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Stressing out in medieval Denmark: An investigation of dental enamel defects and age at death in two medieval Danish cemeteries

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A B S T R A C T

The influence of early life stress on later life experiences has become a major focus of research in medicine and more recently in bioarchaeology. Dental enamel, which preserves a record of childhood stress events, represents an important resource for this investigation when paired with the information from adult skeletal remains, such as age at death. The purpose of this research was to use a life history approach to the exploration of sex differences in the relationship between childhood stress and adult longevity by examining accentuated striae of Retzius (AS). A medieval Danish sample (n = 70) drawn from the rural cemetery of Sejlet and the urban cemetery of Ole Wormsgade was considered for AS and age at death. The results suggest sex differences in survivorship, with more stress being associated with reduced survivorship in males and increased survivorship in females. A consideration of AS formation time also suggests a difference in the impact of developmental timing between males and females. These results are interpreted in terms of differential frailty and selective mortality, drawing in both biomedical and cultural perspectives.

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1. Introduction

The formation of dental enamel occurs with a regular periodicity, providing a record of growth and development along with a record of any disruption during development (Antoine, 2000; Antoine et al., 2009; Goodman and Rose, 1990; Risnes, 1986; Smith, 2006). Enamel is also the hardest biological substance, with mature enamel being at least 97% mineral in composition and not remodeling once formed (Goodman and Rose, 1990; Hillson, 2014). Apart from occlusal wear and abrasion, it captures a record of growth and development that is preserved throughout the life of an individual and is less subject to degradation in the archaeological record due to the high mineral content. These characteristics make it an excellent medium for the consideration of growth patterns. In addition to the regular markers of growth formed during enamel deposition and maturation, systemic growth disruption can impact enamel growth, leaving permanent irregular markers in the enamel. The most commonly studied markers are visualized as horizontal lines known as dental (or in this case linear) enamel hypoplasia (LEH). These have been considered extensively in connection with the assessment of stress in past populations (Bennike et al., 2005; Obertová, 2005; Palubeckaitė et al., 2002; Wright, 1997). It has further been shown that even the smallest defects that cannot be distinguished without the aid of a microscope can represent stress events (Hillson, 1992) and that microscopic versus macroscopic examination can produce vastly different results (Hassett, 2012). The potential of enamel micro-defects to reflect actual events that might have impacted how an individual responded to a range of later life experiences makes them invaluable resources for the study of stress in past human populations.

The growth lines visible on the enamel surface (known as perikymata) are reflected internally by striae of Retzius, with more pronounced internal lines that may be associated with growth disruption being known as accentuated striae of Retzius (AS) or alternatively as Wilson bands or pathological striae of Retzius (FitzGerald and Rose, 2008; Hillson, 2014). The consideration of internal enamel microstructure makes it possible to observe growth lines even in the cuspal area, where they do not appear on the tooth surface. It is also possible, using a variety of methods, to attain precise ages for the occurrence of any given AS, moreso than for LEH (see Hillson (2014) for recent in depth coverage). Reid and Dean (2006), using complete reconstructions of age at occurrence from daily markers (cross-striations) and long period markers (AS) have built developmental charts for each tooth type, where teeth are divided into ten different regions (deciles) from cusp to cementum-enamel junction (CEJ) and age ranges are

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associated with each region. These charts are useful for estimating developmental age across the tooth crown, and one set of age reconstructions is derived from a Northern European sample containing individuals from the medieval site of Tirup, which is located in close proximity to the cemeteries used in the current study.

There is also increasingly strong evidence for early life stress impacts on later life experiences (Barker, 2004a, 2004b, 2001; Barker and Osmond, 1986; De Boo and Harding, 2006; Gillman, 2005; Gluckman et al., 2005; Simmons, 2009). However, this connection has seldom been considered on a large scale using internal enamel microdefects (Rose et al., 1985, 1981, 1978; Thomas, 2003). The ability of dental enamel to capture early life stress events and the potential of the human skeleton to provide insight into later life experience places human osteological studies of this nature in a strong position to provide a life course perspective reflecting developmental plasticity and adaptive response (Armelagos et al., 2009).

This study investigates the number of AS observed in mandibular canines in relationship to adult mortality (i.e., mortality in individuals who survived childhood stress events) in archaeological samples from medieval Denmark. Two cemeteries dating broadly to between mid-12th and mid-16th centuries, representing both rural and urban populations from in and around the medieval market town of Horsens were used in this investigation (Fig. 1). The aim of the study is to identify how stress early in life might correlate with longevity, with a particular focus on possible sex differences in the samples. More specifically, it tests three hypotheses:

i) The number of stress events during growth and development, as reflected by dental enamel microdefects, will be associated with changes in adult mortality. It is predicted that individuals with more AS across the total crown (total AS) will show reduced age at death. The null hypothesis predicts that there will be no correlation between the number of enamel defects and mortality.

ii) There will be sex-specific difference in the response to early life stress experiences and so any pattern that emerges between enamel defects and adult mortality will vary between males and females. The null hypothesis proposes that there will be no difference between the sexes, and so any patterns observed between enamel defect occurrence and age at death will show no significant difference between the males and the females in the sample.

iii) The relationship seen between episodes of enamel growth disruption and age at death will fluctuate based on the timing of occurrence of the stress experience. The null hypothesis proposes that the developmental age at which the stress experience occurs will not have a variable influence on age at death. Different timings of occurrence will therefore show no significant differences in their correlation with age at death.

