhem doubled its population, going from 10,080 inhabitants in 1795, to around 20,000 during the 1850's (Casna et al., 2021; de Vries, 1984, p. 385).

Although some immigrants benefited from their high status, most of Arnhem's inhabitants lived and worked in deplorable conditions. People usually lived in conglomerate spaces, with restricted access to fresh air, clean water and sanitation services (van der Woud, 2010, p. 19; de Vries, 1984). In addition, men, women, and sometimes children, carried out tough manual labor, working from "12 to 20 hours a day" (van der Woud, 2010, p. 316).

It was not until the beginning of the 20th century that conditions started to get better, after multiple health campaigns were carried by the government that improved the sanitization and living conditions of the inhabitants (Osterloh, 2020, p. 29; van der Woud, 2010, p. 317).

Archeological context

Regarding the excavation of the remains, in 2017, the city hall authorities of Arnhem decided to execute a project to redesign the city center of the town. Due to the possibility of causing structural damage to ancient monuments, the commercial archeology company *RAAP* was hired to complete an archeological research project to document, register, and rescue the material heritage found on these structures, including the St. Eusebius Church's cemetery (Baetsen et al., 2018, p. 17; Baetsen & Baetsen, 2020, p. 318).

According to the excavation report from the Arnhem archeological project, 752 burials were exhumated from which 630 were identified as primary burials and 122 were determined as secondary burials. During the excavations, the burials located in the *Oude Kerkhof* (the northern side of the cemetery), were associated with lower socio-economic status (Baetsen et al., 2018, p. 20). In addition, two time periods were identified in this side of the cemetery, the first one dating from 1350 to 1650, and the second one dating from 1650 to 1829 (*idem*, p. 22). The individuals from this sample belong to the latter one, thus, representing the individuals from working classes between the 17th and 19th centuries.

Most of the burial's preservation status was catalogued as "good", meaning that although most of the skeletons presented little signs of fragmentation as well as undamaged cortex and compact bone, not all skeletons were complete (Baetsen & Baetsen, 2020, p. 354).

4.2 Middenbeemster

Historical context

The second sample selected for this study consists of 113 individuals belonging to the skeletal collection of Middenbeemster, retrieved from the Keyserkerk cemetery. This town was one of the firsts to settle in the Beemster Polder, a large portion of land created in the beginning of the 17th Century after draining the Beemster Lake (Falger et al., 2012, p. 202). The inhabitants of Middenbeemster were mostly dedicated to agricultural and livestock activities, specifically dairy farming (Liagre et al., 2022, p. 769). Between the 17th and 18th centuries, the agricultural industrial revolution had not arrived yet to Northern Holland, and the economic activities of rural sites were dependent on traditional methods of farming, involving manual work (Falger et al., 2012, p. 202; Waters-Rist & Hoogland, 2018, p. 107). By 1840, the Beemster community counted 2971 people, most of them belonging to a middle-lower socio-economic class (Casna et al., 2021, p. 894; Falger et al., 2012, p. 210).

Some farms were owned or leased by families, and both men and women were actively involved in the business. Other farms, however, were run by hired workers on a permanent or short-term basis (Waters-Rist et al., 2022, p. 3). Between the 17th and 19th centuries dairy products were already indispensable in Dutch cuisine in the form of cheese and butter, and by the 19th century, the value of these products had increased considerably, making dairy farming a generally economically profitable activity (Wintle, 2000, p. 108). At this time, families had a clear economic advantage over landless workers, as they could benefit directly from their products.

Houses and farms were built in a similar way throughout the Beemster area, with the "typical bell-jar shape" that allowed the storage of hay, and often livestock in winter (Casna et al., 2021, p. 894). Therefore, the living conditions might have been similar for most of the inhabitants regarding their socioeconomic status (Waters-Rist & Hoogland, 2018, p. 110).

According to historic sources, the cemetery of Keyserkerk was in use from 1623 to 1867 and was the only one available for its municipality during most of this time (van Spelde & Hoogland, 2018, p. 325). 01/12/2023 12:59:00Therefore, the individuals from this collection represent not only the population of Middenbeemster, but of all the surrounding towns and villages that conformed the Beemster Polder, including all socio-economic classes.

Archeological context

Due to the renovation of the southern area of the church in 2011, an archeology rescue project took place in order to preserve the historical and cultural value of the archeological materials and human remains buried in the cemetery. The excavation of the Keyserkerk cemetery was carried out by the commercial company Hollandia Archeologen in collaboration with Leiden University, revealing around 488 primary burials and some secondary depositions (van Spelde & Hoogland, 2018).

The excavation identified two different time frames in the cemetery: the oldest period, the "clay phase", and a more recent one identified as the "sand phase". The first one refers to 56 burials dating to the beginning of the 18th century (van Spelde & Hoogland, 2018, p. 326). During this time there was a status distinction between burials, with wealthier people buried indoors, and those of lower socioeconomic status buried in the exterior of the church (van Spelde & Hoogland, 2018).The second phase refers to 432 burials from between 1829 and 1867, when the deposition of human remains inside the church became illegal, and all individuals were buried in the outer cemetery regardless of their socio-economic status, in a substrate of dune sand (van Spelde & Hoogland, 2018, p. 326).

The skeletal collection of Middenbeemster presents a generally good preservation state and completeness of the skeletal material of the individuals, which facilitates the osteological analysis (Lemmers et al., 2013, p. 42). It is important to note that in non-adults, the preservation state and completeness of the skeletal remains was lower than in the adults.

4.3 Osteological data

In the years following the excavation, the skeletal materials from both collections were stored in a laboratory and examined to estimate the age at death and sex of the individuals, along with the recording of any pathological conditions present in bones and teeth. For the collection of Arnhem, the biological profiling of the individuals was conducted by MSc. Willem Baetsen and Dr. Steffen Baetsen as part of the official excavation report, while the Middenbeemster collection, was analyzed by Lemmers and colleagues (2013). Furthermore, both collections were re-assessed by master students of the Laboratory for Human Osteoarcheology from Leiden University under the supervision of Dr. Sarah Schrader and Dr. Rachel Schats.

Estimation of sex

Because the current macroscopic methods for non-adult sex estimation do not provide sufficient accurate results, only the sex of adult individuals was established (Baetsen & Baetsen, 2020, p. 358; Lemmers et al., 2013, p. 36). For both sites the estimation of sex was conducted following the guidelines created by the Workshop for European Anthropologists, which utilizes a scoring system for skeletal features from the skull, mandible, and pelvis (WEA 1980 in Baetsen & Baetsen, 2020, p. 361). In addition, these features were also compared to the standardized method established by Buikstra and Ubelaker (1994) (Lemmers et al., 2013, p. 36).

A combination of other methods was used to increased accuracy of the results. These methods analyze morphological features such as the shape of different landmarks from the *os coxa* following Phenice (1969) and the morphology of the sacrum as described in Bass (1987) (Lemmers et al., 2013, p. 36). Likewise, osteological measures from the humerus were assessed after Stewart (1979) and Steyn and Iscan (1999), as well as measurements from the clavicle and the scapula following McCormick et al. (1991) and Bainbridge & Genovés Tarazaga (1956) (Lemmers et al., 2013, p. 36).

Estimation of age at death

A similar process occurred for the estimation of age at death of individuals; standard WEA (1980) guidelines were used for both collections, as well as other commonly accepted methods. For non-adults, the age-at-death was determined based on the dental eruption of the deciduous teeth following the methodology established by Ubelaker (1978), the dental eruption of permanent teeth based on Moorrees and collegues (1963), the dental measurements of teeth and roots determined by Demirjian et al. (1973) and Liversigde et al. (1999), plus the general ossification of the skeleton, the closure of epiphyses of long bones, and the general length of long bones and clavicle following Schaefer et al. (2009), Maresh (1955) and Black and Scheuer (1996) (Baetsen & Baetsen, 2020, p. 356; Lemmers et al., 2013, p. 36). For adults, age at death was estimated using morphometric features using the pubic symphysis, the auricular surface, and the sternal rib end based on Buikstra and Ubelaker (1994). In addition, other features were used such as the degree of cranial suture closure, and the extent of dental attrition following Meindl and Lovejoy (1985) and Maat (2001) (*ibidem*). Depending on their age-at-death, the individuals were assigned to one of the age categories represented in table 4.1. If a narrower age range could not be obtained, the category 18+ years was used.

Thanks to the efforts to perform biological profiling of the osteological collections, it was possible to determine that in both collections there are individuals of all ages ranging from fetuses (minor or equal to 38 weeks of gestation) to old adults (50 years or older), as well as male and female adults (Baetsen et al., 2018; Baetsen & Baetsen, 2020; Lemmers et al., 2013).

Fetus	<38weeks
Perinatal	38-42weeks
Infant	42weeks-3 years
Child	4-6years
Juvenile	7-12 years
Adolescent	13-18 years
Early Young Adult	18-25 years
Late Young Adult	25-35 years
Mid adult	36-50 years
Old adult	50 years +

Table 4.1: age-at-death categories as established by WEA (1980). Retrieved from Lemmers et al. (2013, p. 37)

4.4 Sample determination and description

Because of the characteristics mentioned before, the samples analyzed for this study were selected from these osteological assemblages. The sociocultural and temporal contexts of Arnhem and Middenbeemster, in addition to the diversity of the sample in terms of number of adult and non-adult individuals, sex, age, and social status, serve as a perfect parameter for assessing the effects of urban living on infant and maternal stress in the Netherlands during post-medieval times.

Although as mentioned before, the Middenbeemster sample presented some issues regarding the preservation and completeness of the skeletal remains of non-adults, teeth were well preserved in adults and non-adult individuals from both collections.

The individuals for this research were selected regarding the completion of the crown in both deciduous and permanent teeth, regardless of sex or social status. Therefore, the individuals that correspond to the age categories of *fetus* and *perinatal* were not considered since at these stages the crowns of all deciduous teeth are not fully developed yet, and therefore no dental pathologies can be observed. In the same way, individuals older than 35 years of age (*mid* *adults* and *old adults*) were excluded from this study, due to the likelihood of developing dental wear at this stage that could invisible the presence of enamel defects. This was also the case for individuals whose age at death could not be determined. The following table (4.2) shows a detailed division of the individuals in the sample:

	Arnhem	**Middenbeemster**	**Total**
Infant	19	34	53
Child	5	5	10
Juvenile	2	14	16
Adolescent	12	6	18
Early Young Adult	10	21	31
Late Young Adults	16	33	49
Total	64	113	177

Table 4.2 Sample size; total number of individuals from Arnhem and Middenbeemster per age-at-death categories

As mentioned before, dental material can be lost from archeological samples for several reasons, and to avoid having individuals with contrasting number of teeth that could skew the data, dental analyses were performed based on the number of teeth rather than on an individual level. The teeth were divided into permanent and deciduous, since non-adults can have both types. It is important to mention that all teeth without a complete crown were excluded from the analysis, including those that were not fully developed, or those with significant tooth.

In total, 3,592 teeth were analyzed; 1,292 from the Arnhem collection and 2,300 from Middenbeemster, as seen in the following table (4.3).

	Arnhem				***Middenbeemster***			
Age-at-death	Deciduous	%	Permanent	%	Deciduous	%	Permanent	%
Infant	226	70%	0	0%	413	77%	8	0%
Child	68	21%	12	1%	49	9%	51	3%
Juvenile	28	9%	16	2%	73	14%	298	17%
Adolescent	0	0%	316	33%	0	0%	148	8%
EYA	0	0%	249	26%	0	0%	524	30%
LYA	0	0%	377	39%	0	0%	736	42%
Total	322	100%	970	100%	535	100%	1765	100%

Table 4.3: Total number of analyzed teeth per site and age-at-death category

4.5 Ethical considerations

The ethical management of human remains is a crucial issue in our discipline. Therefore, since this thesis focuses on the analysis of human remains, it is highly important to touch upon the ethical guidelines that were taken into consideration throughout this research project.

Since the Netherlands has not yet established a legal background for handling human remains, as recommended by Waters-Rist and colleagues (2018), this project was carried out following the “Codes of Ethics and Practice” from the British Association for Biological Anthropology and Osteoarcheology (BABAO, 2019). These guidelines present general recommendations for ethical conduct regarding the "handling, storage and analysis" of human skeletal remains from archaeological sites.

In line with these guidelines, all human remains in the analyzed collections are preserved in appropriate conditions to ensure their physical integrity and long-term survival. During the analysis, no destructive methods were applied, and the skeletal remains were handled with extreme care at all times, avoiding damage and safeguarding their state of preservation. In addition, the ethical conduct protocol was followed during the whole project, always treating the skeletal remains with respect by keeping photographic material to a minimum and maintaining a professional and respectful attitude.

Although to my personal knowledge there is no specific information about the ethical guidelines followed during the excavation of Arnhem and Middenbeemster, it is worth mentioning that since the excavations of the Middenbeemster skeletal collection, a collaborative archeology project has been carried out with the stakeholders. The Middenbeemster community, specifically the Beemster Historical Society, has been actively involved in the research project by contributing archival data on their ancestors, co-organizing outreach activities, and especially by granting us permission to conduct osteoarcheological studies such as this one.

Methods

To analyze the different dental stress indicators, we first established the parameters and dental scoring techniques most suited for this project according to the research questions. Two dental pathologies in both permanent and deciduous teeth were recorded and analyzed: linear enamel hypoplasia and dental caries. For the first one, the main interest relies on the prevalence of enamel defects, the number of episodes per tooth, and their chronological distribution. For the latter one, the main interests rely on the prevalence of carious lesions on deciduous and permanent teeth, and its relation to enamel hypoplasia.

4.6 Scoring and recording LEH

Prevalence and location of LEH

To examine dental defects, all permanent and deciduous teeth were sided and divided by type (lower and upper incisors, canines, premolars, and molars).

LEH was scored in all available teeth and identified based on Goodman and Rose (1990) in the following way: teeth were first gently cleaned with a soft toothbrush to allow for better visibility; then, the buccal (or labial) tooth surface was observed macroscopically in broad daylight and in an oblique angle with the assistance of a LED light and a magnifying glass (10x). The presence of a defect was determined by carefully inspecting the tooth’s surface with a toothpick or fingernail. The severity of the lesion was recorded as “slight”, “moderate”, and “severe”, following Blakey et al., (1994), and Cucina & İşcan (1997). These were recorded on the analysis form following these numeric codes:

0. No LEH
1. Slight LEH (narrow/ shallow lesions only visible with an oblique angle but evident to the touch.)
2. Moderate LEH (relatively wide/deep defects visible to the naked eye, no destruction of the enamel)
3. Severe LEH (major growth arrests with visible destruction of the enamel)
9. Non observable

At least two thirds of the crown must be visible to score LEH, therefore those teeth whose crowns could not be observed due to postmortem damage, severe decay, or dental calculus, were recorded as “Non observable”.

The location of the enamel defects on permanent and deciduous teeth was determined by measuring the distance from the cemento-enamel junction (CEJ[5]) to the middle point of the inferior and superior limits of the enamel defect. This is because the thickness of the defect corresponds to the duration of the stress event, and therefore the middle point of the LEH indicates the average time of formation (Blakey & Armelagos, 1985, p. 373). This was done by using a digital sliding caliper calibrated to an accuracy of 0.01 mm, as suggested by Goodman and Rose (1990).

Chronology of Linear Enamel Hypoplasia (LEH)

Permanent teeth

To determine the temporality of the enamel hypoplasia in permanent teeth, the methodology established by Reid and Dean (2000; 2006) was implemented. These authors divided the height of each tooth into deciles, calculating the age at formation of each of these equal sections in years. Therefore, the total height of each tooth, divided by 10 provides the distance corresponding to a specific age. To consult the measurements used, see Appendix B and C..