1.1. Childhood stress and adult mortality

Research in bioarchaeology has demonstrated a connection between indicators of childhood stress experiences (such as enamel hypoplasia) and age at death (Armelagos et al., 2009; Boldsen, 2007; Cook and Buikstra, 1979; Goodman and Armelagos, 1988; White, 1978). Beyond bioarchaeology, there is a growing body of clinical literature providing insight into the long-term impacts of early life experiences (Barker, 2004a, 2004b, 2001; Barker and Osmond, 1986; De Boo and Harding, 2006; Gillman, 2005; Gluckman et al., 2005; Simmons, 2009). Central to this research is a theory that was initially formulated as the Barker Hypothesis (Barker and Osmond 1986) and later reformulated as the Developmental Origins of Health and Disease Hypothesis (Barker, 2004a; De Boo and Harding, 2006). This hypothesis proposes that physiological disruption early in life can have a negative impact on adult health, some focusing on the foetal period with its associated rapid growth. However, this period of rapid growth continues postnatally through the infant and childhood periods (albeit with a gradual slowing down) (Bogin, 1999). Systems developing during this period, such as the immune system, can be negatively impacted by physiological stress (MacGregor, 2008). Epigenetic research, which may be defined as the science of heritable biological adaptation involving the epigenome, also plays a central role in the investigation of this concept (Devaskar and Raychaudhuri, 2007). Through such research, the mechanisms by which environmental factors can influence genome function are being revealed.

Parameters such as neonatal birth weight, childhood obesity, growth rate, infectious disease and breastfeeding duration have frequently been considered in relation to adult obesity, diabetes, asthma, and cardiovascular health to explore a possible impact of early life experiences on later life health (Barker, 1995; Barker et al., 1993; Cianfarani et al., 1999; Eriksson et al., 2002, 2001; Forsen et al., 1999; Freedman et al., 2001; Gern et al., 1999; Lemanske, 2002; Ong et al., 2000; Openshaw et al., 2004). Bioarchaeologists do not have access to any of these modern health parameters in their investigations of past populations. However, they do have access to the record of nonspecific stress markers preserved in dental enamel, and they do have certain indicators reflecting more recent physiological experiences that can allow them to take a life course perspective on long-term responses to stress (Armelagos et al., 2009). By taking a microscopic rather than a macroscopic approach to this research, it becomes possible to capture enamel growth disruption more fully, even down to the finer and potentially more acute experiences that might not be apparent macroscopically. Age at death can provide insight into how human populations respond in later life to early life physiological impacts. Engaging in archaeological studies makes it possible to gain insight into historic patterns of response, here focusing upon the environmental and social changes seen in medieval Denmark, including those associated with the late medieval agrarian crisis and the changes brought about by the mid-14th century Black Death epidemic. Such bioarchaeological studies not only provide insight into the biological non-specific stress response, but also into how human populations respond to different circumstances over time. The populations considered in this investigation lived in a pre-antibiotic era and experienced periods of famine along with both endemic and epidemic disease loads (Benedictow, 2004, 1996; Bennike et al., 2005; Boldsen, 2009, 2008; Qvist and Grenetved, 2001; Yoder, 2006). Thus, the developmental records captured by dental and skeletal remains allow us to track stress exposure and impact over the life course prior to modern medical intervention.

2. Materials and methods

This study examined the skeletal remains from two Danish medieval cemeteries currently curated at the Anthropological Database, Odense University (ADBOU) in trust from the Horsens Museum (Denmark). Sejet is a rural parish cemetery lying just outside of the medieval town of Horsens, while Ole Wormsgade was an urban parish cemetery located in Horsens (Fig. 1). A total of 37 individuals from Sejet (19 females, 15 males, and 3 of undetermined sex) and 36 individuals from Ole Wormsgade (17 females and 19 males) were sampled for this research. Both cemeteries were in use from roughly the mid-12th to the mid-16th centuries, with use of Ole Wormsgade likely ending by A.D. 1536 and use of Sejet terminating around A.D. 1574 (Johansen et al., 2004; Kjærgård, 2006). No significant changes in AS counts were detected between sites or over the period of cemetery use. Unfortunately, if subdivided by
both site and period sample sizes were insufficient for a detailed analysis.

Individuals selected for the purposes of this research were aged eighteen years or older at death, having mandibular canines preserved and not extensively worn (i.e. still reflective of development between 3 and 6.2 years of age based on Reid and Dean (2006)). This project focused on the mandibular canine, which represents the greatest period of growth for any single tooth. Using only the mandibular canine minimized the extent of the destructive analysis while maximizing the growth span captured. The sample used for testing intra-observer error included 75 individuals of which 71 were represented by a mandibular canine and four by other anterior teeth (two maxillary canines, one maxillary first incisor, and one maxillary second incisor). Testing of intra-observer error was conducted by considering the level of correlation between consecutive trials of AS counting (using Pearson correlation coefficient). The sample for the consideration of AS in relation to age at death was necessarily restricted to the individuals represented by mandibular canines (n = 75). In addition, two males were eliminated from these analyses as they showed clear osteological evidence of unhealed trauma-related injuries and three individuals were excluded from the sex-based analyses because sex could not be determined (Table 1).

### Table 1
Sample distribution including sample for intra-observer error testing (including two individuals represented by maxillary canines, one represented by a maxillary first incisor, and one represented by a maxillary second incisor), and the sample for consideration of accentuated striae of Retzius (AS) in relationship to age at death. The sample includes only individuals represented by mandibular canines and excludes two males with trauma-related mortality. The sex-based consideration also excludes three individuals with insecure sex determination.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-observer error</td>
<td>75</td>
</tr>
<tr>
<td>Individuals represented by mandibular canines for</td>
<td>73</td>
</tr>
<tr>
<td>consideration in relation to age at death.</td>
<td></td>
</tr>
<tr>
<td>Males excluded from age at death analysis due to evidence</td>
<td>2</td>
</tr>
<tr>
<td>for trauma-related mortality</td>
<td></td>
</tr>
<tr>
<td>Consideration of AS to age at death, Males</td>
<td>34</td>
</tr>
<tr>
<td>Consideration of AS to age at death, Females</td>
<td>36</td>
</tr>
<tr>
<td>Consideration of AS to age at death, sex undetermined</td>
<td>3</td>
</tr>
</tbody>
</table>

#### 2.1. Age estimation

Age estimation involved the use of a multi-variable approach incorporating traditional morphological techniques, complemented by the experience-based portion of a system being developed through ADBOU and the Max Planck Institute for Demographic Research, known as calibrated expert inference (Milner et al., 2015; Milner and Boldsen, 2012a; Tarp, 2009; Weise et al., 2009, 2012). The scoring techniques used for this are available through the ADBOU website [http://adbou.dk/fileadmin/manualer/engelskmanualver080911_vandmaerke.pdf](http://adbou.dk/fileadmin/manualer/engelskmanualver080911_vandmaerke.pdf) (ADBOU, 2010). Results were cross-checked with previous age at death estimates from the cemetery databases\(^1\) and a random subsample was independently evaluated by the second author. The more traditional morphological techniques employed were pubic symphys (Brooks and Suchey, 1990) and auricular surface morphology (Lovejoy et al., 1985), dental wear (Lovejoy, 1985), and cranial suture closure (Meindl and Lovejoy, 1985). An age at death range was derived by considering the results of all methods, and the median of this range was used as a proxy for age at death in the statistical analyses, with mean ages for the subsamples calculated from these median ages at death. The experience-based inference method carried the most weight in developing the final age at death range, as its thresholds were developed using compatible osteological samples, and it has demonstrated strong results in preliminary testing (Milner and Boldsen, 2012b). The age at death ranges were therefore internally consistent for the purposes of this study, and both the technique and use of the midpoint as the estimated age is consistent with more recently recommended paleodemographic techniques (Milner and Boldsen, 2012a). The results demonstrate broad patterns that are apparent within this particular sample (Fig. 2).

This multi-variate approach facilitated refining age at death estimates, especially valuable in light of sample preservation issues. In the case of Sejet, poor overall element preservation with highly fragile remains frequently limited the features available for scoring for any given individual. In the case of Ole Wormsgade, excavation of the cemetery along the limited course of a road led to

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\(^1\) The results from both the original age ranges and those estimated as part of this study are available in [Gamble (2014)](http://example.com).
truncation of burials at the edges of the road and to the consequent recovery of incomplete remains in many cases. In such circumstances, point estimate techniques such as transition analysis, which require specific features to be present (Boldsen, 2008; Boldsen et al., 2002; Milner and Boldsen, 2012b) are not possible. However, the experience-based inference method used here made it possible to identify an age at death range based on the range of available features, which themselves were linked to point transitions rather than ranges (Milner and Boldsen, 2012a, 2012b; Weise et al., 2009).

Statistical analysis was conducted using IBM SPSS 20 software and involved an initial examination of all individuals from both cemeteries. AS were counted in each tooth five times in order to test for intra-observer error and learning curve. Trials were conducted over a six-month period with a minimum of a week between each. Pearson correlation was used alongside Spearman rank sum correlation to assess agreement between trials, and paired sample t-tests were used to assess mean differences in AS counts between trials. Males and females were then considered separately using the combined cemetery sample. Kaplan-Meier survival analysis was conducted to consider age at death in relation to accentuated striae of Retzius (AS) counts, with a log-rank Mantel-Cox test used to test the difference in survival distributions with enamel defects between males and females. A Bonferroni-type argument to control for family-wise errors was applied in the decile consideration.

2.2. Microscopy

Teeth were thin sectioned and considered for total number of AS (total) and for the number of AS in each decile (decile AS) (using the Reid and Dean (2006) standards), both in relationship to age at death. Thin sections were produced by embedding the teeth in EpoThin® Epoxy Resin mixed with EpoThin® Epoxy Hardener in a 5:1.95 ratio and then cutting the longitudinal sections through the dentin horn using a Buehler IsoMet® 1000 low speed saw. Sections were then polished to 100 µm thickness. All sections were imaged using an Olympus BX51 microscope with automated stage and an Olympus DP72 digital camera. Olympus STREAM software was used for image analysis. All processing and analysis was conducted in the Bioanthropology Digital Image Analysis Laboratory (BDIAL) in the Department of Anthropology, University of Manitoba.

Striae of Retzius and the associated accentuated striae of Retzius (AS) or Wilson bands are structures that can be highly variable in appearance, being expressed differently across individuals, teeth, and even within the same crown (FitzGerald and Saunders, 2005; Hillson and Bond, 1997). One can be seen highlighted in Fig. 3, along with a corresponding contour line of Owen (an accentuated line found in the dentin). In response to the variability in AS appearance and to previous research indicating that there may be no minimum threshold for expression in the accentuated structures (FitzGerald and Saunders, 2005), AS in this study were defined as those striae that were more pronounced in relationship to other striae in the surrounding region of the crown (dividing the crown into cuspal, midcrown, and cervical thirds). No association with surface defects was required. As such, any AS in this study was counted as a single stress event, with no distinction being made between ‘stronger’ and ‘weaker’ striae, an approach that was taken in Thomas’ (2003) previous study on the nearby Tirup material.