Reid and Dean (2006) calculated these chronological ages of enamel formation for a Southern African and a Northern European sample. However, only the Northern European estimates were used, since the individuals analyzed for this research are biologically closer to this population. With this same principle, the average crown heights to calculate the distance of each decile were retrieved from the averages for Northern European samples calculated by Reid & Dean (2006, pp. 331–332). The crown length used for upper and lower molars corresponds to the highest mesial section of the crown. To consult the measurements used, see Appendix B.

Since this method only provides information about the chronological age formation of molars and anterior teeth, the premolars were not considered for this part of the analysis.

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[5] The CEJ is referred to as the cervical line, or “the inferior border of completely developed enamel”(Blakey & Armelagos, 1985, p. 373).

Deciduous teeth

For deciduous teeth, the age at formation of LEH was first calculated using the following equation adapted from Goodman & Rose (1991, p. 289), and based on the methodology by Blakey & Armelagos (1985, p. 373). Other data proportioned by Liversidge et al.(1993) and Birch & Dean (2014) was used in this calculation as explained in the next lines.

$$\begin{aligned} Age\ at\ formation \\ &= age\ at\ crown\ completion \\ &- \left[\frac{months\ of\ formation}{crown\ height}\right] \times defect\ height \end{aligned}$$

According to this formula, four elements are needed to calculate age at formation of LEH: age at crown completion, months of formation, crown height and defect height.

- The age at crown completion indicates the average age of an individual at the time of full development of each tooth's crown.
- The months of formation refers to the time it takes for the crown of each tooth to fully develop; these timings were retrieved from Birch & Dean (2014).
- The crown height refers to the measurement from the CEJ border to the cuspal end of a tooth. It was calculated based on the average crown heights for deciduous teeth on northern European populations estimated by Liversidge et al.(1993).
- The defect height is calculated from the CEJ border to the central point of the enamel defect as previously mentioned.

Since this research focuses on determining if the formation of the lesion occurred during the prenatal or postnatal period, the crown formation times before birth and after birth were calculated for each type of tooth (first incisors, lateral incisors, canines, and molars) according to Birch & Dean (2014). These authors also estimated the percentage of crown completion before and after birth for each tooth, which we used to determine the pre-natal and post-natal crown heights.

The average crown heights of each tooth were retrieved from Liversidge et al.(1993), who estimated the mean crown measurements for deciduous teeth of northern European populations. To consult the measurements used, see Appendix D.

4.7 Scoring and recording caries

Like with LEH, all available teeth were macroscopically inspected for caries lesions using a LED light and a magnifying glass (10x). A dental explorer was used when narrow cavities were detected in order to determine the severity of the lesion (if the dentine was affected or not).

The severity of the lesion was scored following the methodology established by Hillson (1996). Severity of the lesions was scored for permanent and deciduous teeth in a scale from 0 to 4 in the following way:

0. No visible lesions.
1. Lesions affecting the tooth’s enamel but not the dentine.
2. Lesions affecting the tooth’s dentine.
3. Lesions that have penetrate the pulpar chamber.
4. Lesions that have deteriorated more than 50% of the crown’s surface.
5.

Although lesions scored with a degree of severity of 1 were recorded, they were not taken into consideration for the statistical analyses to avoid interobserver error, as well as confusion with damage occasioned by degradation effects.

Chapter 5: Results

This chapter describes the results obtained from the statistical analyses performed on the data collected for this research. These results will be divided based on the dental pathologies examined on deciduous and permanent teeth for each site, focusing on the differences between age at death categories (intra-group comparisons), and the differences between each site (inter-group comparisons).

The first two sections report the prevalence and average age at formation of enamel hypoplasia per age-at-death category and site, for both permanent and deciduous teeth. Similarly, the third section presents the prevalence of dental caries with respect to age category at death and location for both permanent and deciduous teeth. For permanent teeth, the differences between sexes are shown. Finally, in the last section, an analysis of the relationship between caries and LEH is included.

5.1 Prevalence of LEH

In the following paragraphs the prevalence of LEH for deciduous teeth of Arnhem and Middenbeemster will be shown, followed by the prevalence of LEH for permanent teeth. The columns of the tables in this section include the total number of analyzed teeth, the total number of affected teeth, the percentage of affected teeth regarding the total number of analyzed teeth, the total number of observed LEH lesions, and the average number of LEH per tooth regarding the total number of affected teeth. All of these are presented according to each age-at-death category, as well as per site.

It is important to note that the total number of analyzed teeth might differ from the total number of teeth in the sample, since some of the teeth were excluded from the analysis due to post-mortem damage, high levels of dental calculus, and other conditions that inhibit the observation of dental defects. Another observation to be made is that the total number of LEH might differ from the total affected teeth, since one tooth can have more than one lesion. Because of this, the average number of LEH per tooth was calculated.

Deciduous teeth

Arnhem

As seen in the table below (5.1), 17% of the teeth from the Arnhem sample (54 out of the 322), presented one or more LEH marks. For the infant category, 45 out of the 226 teeth were affected (20%), with a total number of 45 LEH marks, and an average of 1 LEH mark per tooth. For the child category, 7 out of the 68 teeth (10%) were affected, with a total number of 7 LEH marks, and an average of 1 LEH mark per tooth. For the juvenile category, 2 out of 28 (7%) present LEH, with a total number of 2 LEH marks, (1 per tooth).

Due to natural patterns of primary dentition loss, no deciduous teeth were present for adolescents, early young adults, or late young adults, and therefore these tables are non-applicable for these age-at-death categories.

For this site, the infant category presents the highest percentages of LEH (20%) compared to child and juvenile categories (10% and 7% respectively). This difference is not statistically significantly different [X^2 (2, N =322) = 5.50, p = .063].

Prevalence of LEH on Deciduous Teeth: Arnhem

Age-ag-death	***Total teeth***	***Total affected teeth***	***% Affected Teeth***	***Total # LEH***	***Average #LEH per tooth***
Infant	226	45	20%	45	1
Child	68	7	10%	7	1
Juvenile	28	2	7%	2	1
Adolescent	0	0	N/A	0	N/A
EYA	0	0	N/A	0	N/A
LYA	0	0	N/A	0	N/A
TOTAL	322	54	17%	54	1

Table 5. 14 Prevalence of LEH on Deciduous Teeth: Arnhem

Middenbeemster

The table below (5.2) shows the prevalence of LEH in deciduous teeth for the Middenbeemster sample. For this sample 57 out of a total of 535 analyzed teeth were affected with LEH (11%). A total number of 57 LEH marks were recorded, and therefore an average of 1 lesion per affected tooth was calculated. For the Infant age-at-death category, 46 out of 413 were affected with LEH (11%); for the child category, 12% of the teeth were affected (6 out of 49); while for juvenile individuals, 5 out of 73 the teeth were affected (7%). In this case, the total number of LEH is equal to the total affected teeth in each age-at-death category since no more than one lesion per tooth were observed. There seems to be no significant relation between the number of affected and non-affected teeth in relation to the age-at-death [(X^2 (2, N =592) = 1.11, p= .573]

Prevalence of LEH on Deciduous Teeth: Middenbeemster					
Age-ag-death	***Total teeth***	***Total affected teeth***	***% Affected Teeth***	***Total # LEH***	***Average #LEH per tooth***
Infant	413	46	11%	46	1
Child	49	6	12%	6	1
Juvenile	73	5	7%	5	1
Adolescent	0	0	N/A	0	N/A
EYA	0	0	N/A	0	N/A
LYA	0	0	N/A	0	N/A
TOTAL	535	57	11%	57	1

Table 5. 15 Prevalence of LEH in Deciduous Teeth: Middenbeemster

Inter-site comparison:

As seen in the tables above (4.2.1 and 4.2.2) and in the figure below (4.2.1), Arnhem presents a higher prevalence of LEH in deciduous teeth (17%) than Middenbeemster (11%). These numbers appear to be significantly different, showing a dependency between site and affected teeth [X^2 (1, N =857) = 6.13, p= 0.009)].

As for age-at-death categories, the infant individuals from Arnhem present a higher prevalence of LEH in deciduous teeth with 20% of affected teeth compared to the 11% shown in Middenbeemster. The Chi square test demonstrate that these variables are significantly different [(X^2 (1, N =639) = 8.50, p= 0.003)].

The prevalence of LEH in individuals from the child age-at-death category does not differ significantly between sites (10% Arnhem vs. 12% Middenbeemster) [(X^2 (2, N =117) = 0.10, p= .740]. Likewise, juveniles from Arnhem and Middenbeemster present equal percentage of affected teeth (7%) and are not significantly different from each other [(X^2 (2, N =101) = 0.002, p= .958]. Moreover, the average number of LEH lesions per tooth is equal in both sites, with an average of 1 lesion per affected tooth.

In the following figure (5.1) we can observe the different percentages between LEH prevalence for each site. Color coding was used to identify each site in the following way: blue represents Arnhem (AR), and orange represents Middenbeemster (MB).

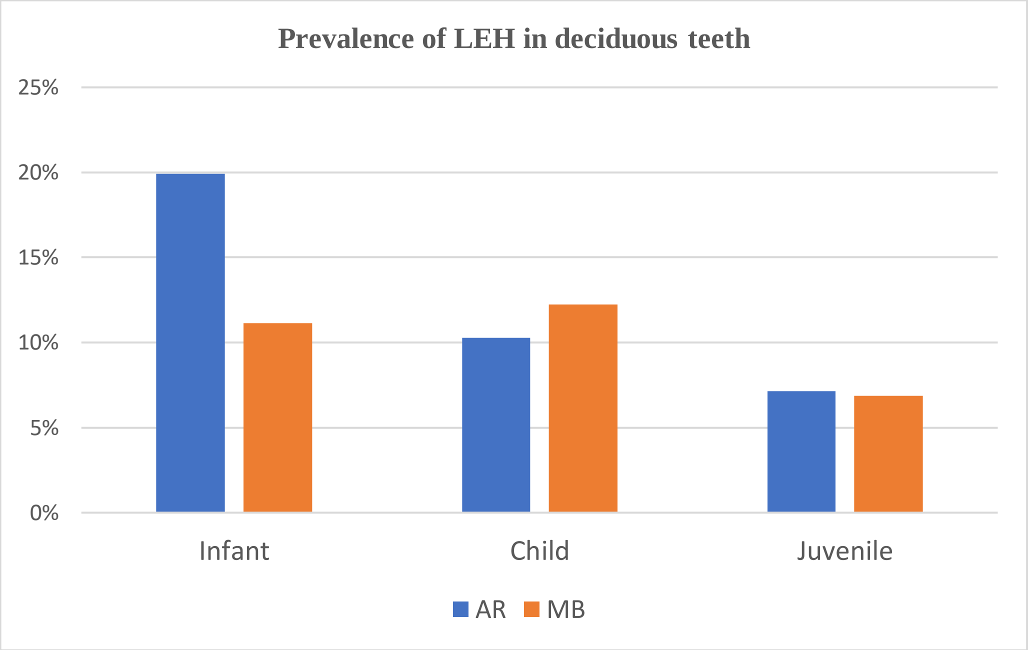

Figure 5. 1 Percentage of prevalence of LEH in deciduous teeth per age-at-death categories and site: Arnhem (AR), Middenbeemster (MB).

Permanent Teeth

Like in the previous section, the tables and figures presented here show the prevalence of LEH in Arnhem and Middenbeemster samples. However, here we focus exclusively on permanent teeth. The total affected teeth, the percentage of affected teeth regarding the total, the total number of LEH, and the average number of LEH per tooth regarding the total affected teeth are presented for both sites according to age-at-death categories; followed by a comparison between sites.

Arnhem

As seen in the table below (5.3), Arnhem's sample is composed by a total of 970 permanent teeth, from which 553 present at least one LEH lesion. This makes for 57% of the total analyzed teeth. The total number of observed LEH lesions was 741, and the average number of LEH per affected tooth was 1.34.

As for age-at-death categories, there were no analyzed permanent teeth for the infant category. For the child category, there were 12 analyzed teeth from which all of them (100%) presented LEH. The total number of observed LEH marks was 13, which is an average of 1.08 lesions per affected tooth. For the juvenile category, there were 16 analyzed teeth, from which 38% presented LEH (6). There were 6 observed LEH marks, which is an average of 1 lesion per tooth. For Adolescents, 316 teeth were analyzed, and 162 presented at least one LEH lesion (51%). There were 196 observed LEH marks, which represents an average of 1.21 marks per affected tooth.

For early young adults, 249 teeth were analyzed, from which 162 were affected (65%). A total of 236 LEH lesions were observed, meaning that there were in average 1.46 lesions per affected tooth. For late young adults, 211 out of the 377 analyzed teeth presented one or more LEH lesions which represents 56%. 290 lesions were detected, with an average of 1.37 LEH marks per tooth.

Between age-at-death groups, the child category presents the highest LEH prevalence (100%), followed by the early young adults (65%), the late young adults (56%), the adolescents (51%), and finally the juveniles (38%). The Chi square test demonstrated that these variables are significantly different [(X^2 (1, N =639) = 13.33, p= 0.003)]. Therefore, there is a significant relation between age at death and LEH prevalence in this site.

Prevalence of LEH in Permanent Teeth: Arnhem					
Age-ag-death	***Total teeth***	***Total affected teeth***	***% Affected Teeth***	***Total # LEH***	***Average #LEH per tooth***
Infant	0	0	N/A	0	N/A
Child	12	12	100%	13	1.08
Juvenile	16	6	38%	6	1
Adolescent	316	162	51%	196	1.21
EYA	249	162	65%	236	1.46
LYA	377	211	56%	290	1.37
TOTAL	970	553	57%	741	1.34

Table 5. 16 Prevalence of LEH in Permanent Teeth: Arnhem

Middenbeemster

As seen in the table below (table 5.4), 1,765 permanent teeth were analyzed for the Middenbeemster sample, from which 1,012 presented one or more LEH marks. This number represents 57% of the total sample. In total, 1,390 lesions were observed, which is equivalent to 1.37 lesions per affected tooth.

As of age-at-death categories, infants present 6 out of 8 permanent analyzed teeth with LEH marks, which makes for the 75%. 7 lesions were observed, and an average of 1.17 lesions per affected tooth was calculated. For the child category, 17 out of 51 permanent teeth presented one or more LEH marks (33%). The total number of registered marks was 26, which represents 1.53 LEH lesions per affected tooth. For juveniles, out of 298 teeth, 130 were affected (44%), and 153 total LEH lesions were registered which represents 1.18 lesions per affected tooth. For adolescents, 49% of the teeth were affected (73 out of 148), and 96 lesions were observed, which indicates 1.32 average lesions per affected tooth. For early young adults, 351 out of 524 teeth were affected (67%), while 496 lesions were registered (1.41 lesions per affected tooth). late young adults showed 59% affected teeth (435 out of 736), with a total of 612 recorded LEH marks. This represents an average of 1.41 lesions per affected group.

When comparing the number of affected teeth between age at death categories, there is a statistically significant difference between these variables [(X^2 (4, N =2735) = 47.57, p= 0.00001)].

Prevalence of LEH in Permanent Teeth: Middenbeemster					
Age-ag-death	***Total teeth***	***Total affected teeth***	***% Affected Teeth***	***Total # LEH***	***Average #LEH per tooth***
Infant	8	6	75%	7	1.17
Child	51	17	33%	26	1.53
Juvenile	298	130	44%	153	1.18
Adolescent	148	73	49%	96	1.32
EYA	524	351	67%	496	1.41
LYA	736	435	59%	612	1.41
TOTAL	1765	1012	57%	1390	1.37

Table 5. 17 Prevalence of LEH in Permanent Teeth: Middenbeemster

Inter-site comparison

Overall, the percentage of LEH on permanent teeth of Arnhem and Middenbeemster is equal (57% in both sites) and does not vary significantly [(X^2 (1, N =4300) = .0.007, p= .931)].

Likewise, the average number of LEH lesions per affected teeth does not vary within sites, with 1.34 for Arnhem and 1.37 for Middenbeemster.