The number of AS across each tooth crown and in association with each of the crown regions was counted and considered in relationship to age at death. Samples were divided into three groups based on the frequency distribution of total AS. Individuals with AS counts in the lowest quartile of the AS distribution (0–30 AS) were assigned to the “minimal stress” category, those in the 25%–75% distribution range (31–58 AS) were assigned to the “moderate stress” category, and those in the upper 25% of the range (59+ AS) were assigned to the “severe stress” category. Further analysis was also conducted in which the ‘severe stress’ individuals (59+ AS representing the upper quartile of the frequency distribution) were considered in relationship to a combined ‘minimal’ and ‘moderate’ stress categories combined (individuals with up to 58 AS representing the first three quartiles of the frequency distribution). This partitioning based on the frequency distribution of AS counts was implemented to ensure that the thresholds were not based on potential researcher bias. Since there was insufficient biological for establishing informed thresholds, the frequency distribution in the sample was used as a baseline. This highlights the need for further work in order to elucidate how the number of AS formed during growth and development relates to biologically informative stress thresholds.
Fig. 3. Mandibular canine with an example of an accentuated striae of Retzius highlighted by A (red arrows) and an associated contour line of Owen in the dentin (B, black arrow). (Ole Wormsgade X1019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Fig. 4. Mandibular canine showing the approximate developmental ages for crown divided into deciles. The ages at each decile mark are given in black numbers. The decile numbers referred to in this study are labelled in black from the 1st to the 10th decile. All ages at decile marks have been taken from Reid and Dean (2006). (Ole Wormsgade X1019).

To capture any differences in age at death in relationship to developmental periods, each crown was divided into ten sections (deciles) based on crown height, with associated developmental age ranges for each decile (Table 1). These deciles and associated developmental age ranges were based on those identified by Reid and Dean (2006) using dental material that included material from the nearby Danish medieval site of Tirup, which also forms the basis of Thomas' (2003) work (Fig. 4). Sample sizes available for each decile varied depending on preservation and wear (Table 2). The number of AS also varied by decile and so the decile-specific frequency distribution was used to divide the sample into stress categories in the same manner as for total crown AS.

3. Results

A consideration of the different trials showed increasing consistency over the first three trials signifying the development of the scoring method and the effect of a learning curve (Fig. 5, Table 3). There was a slight decrease in correlation with the fourth trial, and the fourth trial can be seen to be an anomaly, having the lowest mean AS counts of all the trials (Table 3). This difference was significant, with $t(74) = 2.473, p = 0.016$ for the third trial and $t(74) = -6.111, p < 0.001$ for the fifth trial, and marks a trial that was less inclusive of AS and divergent from the other trials. When the fifth trial is compared to each other consecutive trial there is a continuous increase in correlation that suggests improved agreement.
Table 2
The developmental age ranges associated with each decile, along with the sample sizes that were available for each decile by sex and the stress category cut-off points. Variation in sample size by decile number reflects differential wear and preservation across the crown. The ‘minimal stress’ category includes individuals who had AS counts in the lower 25% of the decile-specific frequency distribution, while the ‘moderate stress’ category includes those with AS counts in the 25–75% range of the decile-specific distribution and those with ‘severe stress’ had AS counts in the upper 25% of the decile-specific frequency distribution range.

<table>
<thead>
<tr>
<th>Decile</th>
<th>Developmental Age (Reid and Dean, 2006)</th>
<th>Female</th>
<th>Male</th>
<th>AS range for the “minimal stress” category</th>
<th>AS range for the “moderate stress” category</th>
<th>AS range for the “severe stress” category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>1.5–1.7</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>2+</td>
</tr>
<tr>
<td>2nd</td>
<td>1.7–2.0</td>
<td>28</td>
<td>27</td>
<td>0–1</td>
<td>2</td>
<td>2+</td>
</tr>
<tr>
<td>3rd</td>
<td>2.0–2.3</td>
<td>33</td>
<td>32</td>
<td>0–2</td>
<td>3–4</td>
<td>5+</td>
</tr>
<tr>
<td>4th</td>
<td>2.3–2.7</td>
<td>34</td>
<td>33</td>
<td>0–4</td>
<td>5–6</td>
<td>7+</td>
</tr>
<tr>
<td>5th</td>
<td>2.7–3.1</td>
<td>33</td>
<td>33</td>
<td>0–3</td>
<td>4–9</td>
<td>10+</td>
</tr>
<tr>
<td>6th</td>
<td>3.1–3.6</td>
<td>34</td>
<td>33</td>
<td>0–5</td>
<td>6–9</td>
<td>10+</td>
</tr>
<tr>
<td>7th</td>
<td>3.6–4.2</td>
<td>34</td>
<td>33</td>
<td>0–5</td>
<td>6–11</td>
<td>12+</td>
</tr>
<tr>
<td>8th</td>
<td>4.2–4.9</td>
<td>31</td>
<td>32</td>
<td>0–5</td>
<td>4–10</td>
<td>11+</td>
</tr>
<tr>
<td>9th</td>
<td>4.9–5.6</td>
<td>29</td>
<td>30</td>
<td>0–3</td>
<td>4–10</td>
<td>11+</td>
</tr>
<tr>
<td>10th</td>
<td>5.6–6.2</td>
<td>26</td>
<td>23</td>
<td>0–2</td>
<td>3–6</td>
<td>7+</td>
</tr>
</tbody>
</table>

over the identification of striae (Table 3). As such, the fifth assessment was used for all further analyses. It should be noted again that the sample here includes some teeth from individuals who could not be used for assessment of age at death.

When the samples from the two sites are pooled, females showed a significantly lower number of accentuated striae of Retzius (AS) than males ($t(70) = 2.648, p = 0.021$). This is probably not fully explained by sex differences in formation time, which are min-

Fig. 5. Scatter plots showing changes in correlation between consecutive trials.
Table 3
Descriptive statistics for each trial presented alongside the correlation statistics. The correlation statistics between trials 3 and 4 show a decrease in correlation from the trial pairs on either side (i.e. 2nd with 3rd trial and third with 4th trial). The comparison of each trial to the last (5th) trial is also shown in the last set of columns. This shows a consistent increase in correlation between trial five and each consecutive trial.

<table>
<thead>
<tr>
<th>Trials</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>75</td>
<td>75.03</td>
<td>39.38</td>
</tr>
<tr>
<td>T2</td>
<td>75</td>
<td>41.01</td>
<td>23.53</td>
</tr>
<tr>
<td>T3</td>
<td>75</td>
<td>42.38</td>
<td>18.00</td>
</tr>
<tr>
<td>T4</td>
<td>75</td>
<td>38.45</td>
<td>20.16</td>
</tr>
<tr>
<td>T5</td>
<td>75</td>
<td>45.41</td>
<td>20.74</td>
</tr>
</tbody>
</table>

The correlation between Trials

<table>
<thead>
<tr>
<th>Trials</th>
<th>Spearman</th>
<th>Pearson</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 to T2</td>
<td>0.409</td>
<td>0.527</td>
</tr>
<tr>
<td>T2 to T3</td>
<td>0.741</td>
<td>0.744</td>
</tr>
<tr>
<td>T3 to T4</td>
<td>0.694</td>
<td>0.718</td>
</tr>
<tr>
<td>T4 to T5</td>
<td>0.863</td>
<td>0.884</td>
</tr>
</tbody>
</table>

The correlation to Trial 5

<table>
<thead>
<tr>
<th>Trials</th>
<th>Spearman</th>
<th>Pearson</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 to T5</td>
<td>0.639</td>
<td>0.703</td>
</tr>
<tr>
<td>T2 to T5</td>
<td>0.706</td>
<td>0.724</td>
</tr>
<tr>
<td>T3 to T5</td>
<td>0.847</td>
<td>0.858</td>
</tr>
<tr>
<td>T4 to T5</td>
<td>0.863</td>
<td>0.884</td>
</tr>
</tbody>
</table>

The statistical analysis shows that there is a significant increase in correlation between each trial and the last trial (5th trial). This indicates a consistent trend in the data. The results suggest that the stress levels increase over time, with the 5th trial showing the highest correlation.

In the severe stress category, the results show a significant difference in survival rates between males and females. The survival analysis for males and females is presented in the chart below.

**Fig. 6.** Survival functions showing the likelihood of females and males surviving to any given age at death in relation to the number of accentuated striae of Retzius (AS) in each canine. Stress thresholds were established based on the frequency distribution of AS with minimum being less than 30 AS, moderate being between 31 and 58 AS, and severe being 59 or more AS. While slightly different trends between females and males are apparent, with more stress experiences being associated with higher age at death in females and the opposite being the case for males, neither of these results was significant for the established stress thresholds.
Table 4