When comparing age-at-death categories, a t-test shows that there is no significant difference between them [t (5) = 0.91324, p = 191].

Infants were excluded from the analysis since in Arnhem there are no permanent teeth present for this category, and the sample size for Middenbeemster is too small (8 teeth). Likewise, the child category was excluded from the analysis since Arnhem sample is composed only by 12 teeth from the same individual, which could skew the results.

For the juvenile category, both sites present similar percentages of affected teeth (38% Arnhem, 44% Middenbeemster), and are not significantly different from each other. Similar thing occurs for the rest of the age-at-death categories, with percentages of 51% in Arnhem and 49% in Middenbeemster for the adolescents, 65% vs. 67% respectively for early young adults; and 56% vs. 57% respectively for late young adult individuals, as observed in the figure below (5.2).

As seen on the tables above (5.3 and 5.4), the average number of lesions per affected teeth varies slightly between age-at-death categories. On one hand, the child category shows a higher number of lesions per tooth on Arnhem (1.08) compared to Middenbeemster (0.51). On the other hand, the juvenile category in Arnhem shows less lesions per tooth than in Middenbeemster (0.38 vs. 0.51 respectively). While adolescents, early young adults, and late young adults show similar ratios for Arnhem and Middenbeemster: 0.62 and 0.65 for the first one; 0.95 and 0.95 for the second one; and 0.77 and 0.83 for the latter one; none of these are statistically different according to the t-test [*t* (5) = 0.91324, p = 191].

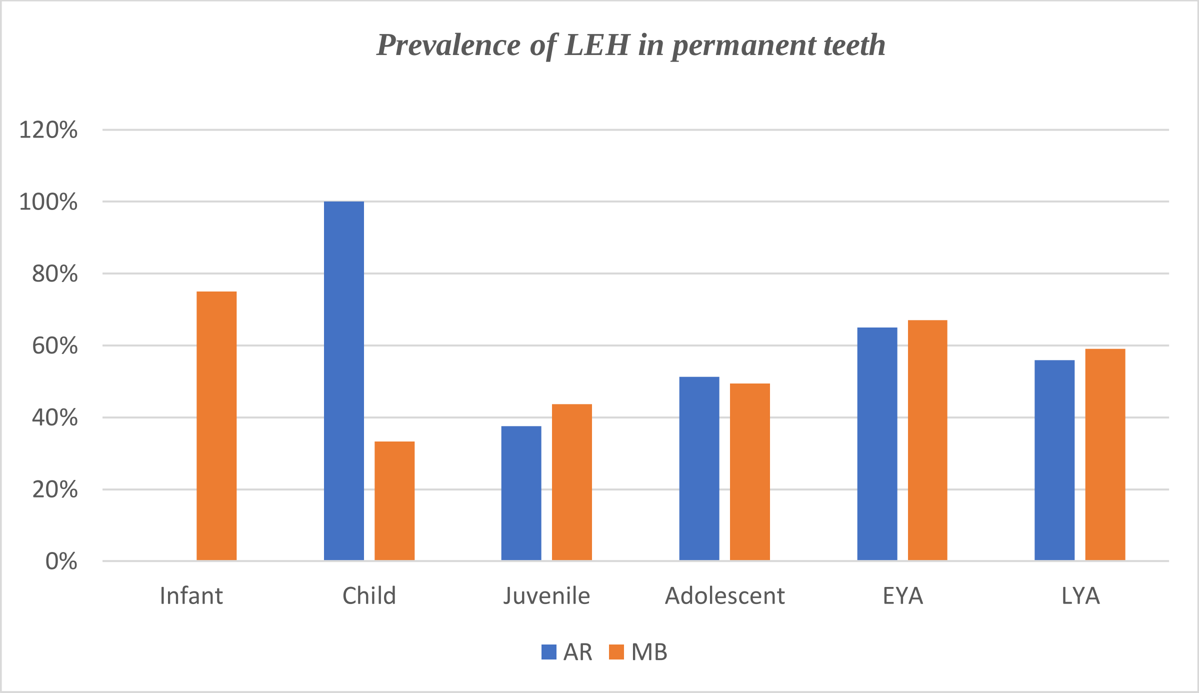

Figure 5. 2 Prevalence of LEH in permanent teeth per age-at-death categories and site. Arnhem (AR), Middenbeemster (MB).

5.2 Chronology of LEH: Age at Formation

In this section, the age at formation of LEH for deciduous and permanent teeth will be presented, as well as a comparison of the differences between Arnhem and Middenbeemster. It is important to consider that age-at-death of the individuals in this section is not relevant, since the LEH lesions could develop at any stage of childhood.

Deciduous teeth

In the following table (5.5) the absolute and relative values of age-at-formation of LEH on deciduous teeth are presented for both sites. As stated in the previous chapter, each tooth was divided into prenatal and postnatal periods according to their growth rate following the methodology by Birch and Dean (2014). Here, the results are presented according to measurement of the lesions regarding the CEJ. Therefore, that absolute number for each site indicates the lesions that could be paired with the prenatal or postnatal period, and the frequency indicates the percentage of lesions per age-at-formation, regarding the total lesions per each site.

In general, the total number of LEH lesions produced during the prenatal period (*in utero*) were 72 (64.9%), while the ones produced during the postnatal period were 39 (35.1%).

Specifically for the Arnhem sample, 54 lesions were observed, from which around 57% of them were produced *in utero* (31), while the remaining 42.6% occurred during the postnatal period. For the Middenbeemster sample, there were 57 lesions observed, from which around 72% (41) were produced *in utero*, while the remaining 28% (16) occurred after birth.

Age-at-formation of LEH in deciduous teeth: Arnhem and Middenbeemster						
Age	***Arnhem***		***Middenbeemster***		***Total***	
	Absolute	***Frequency***	***Absolute***	***Frequency***	***Absolute***	***Frequency***
Prenatal	31	57.4%	41	71.9%	72	64.9%
Postnatal	23	42.6%	16	28.1%	39	35.1%
Total	54	100%	57	100%	111	100%

Table 5. 18 Age-at-formation of LEH in deciduous teeth (prenatal or postnatal) according to site.

To compare the differences between prenatal and postnatal lesions between sites, a Chi Square test was applied, indicating no significant difference between them [(X^2 (1, N =1) = 2.56, p= .109)].

Permanent teeth

As mentioned in the previous chapter, the age-at-formation of LEH lesions in permanent teeth was calculated based on deciles established by Reid and Dean (2006). The table below (table 5.6) shows the number of lesions according to their chronological distribution, divided in age ranges going from 1 year old to 11 years old. This is shown for both sites, as well as for the total. The colors in the table serve as a visual aid to identify the incidence of lesions, going from bright red (highest) to bright green (lowest).

Age range	Arnhem		Middenbeemster		Total	
	Absolute value	Frequency	Absolute value	Frequency	Absolute value	Frequency
1.0 - 1.4	1	0.2%	0	0.0%	1	0%
1.5 - 1.9	10	1.7%	27	2.5%	37	2%
2.0 - 2.4	61	10.4%	125	11.4%	186	11%
2.5 - 2.9	137	23.4%	282	25.6%	419	25%
3.0 - 3.4	88	15.0%	170	15.5%	258	15%
3.5 - 3.9	96	16.4%	179	16.3%	275	16%
4.0 - 4.4	61	10.4%	89	8.1%	150	9%
4.5 - 4.9	61	10.4%	92	8.4%	153	9%
5.0 - 5.4	22	3.8%	65	5.9%	87	5%
5.5 - 5.9	16	2.7%	28	2.5%	44	3%
6.0 - 6.4	8	1.4%	2	0.2%	10	1%
9.4 - 9.9	0	0.0%	1	0.1%	1	0%
10.0 - 10.4	8	1.4%	17	1.5%	25	1%
10.5 - 11.0	17	2.9%	23	2.1%	40	2%

Table 5. 19 Heat map comparing number of lesions regarding age-at-formation of LEH in permanent teeth for Arnhem and Middenbeemster

Arnhem

As observed in table above (table 5.6), in Arnhem, between the ages 1 to 1.4 and 1.5 to 1.9, the incidences of lesions appear low, with only 0.2% and 1.7% of the total lesions formed within these ages. Between 2 to 2.4 years of age, the incidence of lesions begins rise, with 10.4% of the total lesions occurring at this age range. The highest peaks of lesions occur between ages 2.5 to 2.9 years (23.4% of the total lesions), followed by the age range between 3 to 3.4 and 3.5 to 3.9 ages, with 15% and 16.4% of the total lesions occurring during these ages respectively. The incidence of lesions decreases during the ages of 4 to 4.4, and 4.5 to 4.9, each of them with an incidence of 10.4% of the total lesions. The incidence of lesions continues to decrease between the ages of 5 to 5.4 and 5.5 to 5.9 years, with 3.8%, and 2.7% of the total lesions forming during these years. The lowest incidence of lesions occurs between the ages of 6 to 6.4, 9.4 to 9.9, and 10 to 10.4, with 1.4%, 0.0%, and 1.4% of the total lesions developing during these age ranges respectively. The incidence of lesions increases during the age range of 10.5 to 11 years, with 2.9% of the lesions forming during these ages.

Middenbeemster

A similar pattern occurs in Middenbeemster, with the lowest incidence of caries occurring between 1 and 1.4 years, with no lesions registered in these years; followed by an increase between the ages 1.5 to 1.9, when 2.5% of the lesions were formed. Likewise, the highest incidence of lesions occurring from 2.5 to 2.9 years (25.6%). Other ages with high incidences include the years between 2 to 2.4, 3 to 3.4 and 3.5 to 3.9, with 11.4%, 15.5% and 16.3% of the total lesions occurring during these years.

Like in Arnhem, the incidence drops between ages 4 to 4.9, with 8.1% of the total lesions occurring between 4 to 4.4 years, and 8.4% during the period of 4.5 to 4.9 years of age. The incidence of lesions continues to lower between 5 and 5.9 years, with 5.9% and 2.5% of the total lesions developed respectively during these ages. Between 6 and 6.4 years, the incidence of lesions drops to 0.4% of the total lesions associated to these ages. Similarly, the incidence of lesions developed between 9.4 to 9.9 years go as low as 0.1%; while during the ages of 10 to 10.4, 1.5% of the lesions occurred. Finally, between 10.5 and 11 years, the incidence increases with 2.1% of the lesions forming at this age.

Inter-site comparison

Statistic tests were applied to compare the incidence of LEH lesions per age-ranges between each sample. Since the samples are not normally distributed according to Levene's test (*f* = 5.48497, *p*= 0.02781), a Mann-Whitney-U-test was applied showing no significant difference between both samples (*z*= -1.28205, *p*=.20054). This relation can be better appreciated in the figure below (figure 5.3), in which the peaks appear equally distributed for Arnhem and Middenbeemster.

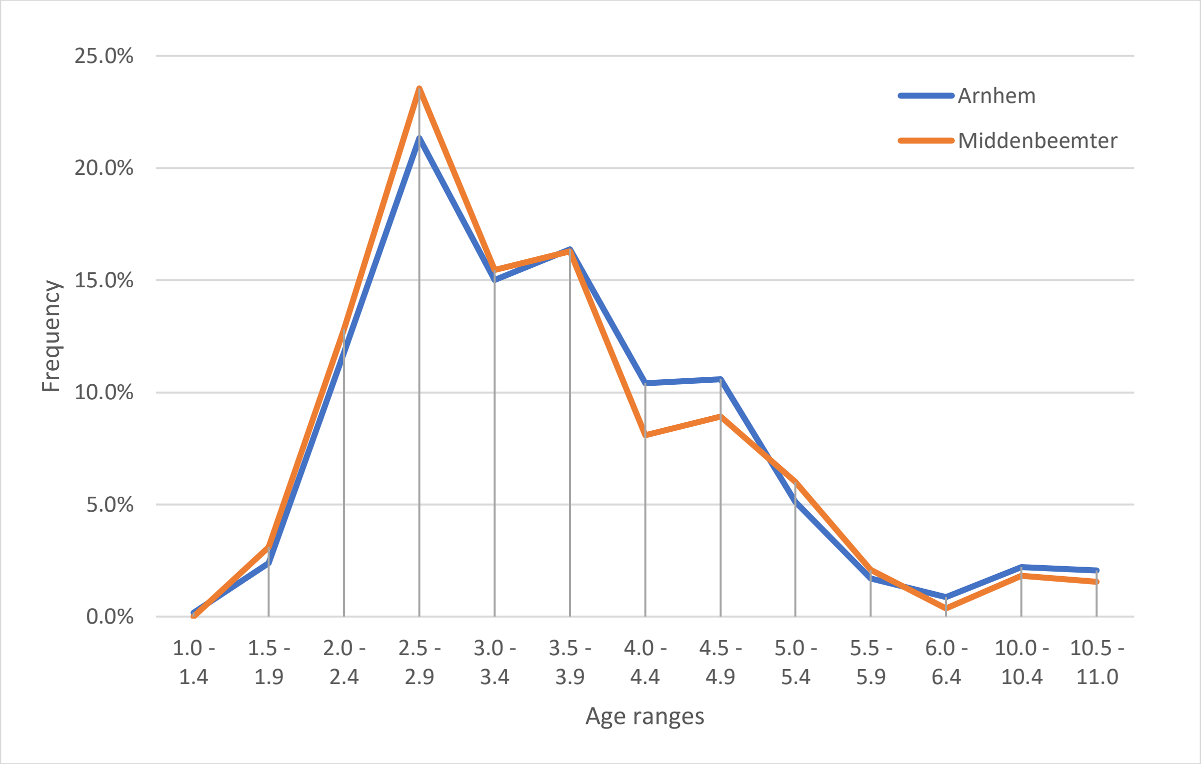

Figure 5. 3 Frequency of LEH lesions in permanent teeth related to their age-at-formation per site.

5.3 Prevalence of Carious Lesions

In this section, the prevalence of caries for deciduous and permanent teeth is presented, considering differences between age-at-death categories and between sites. The following tables include the number of affected teeth and their relative frequency regarding the total number of teeth as well as the number of caries and its frequency regarding the total number of caries observed (within each site). Furthermore, intra and inter-site comparisons were applied, considering the age-at-death categories. It is important to note that the number of affected teeth vary from the number of caries, since a tooth can have multiple lesions.

Deciduous teeth

In the next paragraphs, an overview of the prevalence of caries on deciduous teeth is presented for both sites. Furthermore, intra and inter-site comparisons are made. adolescent, early young adults, and Late Young Adults were not considered for this section, since these individuals do not present deciduous teeth.

Arnhem

Overall, as seen in previous sections, the Arnhem sample is composed by a total of 322 deciduous teeth, from which only 15 (5%) present one or more caries. As seen in the table below (table 5.7), the total number of caries found for this site was 21.

The infant age-at-death category, out of 226 analyzed teeth, 6 presented caries (3%), and there were 8 caries observed, which represents 38% of the total caries for this site. For the child category, 4 out of the 68 analyzed teeth were affected (6%), and 6 caries were registered, or 29% of the total number of deciduous caries in this site. For juvenile individuals, 5 out of 28 teeth presented caries, and 7 caries lesions were detected (33% from the total). When comparing age-at-death categories, there is a statistically significant difference between age-at-death and prevalence of caries [(X^2 (2, N =322) = 13.25, p= .001)].

Dental caries on deciduous teeth: Arnhem					
Age-at-death	***Total teeth***	***Affected teeth***	***%***	***Total # of caries***	***%***
infant	226	6	3%	8	38%
child	68	4	6%	6	29%
juvenile	28	5	18%	7	33%
Total	322	15	5%	21	100%

Table 5. 20 Table of dental caries on deciduous teeth from Arnhem by age at death categories.

Middenbeemster

For Middenbeemster, 535 teeth were analyzed, from which 133 presented one or more caries (25%). As seen in the table below (table 5.8), in total, 232 caries were registered on deciduous teeth of this site.