Mantel-Cox survival analyses testing the relationship between the number of accentuated striae of Retzius (AS) and median age at death in the complete sample, and in each sex. Statistics for the total crown are shown both with the three stress thresholds (minimal, moderate, and severe) and for the two stress thresholds (minimal and moderate combined with severe as a separate category). In the sex-divided sample, it can be seen that males with severe levels of AS experienced significantly reduced survivorship (p = 0.047). The deciles are then shown, with significant results in the 2nd (forming from 1.7–2 years of age), 3rd (forming from 2–2.3 years of age), and 8th (forming from 4.2–4.9 years of age) deciles highlighted by an asterisk.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sex</th>
<th>N</th>
<th>Chi-Square</th>
<th>df</th>
<th>Sig</th>
</tr>
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<tr>
<td>Total AS</td>
<td>Overall</td>
<td>73</td>
<td>1.505</td>
<td>2</td>
<td>0.471</td>
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<td></td>
<td>Females</td>
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<td>2.512</td>
<td>2</td>
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<td>Males</td>
<td>34</td>
<td>4.815</td>
<td>2</td>
<td>0.090</td>
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<tr>
<td>Severe AS (59 +)</td>
<td>Overall</td>
<td>73</td>
<td>0.019</td>
<td>1</td>
<td>0.891</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>36</td>
<td>1.906</td>
<td>1</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>Males</td>
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<td>3.945</td>
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<tr>
<td>1st Decile</td>
<td>Overall</td>
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<td>Females</td>
<td>11</td>
<td>0.273</td>
<td>2</td>
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<tr>
<td></td>
<td>Males</td>
<td>11</td>
<td>0.647</td>
<td>2</td>
<td>0.723</td>
</tr>
<tr>
<td>2nd Decile</td>
<td>Overall</td>
<td>57</td>
<td>5.151</td>
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<td>0.076</td>
</tr>
<tr>
<td></td>
<td>Females</td>
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<td>11.914</td>
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<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>27</td>
<td>0.675</td>
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<td>0.714</td>
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<tr>
<td>3rd Decile</td>
<td>Overall</td>
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<td>1.254</td>
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<tr>
<td></td>
<td>Females</td>
<td>33</td>
<td>6.889</td>
<td>2</td>
<td>0.032*</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>35</td>
<td>1.084</td>
<td>2</td>
<td>0.582</td>
</tr>
<tr>
<td>4th Decile</td>
<td>Overall</td>
<td>70</td>
<td>0.069</td>
<td>2</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>34</td>
<td>1.350</td>
<td>2</td>
<td>0.509</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>36</td>
<td>0.509</td>
<td>2</td>
<td>0.775</td>
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<tr>
<td>5th Decile</td>
<td>Overall</td>
<td>69</td>
<td>2.394</td>
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<td>0.302</td>
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<tr>
<td>6th Decile</td>
<td>Overall</td>
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<td>0.921</td>
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</tr>
<tr>
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<td>Males</td>
<td>36</td>
<td>0.847</td>
<td>2</td>
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<tr>
<td>7th Decile</td>
<td>Overall</td>
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<td>0.646</td>
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<td>0.724</td>
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<tr>
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<td>0.136</td>
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<tr>
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<td>Males</td>
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<td>1.056</td>
<td>2</td>
<td>0.590</td>
</tr>
<tr>
<td>8th Decile</td>
<td>Overall</td>
<td>66</td>
<td>0.604</td>
<td>2</td>
<td>0.739</td>
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<td>0.181</td>
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<tr>
<td></td>
<td>Males</td>
<td>35</td>
<td>7.324</td>
<td>2</td>
<td>0.026*</td>
</tr>
<tr>
<td>9th Decile</td>
<td>Overall</td>
<td>62</td>
<td>1.692</td>
<td>2</td>
<td>0.429</td>
</tr>
<tr>
<td></td>
<td>Females</td>
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<td>1.271</td>
<td>2</td>
<td>0.530</td>
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<tr>
<td></td>
<td>Males</td>
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<td>2.297</td>
<td>2</td>
<td>0.317</td>
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<tr>
<td>10th Decile</td>
<td>Overall</td>
<td>51</td>
<td>0.088</td>
<td>2</td>
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<tr>
<td></td>
<td>Females</td>
<td>26</td>
<td>0.212</td>
<td>2</td>
<td>0.899</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>25</td>
<td>0.608</td>
<td>2</td>
<td>0.738</td>
</tr>
</tbody>
</table>

Based on the analysis, females with more AS in any given decile tended to live longer, while males with more AS tended to have lower ages at death. This pattern was significant in the third decile for females (which represents the developmental ages from 1–2.3 years) and in the eighth decile for males (representing the developmental age range from 4.2–4.9 years). In the case of females from the second decile (developing from 1.7–2 years of age), individuals with moderate stress scores were actually significantly more likely to die at a younger age, and this result was significant when a Bonferroni-type argument was applied (χ² = 11.914, df = 2, p = 0.003) (Fig. 8, Table 4). Given the smaller sample sizes and limitations of adult age at death estimation techniques, the results from the decile analyses should be treated with caution. While they suggest a useful avenue for the exploration of links between developmental patterns of AS and mortality, the lack of consistent significant results in each decile suggests the need for further research. The importance of establishing biologically informed thresholds for stress, rather than using the frequency distributions as a baseline, should also be emphasized.

4. Discussion

This study found significant differences in the sex-based pattern of survivorship in relation to the childhood formation of dental enamel microdefects (represented by counts of AS). Furthermore, it suggests that developmental age is a critical factor in the nature of the relationship between AS and adult mortality. In response to the hypotheses proposed, the null hypothesis that there will be no correlation between childhood stress experiences and age at death is not consistent with the current results. Furthermore, the null hypothesis that there will be no sex-based differences cannot be rejected, as there are differences in the age at death association with AS between males and females. In particular, this is significant in the case of males with severe levels of accentuated striae of Retzius (AS) measured through high counts of AS.

Critically, these results show relationships between microscopic dental enamel defects and mortality that are consistent with previous findings using linear enamel hypoplasia (LEH), including those based on the nearby site of Tirup (Boldsen, 2007). Namely, past investigations of LEH in association with adult mortality risk have found that the presence of LEH has a negative association with age at death in males, but not in females (Boldsen, 2007; DeWitte, 2010a). It is important to note in this respect that LEH capture slightly different dimensions of growth arrest than AS, reflecting episodes of longer duration (chronic as opposed to acute episodes) and biased in expression towards the mid-crown region where LEH are most visible. The LEH data are consistent with scenarios presented by epigenetic research (Devaskar and Raychaudhuri, 2007) by the Developmental Origins of Health and Disease Hypothesis (Barker, 2007, 2004a, 2001; Barker et al., 2002; De Boo and Harding, 2006). In these cases, more stress is often seen to have an adverse impact on later life health (De Boo and Harding, 2006) and a particularly adverse impact on males (Bale, 2011; DeWitte, 2010a; Stini, 1969; Stinson, 1983). While not significant, the positive trend for females in relation to total crown AS count also requires further investigation as it seems to suggest that, in some circumstances
at least, higher AS counts in females are connected to increased survival.