Regarding age-at-death categories, Infants had 55 affected teeth out of 413 analyzed ones (35%). 81 caries (35%) from the 232 lesions mentioned above belong to this age category. For the child category, 35 out of 49 teeth were affected (71%), and 74 lesions were registered, which represents 32% of the total caries for this site. Lastly, for juvenile individuals 43 out of 73 teeth presented dental caries (59%), and 77 lesions were observed, which represents 33% of the total deciduous caries for this site. When comparing age-at-death categories, there is a statistically significant difference between age-at-death and prevalence of caries [(X^2 (2, N =549) = 135.80, p= .001)].

Dental caries on deciduous teeth: Middenbeemster					
Age category	***Total teeth***	***Affected teeth***	***%***	***Total # of caries***	***%***
infant	413	55	13%	81	35%
child	49	35	71%	74	32%
juvenile	73	43	59%	77	33%
total	535	133	25%	232	100%

Table 5. 21 Table of dental caries on deciduous teeth from Middenbeemster by age at death categories

Inter-site comparison

Overall, as seen in the previous table, the Middenbeemster sample presents higher percentages of caries than the Arnhem sample (25% vs. 5% respectively). Likewise, per age category, Middenbeemster presents a higher prevalence of affected teeth in all three of them (13% vs. 3% in infants; 71% vs. 6% in children; 59% vs. 18% in juveniles, for Middenbeemster and Arnhem respectively). According to the Chi square test, the total number of caries in Arnhem does not vary significantly from the ones in Middenbeemster [X^2 (1, N = 148) = 0.010, p = .994]. Furthermore, the relative frequency of caries per age-category regarding the total number of caries per each site does not vary, showing similar numbers per each age category (38%, 29%, and 33% for Arnhem; and 35%, 32%, and 33% for Middenbeemster) [X^2 (1, N = 253) = 0.1229, p = .940]. This can be better appreciated in the figure below (figure 5.4)

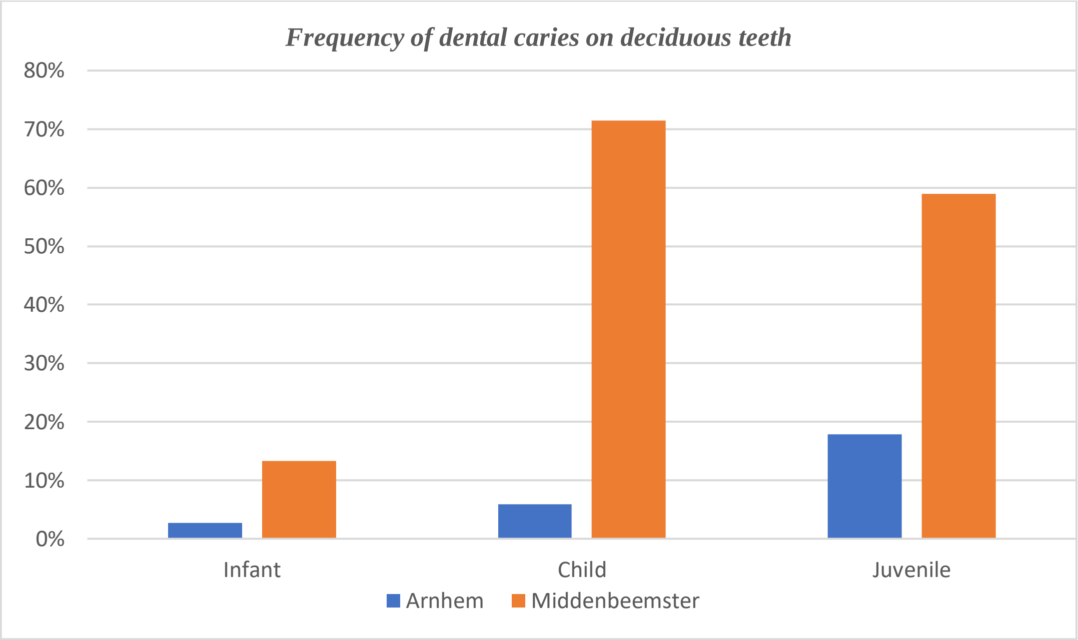

Figure 5. 4: Frequency of dental caries on deciduous teeth per age-at-death categories and site.

Permanent teeth

This section will provide the results of the prevalence of caries on permanent teeth for both Arnhem and Middenbeemster. As with deciduous teeth, the total number of affected teeth and the total number of caries is shown per age-at-death category. For adult individuals, differences by sex are presented. Furthermore, intra and inter site comparisons will be made

Arnhem

As observed on the table below (5.9), Arnhem presents in total 226 affected teeth out of the 970 analyzed ones. In other words, 23% of the sample presented one or more caries. In total, 400 caries were identified for this site.

Regarding age-at-death categories, infants were excluded from the analysis since no permanent teeth were present. For children, 25% of the teeth were affected, and 5 caries were registered, which represents 1% of the total caries detected for this site. For juvenile individuals, out of the 16 present teeth, no lesions were observed. For adolescents, 16% of the teeth were affected, and 71 caries were registered, which represents 18% of the total affected teeth for this site. For early young adults, 66 teeth were affected, which represents 27% of the analyzed teeth for this age category. Furthermore, 30% of the total caries registered for this site, fall withing this age group, with 121 carious lesions. Finally, 28% of late young adults' teeth were affected with one or more caries (106 teeth), and 203 caries were registered. This last number means that 51% of the total observed carious lesions for this site belong to the late young adult age category.

Intra-site comparisons were applied to find the relationship between age-at-death categories and caries from Arnhem. Due to the absence of permanent teeth, infants and children were excluded from the analysis. A chi-square was carried out, considering age-at-death, as well as number of not affected, and affected teeth. This test shows a significant relationship between these variables [X^2 (4, N = 970) = 15.1563, p = .001].

Dental caries on permanent teeth: Arnhem					
Age-at-death	***Total teeth***	***Affected teeth***	***%***	***number of caries***	***%***
Infant	0	0	0%	0	0%
Child	12	3	25%	5	1%
Juvenile	16	0	0%	0	0%
Adolescent	316	51	16%	71	18%
EYA	249	66	27%	121	30%
LYA	377	106	28%	203	51%
Total	970	226	23%	400	100%

Table 5. 22: Table of dental caries on permanent teeth from Arnhem by age at death categories

Middenbeemster

As indicated in table 5.10, for the Middenbeemster sample, 584 out of 1765 examined teeth were affected with one or more carious lesion, making up 33% of the total. Within the affected teeth, 1104 caries were registered for this site.

With respect to the age categories, the Infant category did not present any caries in the 8 permanent analyzed teeth. From the child category, only one permanent tooth out of the 51 examined was affected (2%), and 2 caries were registered for this age category. For Juveniles, out of 298 analyzed permanent teeth, 16% were affected (49). 74 caries were observed for this age category, making up 7% of the total carious teeth for this site. For Adolescents, 16 out of 148 teeth presented caries (11%), and 24 caries were detected, which represents 2% of the total affected teeth. Early Young Adults had 199 affected teeth out of 524 examined ones, which represents 38%. Regarding number of caries, 357 cavities were registered, which constitutes 32% of affected teeth. Lastly, 43% of Late Young Adults' teeth presented one or more caries, and 647 lesions were registered, representing 59% of the total affected teeth.

When analyzing the relationship between age-at-death categories and affected teeth for this site~~,~~ a chi-square test was applied. The categories of Infant and child were excluded from the analysis to avoid biased results. The result shows a significant relationship between these variables considering number of affected teeth vs. not affected [X^2 (4, N = 1757) = 133.07, p = .000].

Dental caries on permanent teeth: Middenbeemster					
Age category	***Total teeth***	***Affected teeth***	***%***	***Total number of caries***	***%***
Infant	8	0	0%	0	0%
Child	51	1	2%	2	0%
Juvenile	298	49	16%	74	7%
Adolescent	148	16	11%	24	2%
EYA	524	199	38%	357	32%
LYA	736	319	43%	647	59%
Total	1765	584	33%	1104	100%

Table 5. 23 : Table of dental caries on permanent teeth from Middenbeemster by age at death categories

Inter-site comparison

To compare the prevalence of caries between Arnhem and Middenbeemster samples, an independence test was carried out between the total affected teeth, and the total number of caries for both sites. A Levene's test was used to proof homogeneity of the variance, however for the affected teeth and total number of caries this was not met (f= 7.94, p= .018; f= 8.09, p= .021, respectively), and therefore a non-parametric version of the test was used (Mann-Whitney-U-test). This test indicated no significant difference between the number of affected teeth from both samples (z= -0.41779. p= .67448), as well as no significant difference between total amount of caries between sites (z= -0.626. p=.528).

Likewise, the distribution of caries between age-at death categories varies significantly between sites [X^2 (6, N = 810) = 103.84, p = .001] This can be better appreciated in the figure below (figure 5.5)

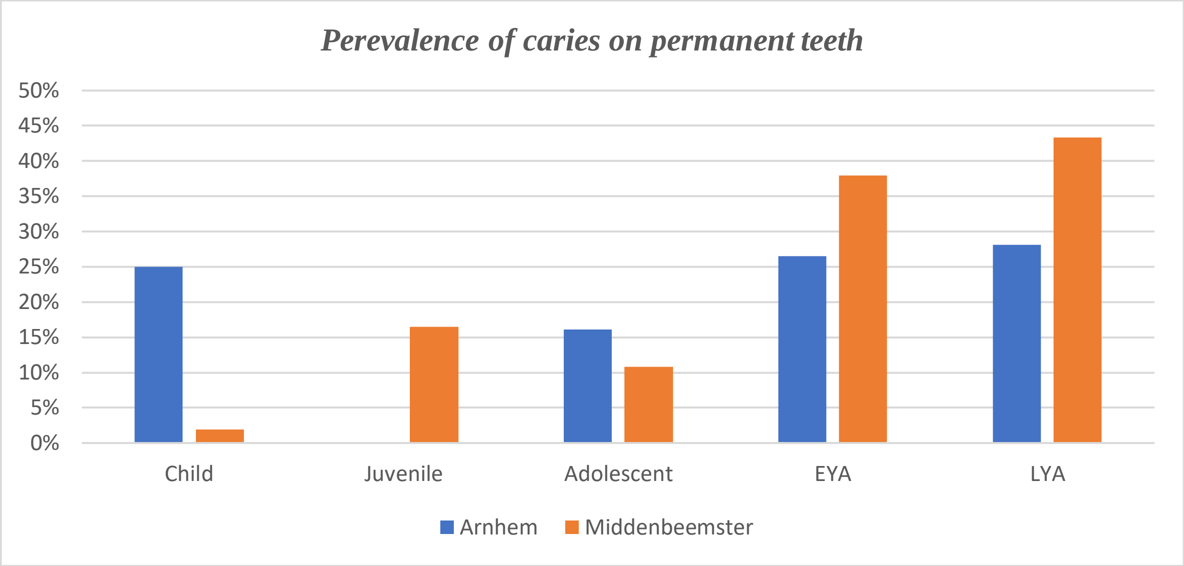

Figure 5. 5 Prevalence of caries on permanent teeth per age at death category and site.

Sex and caries in adults

The table below (table 5.11) presents the prevalence of affected teeth of adult individuals divided by sex and age at death. The total number of teeth might vary from those presented in the previous table, since the individuals whose sex could not be identified were excluded from this specific analysis. As before, the frequency of affected teeth is calculated regarding the total teeth, while the frequency of the total number of caries per age category was taken with respect to the total number of caries by sex group.

Arnhem

As seen in the table 5.11, in Arnhem, for male individuals out of the 317 analyzed teeth, 24% were affected with caries lesions, while for females, out of 309 teeth, 31% presented one or more caries. As of age categories, 29% of the teeth of early young adult males have one or more carious lesions, while for late young adult males, 21% of the analyzed teeth are affected. For early young adult females, 25% of the analyzed teeth present caries, while for late young adult females, the frequency of affected teeth is 37%. According to chi square statistical analysis, there is no significant relationship between sex and age-at-death regarding presence of caries in Arnhem (X^2 (1, N = 172) = 0.149, p = .699).

Middenbeemster

As seen in table 5.11, for the Middenbeemster males, out of 540 analyzed teeth, 179 were affected (33%); for females this number was higher, with 48% affected teeth out of the 690 analyzed. Regarding age categories, male early young adult individuals had 27% affected teeth, while the female early young adults had one or more caries in 45% in the total analyzed teeth for this age group. Male late young adults presented caries 130 teeth out of 356 (37%), while females had caries in 50% of the recorded teeth (189 out of 356). For this site, there is a significant relation between sex and age-at-death regarding presence of caries (X^2 (1, N = 509) = 11.693, p = .0006).

Arnhem						
	Total teeth		**Affected teeth**			
	M	***F***	***M***	***%***	***F***	***%***
EYA	103	146	30	29%	36	25%
LYA	214	163	45	21%	61	37%
Total	317	309	75	24%	97	31%
Middenbeemster						
	Total teeth		**Affected teeth**			
	M	***F***	***M***	***%***	***F***	***%***
EYA	184	310	49	27%	141	45%
LYA	356	380	130	37%	189	50%
Total	540	690	179	33%	330	48%

Table 5. 24 Prevalence of dental caries in males and females per age-at-death categories and site.

Inter-site comparison

When comparing Arnhem and Middenbeemster, we can observe a significant relationship between sex and site code, where females are more affected than males in Middenbeemster (X^2 (1, N = 681) = 3.91, p = .047). This can be better appreciated in the figure below (figure 5.6)

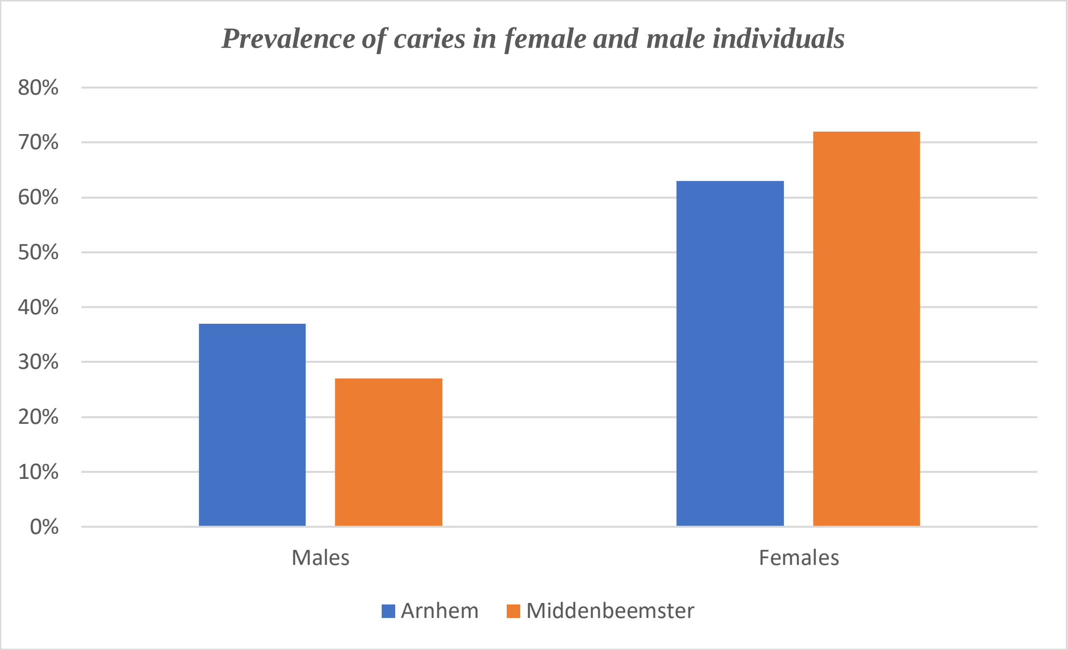

Figure 5. 6 Prevalence of caries on permanent teeth by sex and site.

4.5 LEH and Caries

Deciduous teeth

The table below shows the relation between caries and LEH lesions per site and age at death categories for permanent teeth. The numbers presented in the table refer to teeth with caries and LEH, either caries or LEH, or teeth with no affections (no caries and no LEH); this is presented per age at death category and site. The p-value shows the results from the statistical tests conducted (Chi- square), showing the statistical significance of these relations. For those groups which contained a value of zero in any of the categories, the tests could not be conducted due to statistical incongruences; therefore, the p-value in these cases is non-applicable.

		Arnhem			Middenbeemster		
		LEH	NoLEH	p-value	LEH	NoLEH	p-value
Infant	Caries	2	4	0.75	17	0	n/a
	No Caries	43	177		29	329	
Child	Caries	1	3	0.88	6	29	0.44
	No Caries	6	58		6	14	
Juvenile	Caries	0	5	n/a	1	42	0.17
	No Caries	2	21		4	26	
Total	Caries	3	12	0.73	24	109	0.001
	NoCaries	51	256		33	369	

Table 5. 25 Number of deciduous teeth with LEH and no LEH compared to teeth with caries and no caries per age at death categories and site. The p-value of the X^2 test is shown.

Arnhem

In Arnhem, no relations were observed between caries and LEH in any of the age groups, with all values exceeding the significance level of 0.05 (0.75 for infants, 0.88 for children, and non-applicable for juveniles). Without taking into account age at death categories, the total number of teeth with caries and with LEH adds up to 3, while the number of teeth without caries or LEH equals 256.

Middenbeemster

For Middenbeemster, in the infant category, 17 teeth presented caries and LEH, while 329 had no caries and no LEH. For children, 6 teeth had caries and LEH and 14 teeth no caries and no LEH; for juveniles one teeth presented caries and LEH, and 14 had no caries and no LEH. Finally, the total number of deciduous teeth with caries and LEH for this site is 24, and 369 with no lesions at all.

When disregarding age groups, a significant relationship between LEH and caries can be observed for this site. This relationship shows that teeth with no LEH are more likely to have caries than those with no LEH nor caries (109 teeth with caries and without LEH, 33 teeth without caries but with LEH, and 24 teeth with LEH and Caries).

Permanent teeth

The same Chi square analysis was carried out for permanent teeth as seen in the following table.

		Arnhem			Middenbeemster		
		LEH	NoLEH	p-value	LEH	NoLEH	p-value
Child	Caries	3	0	n/a	0	1	n/a
	No Caries	9	0		9	33	
Juvenile	Caries	0	0	n/a	24	25	0.5
	No Caries	6	10		106	143	
Adolescent	Caries	22	29	0.26	7	9	0.84
	No Caries	140	125		66	66	
EYA	Caries	32	34	0.002	134	65	0.96
	No Caries	130	53		217	108	
LYA	Caries	53	53	0.18	179	140	0.17
	No Caries	158	113		256	161	
Total	Caries	113	128	1	344	240	0.27
	No Caries	494	557		654	511	

Table 5. 26 Number of permanent teeth with LEH and no LEH compared to teeth with caries and no caries per age at death categories and site. The p-value of the X^2 *test is shown.*

Arnhem

For the Arnhem sample, the X^2statistical analysis was not performed in the age groups of children and juveniles due to the lack of permanent teeth.

For adolescents, no significant relation was found between caries and LEH lesions, however, for early young adults the statistical analysis points towards a significant relation between these conditions. According to the table, there were more teeth presenting LEH, but no caries (130) compared to those with caries and LEH (32), those with caries but no LEH (32), and even those without LEH nor caries (52).

Nonetheless, for late young adults no significant relationship was found, as well as for the total permanent teeth analyzed from Arnhem.

Middenbeemster

Like in Arnhem, for this site the analysis was not applied to the infant category due to lack of present data. No significant relationship was found between caries and LEH in any age category, since all values exceed the significance level of 0.05 (juvenile 0.5, adolescent 0.84, early young adult 0.96, late young adult 0.17). Likewise, when disregarding age groups, there was no significant relation found with respect to LEH and caries for permanent teeth on this site.

Chapter 6: Discussion

This chapter presents a complete interpretation of the results obtained in this research. Historical, archaeological, clinical, and osteological sources are used to provide a comprehensive evaluation of said results. In the first section, the findings obtained from the analysis of prevalence and age at formation of linear enamel hypoplasia in permanent and deciduous teeth will be discussed, considering the differences found between the two sites and between age at death categories. Next, the results of the analysis of dental caries in permanent and deciduous teeth will be interpreted in the same way, taking into account the differences between sites and age at death categories. Similarly, the relationship between enamel hypoplasia and dental caries in both permanent and deciduous teeth will be analyzed. Subsequently, age categorical data will be used to examine the outcomes of the two pathologies from the perspective of fragility and survival. Finally, the limitations encountered will be reviewed followed by recommendations for future research.

6.1 LEH: permanent teeth.

Prevalence of LEH

As discussed in previous chapters, the presence of linear enamel hypoplasia has been used as an indicator of physiological stress in archaeological populations (Goodman & Rose, 1990; Hillson, 1996; Ortner, 2003). Historical, and archaeological sources have pointed out the negative effects of urban living on people's health. In fact, post-medieval and early modern European cities tend to have higher levels of LEH than their rural counterparts, as noted by Palubeckaitė et al. (2002) and Primeau et al. (2015).

Nonetheless, the prevalence of LEH in permanent teeth in this research contradicts this pattern, showing an equal incidence of LEH in Arnhem and Middenbeemster for the permanent dentition with high percentages in both sites. This suggests that growing up in the Netherlands between the 17th and 19th centuries represented high levels of stress for children, regardless of whether they lived in the countryside or in the city. It is important to note that since permanent teeth begin to develop from the first year of age, the presence of LEH discussed in this section mainly accounts for physiological stress events occurring during infancy and childhood (between 1 to 6 years).

When comparing prevalence of LEH between age-at-death groups (permanent dentition), infants had a significant higher prevalence of LEH in both Arnhem and Middenbeemster, which indicates that both rural and urban infants between ages 1 to 3 were the most vulnerable group to be affected by physiological stressors.

The findings of this research are in line with those of Schats (2016) and Fernández Sánchez (2017), who observed that already for the medieval period there were no differences between Dutch rural and urban settings in terms of LEH. Likewise, the prevalence of enamel hypoplasia in permanent teeth for Arnhem and Middenbeemster (57% in both sites) is similar to those recorded for the medieval Dutch collections of Alkmaar, Blokhuizen, and Klaaskinderkerke (65.3%, 52.9%, and 53% respectively) (Schats, 2016, p. 161).

As stated by Schats (2016, p. 153), even though the levels of physiological stress appear equal, multiple possible sources of stress could be affecting children in both sites. Although the etiology of LEH has proven to be multifactorial, its presence has been associated with chronic episodes of high fever, vomiting, and diarrhea during childhood, as well as malnourishment and nutritional deficiencies (Bereczki et al., 2018; Ford et al., 2009; Goodman & Rose, 1990; Suckling, 1989). The next few paragraphs provide a review of the possible causes of physiological stress that could have caused any of these symptoms in both urban and rural settings, including infectious diseases, nutritional deficiencies, and breastfeeding and weaning stress.

Infectious diseases

According to historical sources, Dutch cities through the 17th and 18th centuries were highly insalubrious places, especially for the working classes who lived in conglomerate spaces, with restricted access to fresh air, clean water and sanitation services (van der Woud, 2010, p. 19; de Vries, 1984). In Arnhem, unwealthy citizens often lived near sources of toxic fumes such as open sewers, dumps, and factories (Wintle, 2000, p. 19). These living conditions allowed for the spread of contagious diseases, with outbreaks of smallpox, measles, scarlet fever, typhoid, cholera and dysentery occurring frequently, especially among the lower classes (Osterloh, 2020, p. 14; van der Woud, 2010, pp. 92–93; Wintle, 2000, p. 19). According to historic sources, during the 19th century, smallpox was the highest cause of mortality in children (Wintle, 2000, p. 14), and around 1860, physicians were particularly worried about the spread of infectious diseases in Arnhem, indicating that the health of the citizens was in “unfavorable conditions” (Osterloh, 2020, p. 20). Moreover, the skeletal record shows that people in cities were exposed to constant stress due to respiratory conditions; Casna and collegues (2021) found significant rates of chronic maxillary sinusitis in the Arnhem sample related to indoor and outdoor air pollution (fumes from chimneys, factories, dust, etc.), and constant exposure to viruses related to respiratory diseases.

In the countryside, health conditions were no better than in the cities. Between the 17th and 18th centuries, the agricultural industrial revolution had not arrived yet to Northern Holland, and the economic activities of rural sites were dependent on traditional manual work; in Middenbeemster, these were mainly related to the production of cheese and other dairy products (Falger et al., 2012, p. 202; Waters-Rist & Hoogland, 2018, p. 107). It is well known that dairy farming often comes with health hazards, especially for children, as they seem to be at a higher risk to develop gastroenteric infectious diseases associated with exposure to zoonotic pathogens resulting from the contact with cattle, such as salmonellosis, listeriosis, and infections of *Escherichia coli* (Smith et al., 2004, p. 1098). Respiratory conditions were also common in Middenbeemster as shown from osteological analysis; however these are thought to be related to rigid weather conditions, as well as exposure to pollen and allergens, rather than to infectious diseases and pollution (Casna et al., 2021, p. 898).

Other infectious diseases affecting children in rural areas were related to polluted water bodies. As pointed out in the historic record, after big land reclamations from sea or river, such as the one in Beemster, certain areas remained filled with stagnated water (Falger et al., 2012, p. 80). This not only contributed to the spread of polluted water related diseases (cholera, typhoid, dysentery, etc), but also allowed the mosquitoes that transmit malaria to reproduce (Wintle, 2000, p. 16). Although this disease was uncommon in freshwater polders, we cannot rule out the possibility of saltwater intrusions into the polder, which in turn, could have led to malaria outbreaks in Middenbeemster.

In sum, infectious diseases causing high fevers, vomits, and diarrhea were both present in rural and urban areas. In Arnhem, these diseases appear to be more related to airborne infections due to conglomerate living spaces, air, and water pollution; while in Middenbeemster, the main hazards involved gastrointestinal diseases related to cattle and polluted water.

Nutritional deficiencies

Other causes of LEH have been attributed to malnourishment and nutritional deficiencies, specifically a lack of vitamins A, D, and hypocalcemia (Nikiforuk & Fraser, 1981; Purvis et al., 1973). Urban citizens were thought to have a richer diet than people from the countryside since bigger trading points allowed for higher food availability. However, this was not true for most citizens, as malnourishment and famine were prevailing conditions among the lower classes with a diet dependent mostly on bread, potatoes, and occasionally dairy products (Wintle, 2000, p. 60).

Osterloh (2020, p. 19) emphasizes the presence of highly malnourished children in Arnhem, by analyzing a report to King William III on the living conditions of the city, in which the author notices “pale, hollow-cheeked, pot-bellied children” with “swollen bodies” and “slender legs”. This could be related to the many food crisis occurring during the 18th and 19th century, such as the potato blight, a major crop failure occurring during the 1840’s that caused famine all over the country for several years (Wintle, 2000, p. 55). In accordance, the report by Baetsen and Baetsen, (2020, pp. 501–502) shows skeletal signs of nutritional stress in 15 juvenile individuals from the analyzed sample, including the presence of cribra orbitalia, hyperostosis porotica, and skeletal signs of vitamin D deficiency rickets.

Although it is thought that in Middenbeemster the socioeconomic status of the inhabitants was higher than the ones from Arnhem, and therefore lack of food due to poverty was uncommon, historic sources confirm periods of food scarcity related to crop and cattle plagues during the18th and 19th centuries. For instance, between 1744 and 1769, the Beemster inhabitants were hit by rinderpest[6], wiping out more than half of their cattle (Falger et al., 2012, p. 205). This represented a serious food and economic crisis for the community, as cattle were not only the main economic source, but also an important supply of basic supplements, such as milk, cheese, butter, and meat (Veselka et al., 2015, p. 668; Waters-Rist & Hoogland, 2018, p. 110). Like Arnhem, Middenbeemster was affected by the potato crisis during the 1840’s, as well as many other rye and wheat failures that followed (Falger et al., 2012; Wintle, 2000, p. 56).

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[6] This disease is defined as “an acute infectious disease of ruminant mammals (such as cattle) that is caused by a morbillivirus” (Merriam-Webster. (n.d.). Rinderpest. In Merriam-Webster.com dictionary).

In addition, signs of nutritional stress in non-adult individuals from Middenbeemster have been previously identified in the osteoarcheological record. Veselka and collegues (2015) noted rickets in 13.6% of the infants and children (ages 0 to 6), suggesting that vitamin D deficiency was not uncommon. Other skeletal non-specific signs of nutritional stress in the Middenbeemster collection include short stature, cribra orbitalia, porotic hyperostosis, and cribra femora (Lemmers et al., 2013, p. 113).

Chronology of LEH on permanent teeth.

The results from this research show an equal distribution of age at formation of LEH on permanent teeth in Arnhem and Middenbeemster, with a higher occurrence between 2 and 3 years. This supports the previous statement that children were equally vulnerable to physiological stressors in both Arnhem and Middenbeemster. Similar studies from other European medieval and post-medieval populations have found parallel results, with peak age for LEH development ranging between 2 to 4 years (e.g Garcin et al., 2010; Obertová, 2005; Palubeckaitė et al., 2002).

To analyze LEH formation patterns special attention must be taken before drawing conclusions, since the data could be biased by several factors (Katzenberg et al., 1996, p. 184; Temple, 2020). First, as indicated by Hillson (2014, p. 195), macroscopic methods might over or underestimate the number of defects on the crown due to normal patterns of tooth geometry and dental wear. Therefore, the absence of LEH lesions between 1 and 1.9 years in this research, could be the result of underscoring early lesions in the occlusal area, as defects are more susceptible to dental microwear and harder to observe in this area.

Second, as mentioned previously, there is no biological weaning age, since this is established by social and cultural practices; thus, no assumptions should be made about weaning ages without the appropriate data (e.g. Katzenberg et al., 1996, p. 184).With this said, some interpretations can be made following the findings of this research. According to historians, because of differences in catholic and protestant beliefs, breastfeeding practices widely varied in the Netherlands regionally, and little information is known about the duration of breastfeeding in some regions (Wintle, 2000, p. 20). However, isotopic, and historical data from other contemporaneous sites in Europe indicate differences in breastfeeding and weaning practices between rural and urban areas. For Late Medieval urban societies in continental Europe, Fulminante (2015, p. 42) found shorter breastfeeding and weaning times compared to rural sites, with a breastfeeding duration of around 1 to 3 years, and weaning lasting between 5 and 24 months. Likewise, when studying post-medieval British populations, Britton et al. (2018, p. 515) found a short weaning process, starting soon after birth and completed around the first year of life.

Assuming a relationship does exist between LEH and weaning stress, the data from this research goes in line with a breastfeeding duration of 1 to 3 years. The increase of LEH at 2 years (24 months) could indicate the start of the weaning period, with highest incidence between 2.5 and 2.9 years (30 to 34 months), representing post-weaning stress. However, within this interpretation, this data indicates no difference between rural and urban breastfeeding and weaning practices.

Nonetheless, the breastfeeding hypothesis contradicts the results from isotopic research carried in the Middenbeemster sample, which indicate that breastfeeding was "of very short duration, or completely absent" in this rural context (Waters-Rist et al., 2022, p. 18). According to Waters-Rist and collaborators (2022, p.10), 5 of the 20 individuals analyzed between 1 and 11 months of age were categorized as being in the weaning stage as of 1 month of age, while the other 15 individuals "lack isotopic evidence suggestive of breastfeeding or weaning," indicating that they were not breastfed. The same occurred with children aged 2 to 6 years old, of which only 3 of the 31 individuals analyzed were categorized as being in the weaning stage, while the rest were not breastfed (*idem*, p. 10). Following these data, the LEH peak between 2.5 and 2.9 years rather than being related to post-weaning stress, could be linked to the other sources of stress listed above (e.g., nutritional stress and infectious diseases), as breastfeeding did not occur in most cases.