It is possible that these differences can be explained by some aspect of developmental programming from early life stress experiences that had a negative impact on male survivorship but was beneficial to females in some instances. It is also possible that the observed pattern reflects a differential stress response by females that is also connected to lower frailty as defined by Vaupel (1990, 1988) and Vaupel et al. (1979) and discussed by Wood et al. (1992). Alternatively, cultural factors involving different treatment of males and females in these populations may have influenced these results. Finally, it is important to emphasize again that the individuals included in this study were all adults, i.e. the survivors of childhood stress events. It is therefore also possible that more frail females were less likely to survive until adulthood, making the females in this sample already the more robust in the population for this sex. A consideration of these different explanations therefore seems pertinent.

In her study of AS in the nearby contemporary Danish site of Tirup, Thomas (2003) found a complex relationship between AS and mortality that seemed to be dependent on the severity of AS. Thomas (2003) distinguished between ‘weak’ and ‘strong’ AS in her analysis and found that largely, increased numbers of AS were associated with a greater risk of dying before the age of thirty. However, weak AS developing before the age of seven were associated with a reduced risk of dying between the ages of seven and thirty. When Thomas (2003) considered survivorship in relation to different segments of the crown, she found that individuals with more strong AS forming in enamel in the age interval from 2–4 years had a lower hazard of dying within that age interval. Strong AS forming in the 1–2 year intervals and in the 4–7 year intervals for enamel development showed the opposite effect. Thus, defects forming at certain developmental times seemed to have a differential impact on mortality, results that complement the results from this study. Thomas (2003) suggested that a possible explanation for this pattern was that individuals who exhibited weak AS and those who exhibited strong AS for the same age range were likely subjected to similar illnesses but that the more robust individuals were not as severely impacted and so only developed weak AS. This would be consistent with those individuals then being more likely to live longer (Thomas, 2003). Alternatively, she notes that it is possible that the weak AS mark less severe stressors that served to strengthen an individuals’ ability to adapt rather than to weaken their ability to deal with conditions through life.

There are two core differences between Thomas’ (2003) work and that in the current study. The first is that Thomas (2003) considered children and adults, but due to attrition at Tirup only captured the adult cohort up to the age of 30. This means that while Thomas focused upon non-survivors as well as some survivors of childhood, the current study focused upon the latter. The second difference is that Thomas (2003) was not able to consider sex differences in her sample. If females in Thomas’ (2003) sample expressed increased numbers of weak striae, it may be that these two factors (i.e., weak AS being more common in females and reflecting a different stress response) are connected in the positive relationship between weak AS and mortality.

Thomas’ (2003) findings are critical as they highlight the possibility that AS can be related to positive as well as negative later life experiences and that this relationship might be variable with age at defect formation. The results from the current study also support Boldsen’s (2007) and DeWitte’s (2010a) findings of sex-specificity of some of these relationships, with the negative trend in males being significant. It is possible that the lack of any significant effect for the overall sample in this study reflects a summation of complementary trends in males and females, a critical point for studies that focus on combined-sex samples. Unfortunately, some of the mechanisms behind sex differences in these experiences are still poorly understood, and there are few studies of differences in developmental timing between the sexes in early forming physiological systems. Broadly, males are seen to be less buffered than females.
Fig. 8. Survival functions showing variability between deciles with significant results. The second decile results developmental age range from around 1.7–2.0 years of age (Reid and Dean 2006) show that females with moderate stress had significantly reduced survivorship ($\chi^2 = 11.914$, df = 2, $p = 0.003$). Females with severe stress in the third decile (developmental age range from around 2.0–2.3 years of age (Reid and Dean 2006)) had significantly increased survivorship ($\chi^2 = 6.889$, df = 2, $p = 0.032$). Finally, while females in the eighth decile (developmental age range from around 4.2–4.9 years of age (Reid and Dean 2006)) expressed no significant impact on survival by the number of accentuated striae of Retzius (AS), males with higher AS counts in this decile showed reduced survivorship ($\chi^2 = 7.324$, df = 2, $p = 0.026$).
in utero, with higher early frailty having often been linked to largely biological factors (Guerra-Silveira and Abad-Franch, 2013; Stinson, 1985). This may be related to the documented variability in sex-based fetal responses to maternal stress (Weinstock, 2007); early development of such differences can also have a significant impact later life health outcomes (Bale, 2011). Increasingly, biological sex and sex hormones are being associated with variable physiological reactions to environmental stressors in humans and in non-human animal models (Ahmed et al., 1985; Beagley and Gockel, 2003; Bouman et al., 2005; Carey et al., 2007; Klein, 2000; Lang, 2004; Verheul, 2001; Wang et al., 2006). Furthermore, females have been shown to mount stronger humoral and cell-mediated immune responses (Shames, 2002; Verheul, 2001) leading to a more robust response to a range of infectious diseases (Beagley and Gockel, 2003; Guerra-Silveira and Abad-Franch, 2013; Roberts et al., 1996; Stini, 1985; Stinson, 1985).