Many authors suggest that due to a still developing immune system, individuals in the first years of life are more vulnerable to stressors than in other periods of their life (Goodman & Armelagos, 1989; Lewis, 2006, p. 15, 2018, p. 5). Interestingly, in their study about vitamin D deficiency in the Middenbeemster collection, Veselka and collegues (2015) found that individuals between 0 to 3 year old were the most affected with rickets. This goes in accordance with the data presented in this research, which points towards a peak vulnerable period between 2 and 2.9 years. Therefore, linear enamel hypoplasia seems to be related to nutritional deficiencies, rather than to weaning stress in this collection, contradicting the weaning hypothesis.

6.2 LEH: deciduous teeth

Prevalence of LEH

Like on permanent teeth, the prevalence of LEH on deciduous teeth could be related to the multiple environmental, infectious, and nutritional stressors discussed above (infectious diseases related to air and water pollution, malnourishment, nutritional deficiency, etc.), exacerbated by poor living conditions due to lower socioeconomic status. However, because primary dentition starts to develop during the first months of pregnancy and concludes around the ninth month after birth, the presence of enamel hypoplasia in deciduous teeth is directly related to maternal and infant stress (Lacruz et al., 2017, p. 941; López-Lázaro et al., 2022; Temple, 2020). According to the results of this research, the prevalence of LEH on deciduous teeth appears to be significantly higher in Arnhem than in Middenbeemster (17% vs. 11%). Likewise, between age at death categories, only infants in Arnhem present a higher prevalence of LEH in deciduous teeth compared to those in Middenbeemster. Therefore, the variation between the prevalence of LEH in rural and urban sites appearing only in deciduous teeth suggests that infants living in Dutch cities during the post-medieval period were more vulnerable to physiological stress than their rural counterparts.

Chronology of LEH

The data from this research shows a higher frequency of deciduous LEH lesions produced during the prenatal period than the postnatal period in both Arnhem and Middenbeemster. This suggests that in both sites, individuals were exposed to more chronic physiological stress during the prenatal period than in their first year after birth. This goes in line with the results by Blakey and Armelagos (1985), who found that in a precolonial population from Illinois (950-1300 A.D), 71% of deciduous LEH corresponded to the intrauterine period, which indicates a direct relation to maternal health status (*idem*, p. 375). Nevertheless, other studies have found contrasting results, with higher incidence of deciduous LEH during the postnatal period than the prenatal (López-Lázaro et al., 2022), demonstrating that the prenatal and postnatal occurrence of deciduous LEH is population specific and context dependent. For both Arnhem and Middenbeemster, deciduous LEH frequency is higher than permanent

LEH, associated directly to maternal stress. Moreover, the higher prevalence of deciduous LEH in Arnhem compared to Middenbeemster suggests that urban environments were tougher specifically for pregnant mothers during the post-medieval period.

Maternal stress

To find the cause of the higher incidence of deciduous LEH in Arnhem related to maternal stress, we must investigate the differences between the daily lifestyle and wellbeing of urban and rural Dutch women from this time. During the pre-industrial revolution (1600- 1800 A.D), multiple historic sources account for women in cities participating in the labor market, specifically in the textile industry (Schmidt & van Nederveen Meerkerk, 2012). In factories, working conditions were tedious, with daily schedules ranging from 12 to 20 hours involving manual work (van der Woud, 2010). Textile industries had often the worst environment, since workers were constantly exposed to toxic chemicals in unheated, damp, and dark mills (Wintle, 2000, p. 66).

Osteological data suggest that working conditions for farmers were no better in terms of physical exertion. Palmer et al., (2016) analyzed activity markers in the skeleton of the Middenbeemster individuals, revealing high activity levels for both men and women, which indicates that daily lifestyle in rural areas involved heavy physical labor for both sexes. Nonetheless, historians argue that although both industrial and agricultural activity entailed long and heavy workloads, the health hazards in the industrial sector were much worse, at least until the second half of the 19th century, when regulations started to be implemented (Wintle, 2000, p. 66). These poor working conditions must have repercussed in the physiological health of pregnant women from Arnhem and their offspring.

Other factors to take into consideration are nutritional deficiencies involving a lack of vitamin D in mothers, since LEH development in deciduous teeth has been linked to maternal vitamin D insufficiency during pregnancy, as well as hereditary vitamin D dependency rickets (Purvis et al., 1973; Reed et al., 2017). Although no signs of osteomalacia (the adult form of rickets) have been identified in the Arnhem sample according to the studies made by Baetsen and Baetsen (2020), medical sources indicate that mild degrees of vitamin D deficiency in mothers can have repercussions in fetal growth, including the development of LEH on deciduous teeth (Beckett et al., 2022; Reed et al., 2017). Because of their living and working conditions, it is highly possible that women in cities were not receiving enough sun exposure, especially during winter months. Therefore, pregnant women might have been affected by a milder degree of vitamin D deficiency.

On the contrary, by working on the fields, women from Middenbeemster were likely to receive sunlight in their hands and face, which would have been enough to avoid vitamin D insufficiency (Veselka et al., 2018). Other sources of vitamin D that can be found in animal foodstuffs include salmon, herring, fish oil, beef offal, and egg yolk; all of which could have been present in the Dutch diet (Waters-Rist & Hoogland, 2018, p. 110). However, the low socioeconomic status of the Arnhem women analyzed in this study could have prevented them from frequently consuming these types of foods (Wintle, 2000, p. 59).

In sum, maternal stress due to constant exposure to infectious diseases along with mild vitamin D deficiency related to deplorable living and working conditions could be an explanation for higher rates of deciduous LEH in Arnhem children.

6.3 Dental caries: permanent teeth

Regarding permanent teeth, this research has shown a significantly higher prevalence of caries in rural Middenbeemster than in urban Arnhem (33% vs. 23%). Similar rates of caries have been found in other post-medieval settlements across Europe. In post-medieval Britain a caries frequency of 21.5% was recorded among the urban lower classes, while 26.5% carious teeth were recorded for the urban middle class group (Mant & Roberts, 2015, p. 205). Likewise, in an urban lower-class population from post-medieval East London, around 28% of teeth presented caries (Smith, 2019, p. 722). In the same way, this study agrees with the results from previous research on urbanization and oral health in medieval and post-medieval Europe, in which higher caries rates have been recorded for rural population (e.g Griffin, 2017; Pitts & Griffin, 2012). However, the results presented in this research contradict the findings of Schats et al. (2021) who found higher frequencies in a Dutch urban late medieval site of Alkmaar compared to the rural village of Blokhuizen (16% and 7.4%). This suggests a possible shift in caries pattern in the Netherlands from medieval to post-medieval times.

Since dental decay is related to a diet high in carbohydrates and low in protein and fiber, to understand the difference between caries frequencies in post-medieval Arnhem and Middenbeemster we must dig into the dietary pattern of these populations. As mentioned previously, Dutch diet between the 1600’s and 1800’s relied on many foods high in carbohydrates, such as rye or wheat bread, and starchy vegetables like potatoes, carrots, and beets (Wintle, 2000, p. 60). Protein intake was based on meat stuffs (beef, lamb, pork, poultry, rabbit, and hare), fish (salted herring and dried cod), dairy products (milk, cheese and butter), and chicken eggs and fish roe (Adamson, 2004, p. 142). Importantly, these products were consumed less among the lower classes. In addition, the wide spread of sugar during the pre-industrial period has been associated with the increase in caries in multiple European populations during this time (Lanfranco & Eggers, 2012, p. 12; Mant & Roberts, 2015, p. 201). In the Netherlands and other European countries, the rise of refined sugar began in the 15th century as an elite product, and around the 17th century, it became more popular and accessible among the middle classes (Mintz, 2018, p. 92; Wittwer-Backofen & Engel, 2018, p. 88). By the first half of the 19th century, sugar beet started to be produced in the Netherlands, which became a cheaper alternative to sugarcane (Wintle, 2000, p. 152).

Although less information is available about specific diets in rural and urban post-medieval sites, isotopic data confirms that in Middenbeemster the diet consisted of mainly rural foods including "crops, vegetables, and less commonly animal protein from domesticated fowl/eggs and terrestrial herbivores" (Waters-Rist et al., 2022, p. 17). Although isotopic values do not permit to distinguish between protein from meat and from dairy, the high levels of caries found in this study suggest that the protein intake must have been mainly from soft dairy products (butter and cheese), and less frequent from meat. Reiterate that Beemster was a dairying village and is known for their cheese production, so this makes sense. Likewise, high frequency of caries suggests that inhabitants from Middenbeemster were not only consuming starchy vegetables and bread, but also sucrose from refined sugar or syrups.

In Arnhem, isotopic studies carried out by Baetsen and Baetsen (2020, p. 622) point towards a more mixed diet composed of both low trophic level terrestrial foods such as vegetables, meat or dairy products; and higher trophic level foods, such as sea water fish. It is important to note that these studies were carried out on a larger sample of skeletal remains, which might have included individuals from higher social strata, and therefore, a more mixed diet is represented.

However, the lower frequency of caries in Arnhem compared to the rural sample are in line with isotopic data, demonstrating that the diet of urban individuals was more heterogeneous, or at least less cariogenic than the one of Middenbeemster. The city's location near the river could explain a higher consumption of marine products, since this allowed fish to reach the market (Baetsen and Baetsen, 2020, p. 623). Due to the low salaries of the individuals studied in this sample, it is not likely that these people were frequently consuming meat and fish; it is more likely that the low caries rates in Arnhem are due to a lower consumption of refined sugars, as they could hardly finance it. The economic situation of farmers on the other hand, could have allowed them to afford this good, which was already an essential in European kitchen (Mintz, 2018, p. 98; Wintle, 2000, p. 303).

In addition, the lower caries frequencies in Arnhem compared with Middenbeemster could reflect higher rates of dental calculus[7], since these two pathologies result from counteracting chemical processes (Keyes & Rams, 2016, p. 2). Dental calculus is associated with a diet combining proteins and carbohydrates, creating a deposit of plaque on the tooth, while dental caries is related to high consumption of single carbohydrates that demineralize the enamel (Griffin, 2017, p. 351; Lieverse, 1999, p. 219). Therefore, teeth affected by dental calculus are not affected by dental caries.

In the same way, lower rates of caries might reflect a more abrasive diet, since dental wear and caries are negatively correlated (Maat & van der Velde, 1987, p. 292).

Regarding access to dental healthcare, historical sources mention that dentistry was not a common practice in post-medieval times, and the most effective form of curing cavities was tooth extraction usually performed by itinerant medical practitioners, barbers, or even at home with the help of medical advice books (Huisman, 2003, pp. 287–288). Due to the rise in dental decay during the 18th century, dentistry started to gain popularity, especially in the cities. This could be an explanation for the lower caries rates in Arnhem compared to Middenbeemster. Nonetheless, it is important to note that proper care of cavities in shape of fillings were only accessible to rich people, and working classes were restraint to teeth extractions.

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[7] Dental calculus is an oral condition defined as “mineralized dental plaque that adheres to tooth surfaces” (Lieverse, 1999, p. 219).

6.4 Dental caries: deciduous teeth

For deciduous teeth, the results from this research show significantly lower percentages of caries in urban Arnhem than in rural Middenbeemster (5% vs. 25%). Likewise, per age at death category, Middenbeemster presents a statistically higher prevalence of affected deciduous teeth in infants, children, and juveniles. The percentage of caries is lower in deciduous teeth than in permanent teeth for both sites, which contradicts other studies by Halcrow et al. (2013) who found a higher percentage of caries in deciduous teeth than in permanent teeth in relation to weaning practices and dietary factors.

The etiology of caries in deciduous teeth has been associated with several factors including nutritional deficiencies, maternal oral health, and weaning diets, to mention some (Nicolau et al., 2007; Schroth et al., 2014; Tanner et al., 2022). In the next few paragraphs, we will analyze the differences between Arnhem and Middenbeemster in terms of possible etiologies for early childhood caries.

According to clinical studies, early childhood caries can be related to vitamin D deficiency during pregnancy (Beckett et al., 2022; Schroth et al., 2014; Suárez-Calleja et al., 2021). This could be an explanation for the appearance of dental caries in Arnhem children, as women were most likely to present mild levels of vitamin D. However, the high frequency of caries in children from Middenbeemster does not support this theory, since as stated before, women in rural environments were likely to maintain sufficient levels of vitamin D due to sun exposure.

Dietary factors involving highly cariogenic weaning foods have also been attributed to early childhood caries (Davies, 1998, p. 114; Seow, 2018, p. 943; Suprabha et al., 2022, p. 4). Historical sources mention that in the Netherlands, it was common practice to feed infants with animal milk from a very early age; weaning foods included sheep’s, cow’s or goat’s milk diluted in water, buttermilk with buckwheat flour, or porridge, all to which sugar or syrup was added (Hofstee, 1983, pp. 10–11; Waters-Rist et al., 2022, p. 19). In addition, isotopic studies on the Middenbeemster sample present elevated $\delta^{13}C$ values for infants, which are related to C_4 plants such as sugarcane (Waters-Rist et al., 2022, p. 19). The high rates of caries in deciduous teeth in Middenbeemster suggest a high cariogenic weaning diet composed by simple carbohydrates and starchy foods such as the ones listed before. Again, it is possible that because of a higher socioeconomic status, people in Middenbeemster were able to afford sugar in larger quantities compared to the Arnhem individuals. Thus, a difference in weaning diets could explain the variation between percentage of deciduous caries between sites.

Maternal oral health and caries

Maternal oral health is another factor implicated in the development of early caries in children according to multiple clinical research, as mothers can transmit the bacteria responsible for caries directly to their infants (Crall & Forrest, 2018, p. 307; Davies, 1998, p. 113; Seow, 2012, p. 162). Although no genetic relations can be established between adult women individuals and children from macroscopic analyses, the cohort of female adult individuals analyzed in this research belongs to biological fertile age-groups (18-35 years old). Therefore, the prevalence of caries in female permanent young adult teeth could be representing maternal oral health. The higher rates of carious lesions in Middenbeemster females compared to the Arnhem female individuals could be representing the mother-infant nexus in terms of oral health and could be attributed to another possible cause for higher rate of early childhood caries in Middenbeemster.

6.5 LEH in relation to caries

Multiple archaeological and clinical studies have found a correlation between LEH and caries in deciduous teeth (e.g Cook & Buikstra, 1979; Halcrow & Tayles, 2008; Infante & Gillespie, 1977; Targino et al., 2011; Vargas-Ferreira et al., 2015). As explained by Vargas-Ferreira and colleagues (2015), enamel hypoplasia increases the probability of dental decay especially in deciduous teeth due to lower levels of mineralization. Likewise, the concave grooves of hypoplastic defects causes dental plaque to accumulate, resulting in carious lesions (Vargas-Ferreira et al., 2015, p. 543).

Nonetheless, the results from this research did not find any positive association between caries and LEH in either permanent or deciduous teeth. In fact, in deciduous teeth, a negative relationship between caries and LEH was found. However, this does not mean that such relationship was not present since severe caries often prevent the observation of enamel hypoplasia due to enamel destruction. By studying deciduous teeth from an archaeological population of East Asia, Halcrow and Tayles (2008) had already noted a negative correlation between these two pathologies, attributed to the nature of these oral conditions. As mentioned before, in the buccal area of teeth, the grooves caused by enamel hypoplasia create perfect environments for the accumulation of dental plaque, resulting in the demineralization of the enamel and the propagation of carious lesions (Halcrow & Tayles, 2008, p. 2219). Thus, caries recorded in the buccal and labial areas of teeth (where enamel hypoplasia develops), might be masking LEH lesions, explaining the negative association between caries and LEH in deciduous teeth in this research.

Regarding permanent teeth, this study did not find any association between dental caries and LEH lesions. Although some dentistry studies have found slight correlations between these two pathologies in permanent teeth (see Fotedar et al., 2014; Idiculla et al., 2011), there is still not a clear explanation for this association. In addition, dental caries seems to have a stronger relation to other enamel defects. For instance, a longitudinal study carried out in modern samples tested if dental caries in deciduous teeth could be considered a "risk factor" for developing enamel hypoplasia in the permanent dentition (Broadbent et al., 2005, p. 261). These authors found no relation between dental caries in deciduous teeth and enamel hypoplasia; nonetheless, dental caries were associated with other enamel defects such as enamel opacities, product of mineralization issues (Broadbent et al., 2005, p. 264). Similarly, Halcrow and Tayles, (2008, p. 2219) observed the association between caries and opacities in deciduous teeth; however, they did not take it into account for their analysis.

The lack of information on the relationship of these two oral conditions in permanent teeth demonstrate that there is still a gap of knowledge in this area, pointing to the importance of investigating the relationship between oral pathologies at a deeper level, expanding the research into other types of enamel defects.

6.6 Oral stress indicators in terms of frailty and survivorship

In order to understand the effects of urban living in terms of frailty, age-structured data will be used to provide an interpretation of frailty patterns in Arnhem and Middenbeemster. By analyzing stress markers in permanent and deciduous teeth we will compare those individuals who survived physiological stressful events into later stages in life vs. those who did not.

Frailty and survivorship through LEH

Several studies have evaluated the relation between the prevalence of LEH and age-at-death, demonstrating that individuals with childhood stress died at a younger age, and therefore were more frail than individuals who show no signs of LEH (e.g Boldsen, 2007; Cook & Buikstra, 1979; Goodman & Armelagos, 1989; Ham et al., 2021; Stodder, 1997). Nonetheless, the osteological paradox points that individuals with skeletal manifestations of stress were able to survive chronic episodes of stress, and were more resilient than those individuals who died before developing a skeletal response to a disruptive impact (Wood et al., 1992, p. 362). Some studies have shown this pattern on enamel hypoplasia lesions, where higher averages of LEH are found among individuals of older age groups compared to younger individuals (e.g Bennike et al., 2005). The LEH frequencies recorded in this study have yielded similar results, showing higher frequencies of LEH in the permanent dentition than in the deciduous one for both sites (57% permanent vs. 11% deciduous in Middenbeemster and 57% permanent vs. 17% deciduous in Arnhem).

However, when analyzing LEH by age-at-death categories the pattern shows the opposite, as significantly higher LEH rates were recorded for infants in both sites for the permanent dentition, compared to children and juveniles. For the deciduous dentition, specifically infants in Arnhem show a higher percentage of LEH compared to Middenbeemster individuals. This suggests that at this site, higher prevalence of LEH in deciduous teeth is related to frailty, rather than resiliency, since individuals who died as infants were clearly more vulnerable than those who died in later stages of childhood.

This could be partly due to maternal stress in-utero, as around 65% of the total deciduous lesion from both sites were developed during the gestational period. Nonetheless, the high LEH rates in infants from Arnhem could indicate constant exposure to maternal stress in utero, resulting in premature death. Meanwhile, the lower LEH rates in deciduous teeth of infants from Middenbeemster could be indicating death related to post-natal diseases that did not leave a skeletal mark.

In line with the DOHaD hypothesis defined in chapter 2, the first 1000 days after conception are a crucial determinant for an individual’s life (Barker, 2012, p. 186; Gamble & Bentley, 2022). This is confirmed by the prenatal and postnatal frequencies of LEH lesions in deciduous teeth, demonstrating how early exposure to physiological stress during gestation or after birth, can result in premature death.

Going back to the osteological paradox, when analyzing the adult dentition, individuals who died as early young adults and late young adults showed a higher prevalence of LEH than those who died as juveniles and adolescents at both sites. Since early childhood and adolescence have been described as critical periods of vulnerability in an individual's life, these results could be representing those individuals who were less frail during early childhood, but more frail during early adolescence; while individuals who survived into adulthood were more frail during childhood, but less frail through puberty and adolescence.

According to Lewis (2018, p.6), as the immune system develops from age 2, breast milk is no longer enough to supply the necessary immune defenses; hence, passive immunity stops being effective, which represents a high vulnerability period for children. The age at formation of LEH recorded in this research confirms this, with higher LEH rates occurring between ages 2 and 4.

In addition, Lewis (2018, p.6) mentions that during pubertal growth, another vulnerability period occurs, since the demand for resources creates a "trade-off effect" between the immune system and physiological development. This is also shown in the chronology of LEH frequencies, as there is a sudden increase of LEH lesions between ages 10 and 11.

Following the concept of selective mortality, individuals with more LEH lesions would be more resilient than those with less lesions, however this study has shown no relation between number of defects and age-at-death in neither permanent nor deciduous teeth, suggesting that the number of defects does not imply "a longer (or shorter) lifespan", as stated previously by Cucina (2011, p. 113).

Frailty and survivorship through caries

Dental caries has been established to be multifactorial and complex interaction between risk and protective factors related to cultural and biological elements such as diet, social status, oral hygiene, hormonal fluctuations, and immunological resistance, to mention a few (Crall & Forrest, 2018; Hillson, 1996; Lukacs, 2011). In terms of frailty, although dental caries is highly associated to dietary patterns, due to the infectious character of the disease, people with compromised immune system are more likely to be affected by dental decay along with other systemic diseases (DeWitte & Bekvalac, 2010, p. 5).

DeWitte & Bekvalac, (2010) found that in medieval London, adult individuals with dental caries and periodontal disease had a higher mortality risk than their peers without these conditions. The present study did not find any association between dental caries and LEH in permanent teeth, which could be indicating that dental caries in adults was related to dietary patterns, rather than to immunological deficiencies.

Moreover, multiple studies suggest that women are biologically predisposed to develop dental caries due to the hormonal fluctuations related to their reproductive cycle (see Lukacs, 2008, 2011; Lukacs & Largaespada, 2006). The production of estrogen during pregnancy and menstrual cycle alter saliva composition and create immunological stability, leaving females more exposed to external stressors, such as the bacteria responsible for dental caries (Lukacs, 2008, p. 905). The higher prevalence of caries in women in both Arnhem and Middenbeemster could be reflecting this predisposition. Nonetheless, Middenbeemster women present higher rates of caries compared to Arnhem women, which might be related to the dietary differences mentioned earlier. This indicates that apart from immunological and biologically related factors, diet is still a major component of dental caries formation.

Regarding age-at-death, it must be taken into account the fact that dental decay is a progressive pathology, hence, older individuals are expected to have higher frequencies of caries (Hillson, 1996). The frequency of caries lesions in permanent teeth goes accordingly, showing a significant increase in caries depending on the age-at-death in both sites. This could also be analyzed within the parameters of the osteological paradox, in which older individuals are expected to have more lesions than younger individuals as a sign of resiliency; thus, older individuals lived long enough to develop dental caries.

Specifically for deciduous teeth, clinical research has found that developing caries during early childhood can be associated to malnutrition. Often, pain caused by carious lesions prevents children from eating nutrient-rich foods, leading to undernutrition (DeWitte & Bekvalac, 2010; O'Sullivan et al., 1992, p. 26; Tanner et al., 2022, p. 115). In other cases, high rates of dental caries in children have been associated to other forms of malnutrition such as obesity, characterized by the overconsumption of "low-nutrient-dense foods" (often cariogenic), resulting in nutritional deficiencies (Mobley et al., 2009, p. 410).

This study has shown that in general, non-adults from rural Middenbeemster had a significantly higher rate of dental caries compared to individuals from Arnhem. As mentioned before, it is possible that the weaning diet of the infants of Middenbeemster consisted of highly cariogenic foods, in form of sugar sweetened beverages. High consumption of "low-nutrient-dense foods", along with an "inadequate intake of fruits and vegetables”, such as the diet of the infants in Middenbeemster, have proven to cause malnutrition (Mobley et al., 2009, p. 411).

In addition, isotopic data of Middenbeemster suggests that breastfeeding was not a common practice in this site (Waters-Rist et al., 2022, p. 2). Since breastfeeding is extremely important in developing immunological resistance (see Lewis, 2018, p. 7), the absence of this practice combined with a nutrient-poor diet, might have prevented Middenbeemster infants from acquiring the immunological and nutritional benefits needed for their development, resulting in a higher prevalence of dental caries due to immunological deficiencies and malnutrition.

However, the lower levels of caries in non-adults from Arnhem should not be interpreted as a sign of better health, as higher levels of LEH in deciduous teeth in this site prove the opposite. Interestingly, contrary to permanent teeth, caries in deciduous teeth did not present an age-related pattern. This means that in deciduous teeth, apart from the differences in dietary practices mentioned before, caries could also be representing heterogenous frailty between individuals, in the sense that those who do not present caries lesions might have died before they could develop this condition. Thus, the higher prevalence of caries in deciduous teeth of individuals from Middenbeemster, along with the lower prevalence of LEH compared to individuals from Arnhem, could be seen as a sign of resilience.

6.7 Limitations and future research

Limitations

Although this study succeeded in overcoming some of the limitations imposed by the Osteological Paradox by including non-adults and using age-categorical data, the following theoretical and methodological limitations were encountered.

Regarding theoretical aspects, we must consider that the sample analyzed is by no means a representation of the whole living population, since several factors such as population growth and variation might produce biases. For example, this research assumes that every individual grew up in the same setting, without considering possible migrations into the rural or urban environment childhood. This is related to the Osteological Paradox limitation of demographic non-stationary discussed in chapter 2. In addition, due to severe dental wear, only non-adults and young adults were considered for this analysis, leaving out important information from older individuals.

Another important point that must be considered as a limitation for the interpretation of the data is the disparity of information available between Arnhem and Middenbeemster. For this latter site there is a large amount of historical, archaeological, isotopic, and osteological data, product of the multiple investigations carried out over the years. However, these studies have not yet been carried out for Arnhem, leading to problems when comparing sites. Similarly, there is a lack of historical and archaeological information available about oral hygiene practices in the Netherlands during post-medieval times, which limits our interpretations of the results, especially for dental caries.

Regarding methodological aspects, macroscopic methods have shown to under or overrepresent LEH lesions. As stated by (Hillson, 2014, p. 196) when calculating chronology of LEH, the lesions on the occlusal part of the tooth are often non-observable with macroscopical techniques, which could result in an absence of LEH lesions associated to early years of life. In addition, it can be challenging to distinguish between typical tooth growth patterns such as perikymata spacing and a hypoplastic defect when scoring mild LEH lesions macroscopically. Microscopic methods have successfully overcome this limitation, as pathological conditions can be easily distinguished at this level. Similarly, as mentioned previously, caries on the buccal surface of deciduous teeth (circular caries) might be underrepresenting some LEH lesions. This is specifically important for Middenbeemster, where the caries prevalence is higher and the LEH is lower. Analyzing the location of caries could help to solve this limitation, since “circular caries” could be considered as enamel hypoplasia.

Among other methodological issues encountered include the lack of a standardized techniques to analyze dental caries and enamel hypoplasia in archaeological populations for both deciduous and permanent teeth, which resulted problematic when comparing results with other studies previously published. Furthermore, due to time constraints, this research did not consider severity and location of lesions; yet, as mentioned by DeWitte and Stojanowski (2015), including these variables would yield important information about frailty of the individuals. For similar reasons, interobserver error was not calculated, but should be considered to minimize the possibility of error.

Future research

To overcome the methodological limitations noted above, several factors must be taken into account for future investigations. First, regarding LEH lesions, both microscopic and macroscopic techniques should be employed in order to understand the variability between the two techniques, providing a clearer pattern of age at formation of LEH lesions. This should be done considering intra and inter observer error.

Moreover, the severity and location of LEH and carious lesions should be included in the analysis. This way, it would not only be possible to identify carious lesions related to LEH markers in both permanent and deciduous teeth, but we would have a more complete understanding of the role of oral pathologies in morbidity and mortality patterns of individuals.

Similarly, it is recommended that future investigations examine other oral pathologies such as periodontal disease, dental wear, and dental calculus, as well as other enamel defects, such as hypomineralizations and opacities. An integrative approach to these pathologies would allow us to establish relationships between oral conditions which would help us differentiate diet-related patterns from frailty patterns more clearly. Additionally, it would be interesting to compare the results of this research with other skeletal markers of stress.

In addition, this project has demonstrated that an interdisciplinary approach is essential to understanding the bodily experiences of individuals in the past. Therefore, future investigations should focus on integrating isotopic, archaeological, osteological, and historical data. For instance, carrying out isotopic analysis of Arnhem to investigate dietary and weaning patterns, such as those previously done in Middenbeemster, could complement this research by providing information on frailty and LEH related to dietary deficiencies. Finally, integrating into our research other molecular techniques, such as proteomics and DNA analysis, along with historical sources, could also add relevant information about dietary practices and frailty.

Chapter 7: Conclusions

In general, this research investigated the effects of urban living on non-adults and maternal health in the Netherlands during post-medieval times, within an osteoarcheological framework. This study has demonstrated that by using skeletal data we can come across the bodily experiences of non-adults, providing important information about the relationship between maternal and infant stress.

Regarding the main research question (how did urban life affect the health of non-adults and maternal health in the Netherlands in the post-medieval period?), the results of this research have revealed a small part of the many ways in which the transition to urban life affected the health of people in the past. Specifically for infants and maternal health, the prevalence and age-at-formation of LEH on deciduous teeth has shown that urban living had a negative impact specifically on infants and mothers, as these lesions reflect prenatal maternal stress. However, if infants managed to survive these periods of stress, during the following years of life (3-6), urban life does not appear to have a greater negative impact than rural life, since the prevalence and age-at-formation of LEH on permanent teeth have shown that non-adults living in urban environments were equally stressed than non-adults growing up in rural settings. This has also rejected the post-weaning stress hypothesis, as chronology of LEH does not correlate to breastfeeding and weaning periods.

In addition, the prevalence of caries on permanent teeth has shown that this oral condition can be linked to dietary patterns, rather than physiological stress, as rural individuals with a diet rich in starchy and sugary foods presented more caries. Therefore, urban living did not seem to have a negative effect on this pathology, as both non-adults and adults from rural Middenbeemster present higher caries rates than their urban peers. Nonetheless, prevalence of caries on deciduous teeth has shown that this condition can be used as a proxy to determine resiliency of non-adult individuals, as well as the maternal- infant link after birth.

Although no association was found between caries and LEH in this research, further investigation is needed to understand the complex interaction between these two pathological conditions. Finally, in terms of frailty and survivorship this research has shown that by using age-categorical data we can overcome some of the limitations presented by the osteological paradox, by analyzing individuals who survived episodes of stress vs. those who did not survive.

In sum, the following conclusions can be drawn from this study:

- Prevalence and age-at-formation of LEH on permanent teeth has demonstrated that in the Netherlands during post-medieval times, children living in urban environments were equally physiologically stressed than children growing up in rural settings, with a higher vulnerability between 2 and 4 years.
- In this population, the formation of LEH does not seem to be related to post-weaning stress, as seen by the age-at-formation of LEH.
- Prevalence and age-at-formation of LEH on deciduous teeth has shown that urban living in the Netherlands during post-medieval times had a negative effect on infants and children, as seen from the age-at-formation of LEH linked to prenatal maternal stress.
- Prevalence of caries on permanent teeth has shown that this oral condition can be linked to dietary patterns, rather than physiological stress, as seen from the higher prevalence of dental caries in rural populations compared to urban populations.
- Prevalence of caries on deciduous teeth has shown that this condition can be used as a proxy to determine frailty and resiliency of non-adult individuals, as the progressive character of this condition accounts for those individuals who lived long enough to develop caries compared who those who did not.
- Further investigation is needed to understand the complex interaction between oral pathologies such as linear enamel hypoplasia and dental caries.

Abstract

Urbanization and urban living not only reshaped the social, cultural, political, and economical aspects of European societies, but also had a great impact on people's health. These effects can be observed in the archaeological record through the analysis of skeletal remains from urban sites by comparing them to their rural counterparts. Although multiple studies have focused on investigating the effects of urbanization and urban living, few have researched the impact of urban living on infants, children, and maternal health. By investigating the effects of urban living in non-adults through the analysis of dental stress markers such as linear enamel hypoplasia and dental caries, not only we overcome the preservation issues of working with non-adult remains, but we can also provide information about the nexus between maternal and infant health from a perspective of frailty and survivorship.

Because teeth do not remodel since their formation (in utero for deciduous teeth and during the first years of life for permanent teeth), analyzing time at formation of dental stress markers such as linear enamel hypoplasia in both deciduous and permanent teeth, opens the possibility to assess infant and maternal stress during specific periods of time. In addition, due to the association between dental caries and dietary patterns, this disease can be analyzed as a proxy for malnutrition, providing information frailty regarding oral health.

Therefore, this research focuses on assessing the effects of urban living in infant and maternal health during post medieval times in the Netherlands through the comparison of oral stress indicators from a rural (Middenbeemster) and an urban (Arnhem) collection of post-medieval Dutch non-adults and adults. To do so, frequencies and age-at-formation of linear enamel hypoplasia, as well as frequency of dental caries were recorded and analyzed for permanent and deciduous teeth, using age-categorical data to compare them between rural and urban individuals. The results from this research demonstrate that urban living had a negative impact on infant and maternal health, as found from the dental markers associated to the prenatal period. However, during the following years of life (3-6), urban life did not have a greater negative impact than rural life, as prevalence of enamel hypoplasia indicates that both non-adults living in urban environments were equally stressed than non-adults growing up in rural settings. In addition, this research has shown that analyzing dental caries in deciduous teeth can demonstrate the maternal-infant relationship after birth, and that dental caries can be used as a proxy to determine frailty and resiliency of non-adult individuals.

Key words:

Urbanization, Post-medieval, Frailty, Dental stress markers, Infant and maternal health

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Appendix A:

This images represent the maps of Arnhem and Middenbeemster. The first two images (A.1 and A.2) are from Arnhem before urbanization (1649) and during the urban expansion (1853). The third image (Figure A.3) is a map of Middenbeemster in 1658 (46 years after its creation in 1612).

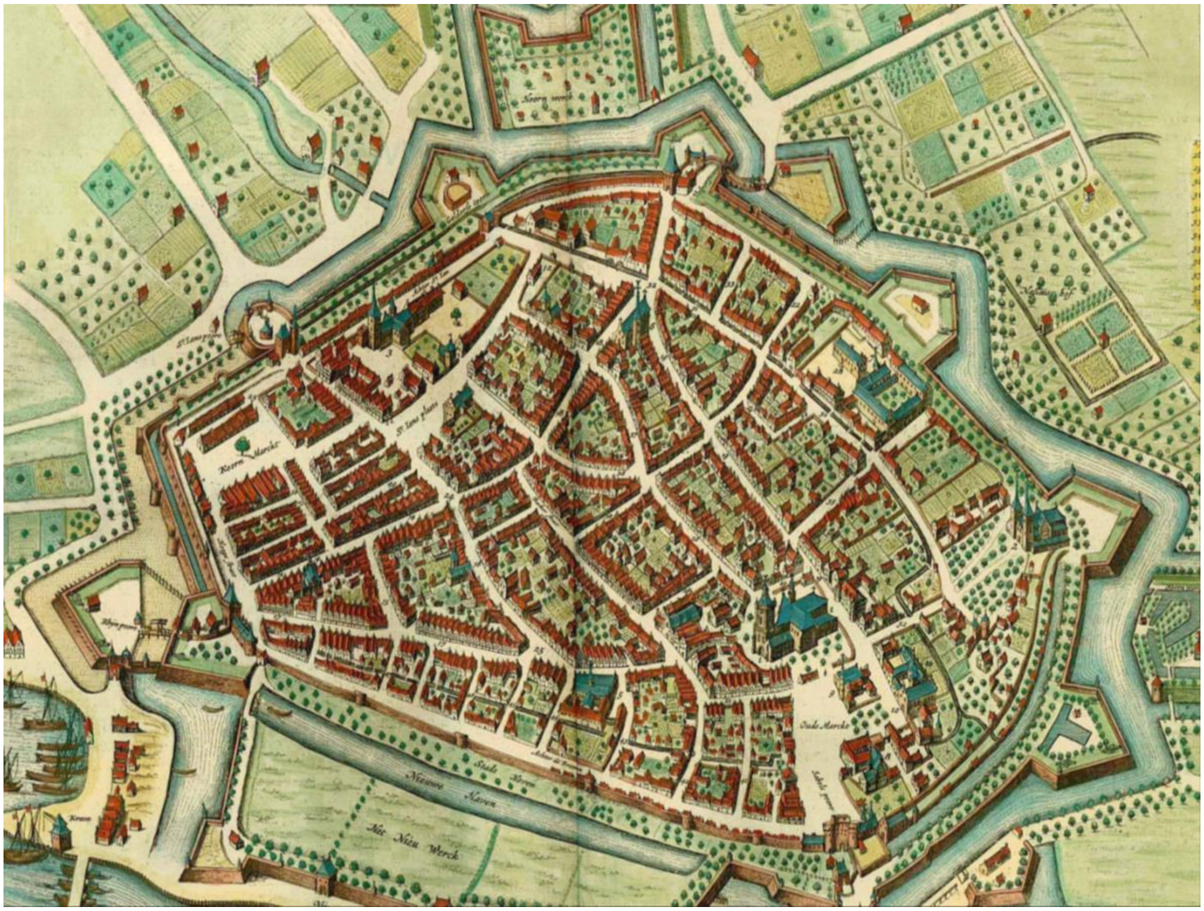

Figure A 1: Map of Arnhem (1649 A.D) by J. Blaeu. Image retrieved from sandeusmaps.com

Map of Arnhem after urbanization (1850)

The yellow areas represent streets to be constructed. Compared to the previous image, houses have occupied the green areas, and extended beyond city walls.

Figure A 2 Map of the urban expansion plan of Arnhem in 1853 by D,A. Thieme. Retrieved from Gelders Archive, 1506 – 8428 (mijngelderland.nl)

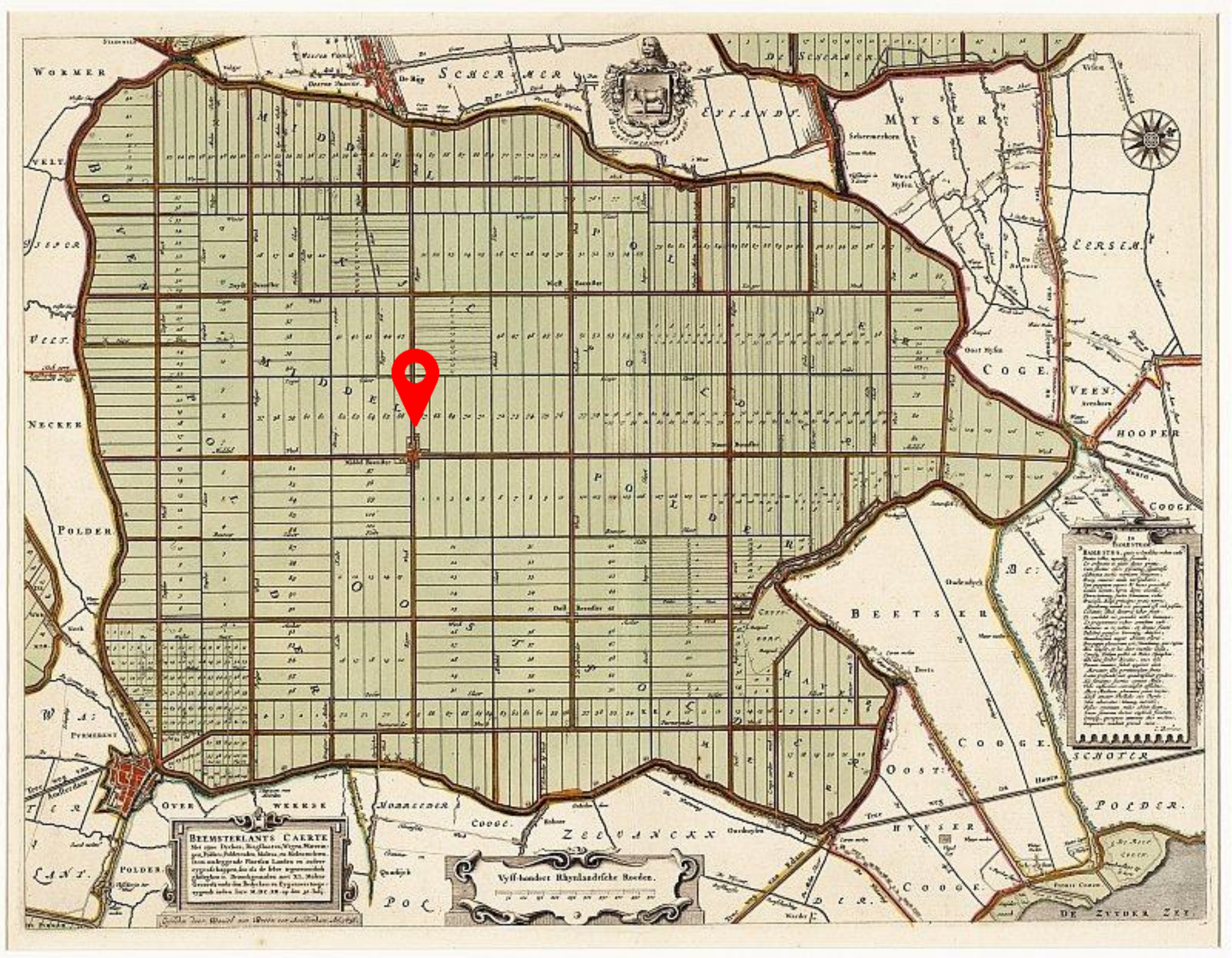

Figure A 3 Map of the Beemster Polder in 1658. The arrow indicates the location and size of Middenbeemster in relation to the Polder. Image retrieved from https://exploringthenetherlands.com/wpcontent/uploads/2020/12/Beemsterlants_Caerte_Daniel_van_Breen_1658.jpg

Appendix B:

This table was used to calculate the deciles to establish the age at formation of LEH on permanent teeth following the formula and data provided by Reid and Dean (2006). The average crown heights used to calculate the distance of each decile were retrieved from the averages for Northern European samples calculated by Reid and Dean (2006, pp. 331–332). Each number indicates a tooth following the numbering system established by the Federation Dentaire Internationale (FDI), in which the mouth is divided into four segments (top right, top left, bottom right, and bottom left). The numbers 11-18 represent the 8 teeth from the top right segment, while the numbers 31-38 represent the 8 permanent teeth from the left bottom segment.

For the deciles, the average crown height was divided into 10. The deciles from the teeth numbered 21-28 were calculated using the same measurements as 11-18, while the deciles from teeth 41-48 were calculated using the measurements from 31-38.

Tooth #	***Average crown height (mm)***	***Deciles mm***
11	13.63	1.363
12	11.6	1.16
13	11.24	1.124
14	N/A	N/A
15	N/A	N/A
16	9.08	0.908
17	8.88	0.888
18	8.85	0.885
31	10.31	1.031
32	10.06	1.006
33	11.57	1.157
34	N/A	N/A
35	N/A	N/A
36	8.99	0.899
37	8.65	0.865
38	8.92	0.892

Table B 1: Table used to calculate the deciles to establish chronology of LEH on permanent teeth

Appendix C:

This table was used to establish the age at formation of LEH on permanent teeth parting from the deciles established by Reid and Dean (2006) (see figure 3.5). Each LEH measurement was matched to the closest decile, and with that, an estimated age-at-formation could be calculated.

The numbers represent a tooth following the numbering system of the FDI as mentioned before.

Age according to each decile per tooth (permanent)						
	11, 21	**12, 22**	**13, 23**	**31, 41**	**32, 42**	**33, 43**
Age (yrs)	5	5.1	5.3	3.8	4.2	6.2
	4.4	4.6	4.8	3.4	3.7	5.6
	3.9	4.1	4.3	3	3.3	4.9
	3.4	3.7	3.8	2.6	2.8	4.2
	2.9	3.3	3.4	2.3	2.4	3.6
	2.4	2.9	3	2	2.1	3.1
	2	2.7	2.7	1.7	1.8	2.7
	1.8	2.4	2.4	1.5	1.5	2.3
	1.6	2.2	2.2	1.3	1.3	2
	1.3	2	1.9	1.1	1.1	1.7
	1.1	1.8	1.7	1	1	1.5

Table C.1 Age correspondent for each decile per tooth of LEH chronology for permanent teeth (Incisors and canines)

Age according to each decile per tooth (permanent)					
	17, 27	**18, 28**	**36, 46**	**37, 47**	**38,48**
Age (yrs)	6.3	11.4	3.3	6.2	11.2
	5.9	10.9	3	5.9	10.9
	5.6	10.6	2.7	5.6	10.5
	5.3	10.3	2.4	5.3	10.3
	5.1	10.1	2.1	5	10
	4.9	9.9	1.9	4.9	9.8
	4.8	9.8	1.8	4.7	9.7
	4.6	9.7	1.6	4.6	9.6
	4.5	9.6	1.5	4.4	9.5
	4.4	9.5	1.4	4.3	9.4
	4.3	9.4	1.3	4.2	9.3

Table C. 2: Age correspondent for each decile per tooth of LEH chronology for permanent teeth (Molars)

Appendix D:

Similarly, this table was used to calculate the stage at formation of LEH for deciduous teeth (if the lesion was formed prenatal or postnatal).

The tooth numbers follow the numbering system from the FDI for deciduous teeth. In this case, the mouth is also divided into four planes of 5 teeth each; therefore 51-55 correspond to teeth from the top right plane, 61-65 top left, 71-75 bottom right, and 81-85 are bottom left.

The average crown heights of each tooth were retrieved from Liversidge et al.(1993).

The tooth numbers indicate

The crown formation times before birth and after birth were calculated according to Birch & Dean (2014).

Tooth #	*Average crown height (mm)*	*Total formation time (months)*	*Prenatal formation time (months)*	*Postnatal formation time (months)*	**Prenatal growth mm**	**Postnatal growth (mm)**
51	6.17	7.9	4.74	3.16	3.70	2.47
52	5.92	8.18	4.48	3.7	3.24	2.68
53	7	14.13	4.22	9.91	2.09	4.91
54	5.86	10.71	4.61	6.1	2.52	3.34
55	6.33	16.64	3.86	12.77	1.47	4.86
61	6.17	7.9	4.74	3.16	3.70	2.47
62	5.92	8.18	4.48	3.7	3.24	2.68
63	7	14.13	4.22	9.91	2.09	4.91
64	5.86	10.71	4.61	6.1	2.52	3.34
65	6.33	16.64	3.86	12.77	1.47	4.86
71	5.26	7.9	4.74	3.16	3.16	2.10
72	6.03	8.18	4.48	3.7	3.30	2.73
73	7.01	14.13	4.22	9.91	2.09	4.92
74	6.41	10.71	4.61	6.1	2.76	3.65
75	6.31	16.64	3.86	12.77	1.46	4.84
81	5.26	7.9	4.74	3.16	3.16	2.10
82	6.03	8.18	4.48	3.7	3.30	2.73
83	7.01	14.13	4.22	9.91	2.09	4.92
84	6.41	10.71	4.61	6.1	2.76	3.65
85	6.31	16.64	3.86	12.77	1.46	4.84

Table D 1: Measurments for calculation of LEH chronology for deciduous teeth.