The late medieval agrarian crisis in association with episodes such as the Great Bovine Pestilence and consequent periods of famine (Hybel and Poulsen, 2013; Yoder, 2006) may have interacted in a complex fashion with individual frailty for the populations in this study. Evidence points to increased morbidity and mortality prior to Black Death in England, along with selectivity in response to pre-existing health conditions as part of the Black Death epidemic (DeWitte, 2015, 2010b; DeWitte and Hughes-Morey, 2012; DeWitte and Wood, 2008; DeWitte and Slavin, 2013). It is possible that less frail individuals who survived more stress events were able to mount a stronger immune response to pathogens later in life, thus conferring an advantage that would contribute to greater longevity.

In this situation, the difference seen between males and females might lie in sex-based differential frailty, but also in the possibility of differing developmental schedules between the sexes (Bale, 2011; Buss et al., 2009; Cohen Hubal et al., 2008; Selevan et al., 2000; Taylor, 1969; Uekert et al., 2006). These differing trends have two primary implications. The first is that slower developing systems have more time to sustain stress, being in a particular immature state for longer (Weinstock, 2007) and the second is that the more developed a physiological system is, the more capable it may be of coping with stressors. External stressors will have the most impact on developing (as opposed to mature) systems. If there is sex-specificity in developmental timings for a given physiological system, then males and females may also exhibit differential frailty according to timing of development for any given system.

While neonates do have an active immune system, it is not fully mature at birth and so infants are more susceptible to infection than adults (Burns-Naas et al., 2008; West, 2002; Ygberg and Nilsson, 2012). If the female immune system, consistent with other systems (Buss et al., 2009; Taylor, 1969; Uekert et al., 2006; Weinstock, 2007), develops faster than the male immune system, it may be better able (at an earlier developmental age) to mount an active response and to start building up a strong immunity to pathogens in the environment. In this case, males will be more reliant on the various protective factors conferred by breast milk and less able to develop their own active resistance until a slightly later age. On the other hand, females will be better able to combat infection and to develop an active immune response, which will be beneficial later in life if they are strong enough to survive.

The appearance of sex differences in the decile analysis might be suggestive of sex-specific developmental schedules affecting the impact of early physiological stress episodes on later life. While these results must be treated with caution due to smaller sample sizes and general insignificance under a Bonferroni-type argument, the female pattern found in the second decile was significant even under the Bonferroni-type argument and further highlights the complexity of these patterns. It is possible that several factors are interacting to form this pattern, and it is thus useful reflect upon selective mortality and individual frailty in Wood et al.’s (1992) ‘Osteological Paradox.’ We might explain individuals exhibiting low levels of stress (as represented by AS counts) as having increased survivorship due to low impact from stressful stimuli during growth and development (Rose et al., 1983). It is also possible that these individuals were less frail from the outset and so were not as severely impacted by stress experiences during childhood (Wood et al., 1992). Adults who experienced more episodes of stress (as marked by AS counts), surviving longer into adulthood, might be explained through selective mortality during childhood (i.e., these adults were still the survivors of childhood stress events). What, then, about individuals with moderate stress who do not survive as long either as those individuals with less or as those individuals with more AS? Perhaps these individuals were not impacted sufficiently severely during childhood to die at that point, but yet the health impact of their stress experiences influenced adult mortality.

The potential effects of cultural differences in stress exposure for male and female children over the life course may also be informative. If such differences existed, we might propose a situation in which females were less culturally buffered during childhood from situations likely to result in physiological stress (DeWitte, 2010a; Stinson, 1985). Such situations could take a range of forms including poorer nutrition, increased exposure to infection, and different levels of care should they contract some infection. We might see girls being less likely to survive childhood unless they were particularly robust, i.e. the frailer females would be less likely to survive childhood and make it into the adult sample used in this study. Males on the other hand, if they were more buffered due to cultural factors as has been seen at times in both modern (Chen et al., 1981; Hrdy, 1990; Sen and Sengupta, 1983) and past cultures (Cohen and Bennett, 1993), might see greater survivorship of frail individuals into the adult population. Under this situation, we might see male adults with more indicators of childhood stress (represented by AS) who are at more risk of dying at an earlier adult age, whereas the already selected female population would be less affected by stressors as adults.

This scenario is consistent with the survival analysis in this study, which isolates severe AS (marked by high AS counts) from the rest of the sample. In this case, the individuals who might be most affected in the female sample would be those who sustained sufficient stress to see some impact later in life, but not enough to be selected out of the population in childhood. The significance of the reduced survivorship for females with moderate AS in the age interval from 1.7 to 2 years of age (Reid and Dean, 2006) is unclear. We may be seeing sex-specific effect of variable weaning times (Rose et al., 1985, 1981), although Katzenberg et al. (1996) caution against taking an overly simplistic interpretation to a nonspecific stressor such as enamel defects, and Thomas (2003) has stressed an epidemiologic interpretation for the Tiburp material. Furthermore, the age range from 1.7 to 2 years should be treated as an approximation, being based on the Reid and Dean (2006) averages. Refinement of this estimate through the direct application of histological methods for counting short-period (circadian) markers (cross-striations) could allow for further insight. Unfortunately, a decided absence of direct evidence from historical sources or bioarchaeological studies for differential treatment of boys and girls for this period in Denmark also limits our current ability to tease this scenario apart. Children are seldom the subject of historical documentation and the limitations of the sex estimation in subadult skeletons restrict bioarchaeological inferences. While Yoder (2006) found no sex difference in adult diet based on stable isotopes for medieval Danes, this does not preclude different treatment of children. The existence of such differences thus should be considered as a possible interpretation, but further supporting evidence is required.
The use of an inclusive definition for AS in this study may mean the conflation of ‘weak’ and ‘strong’ AS, as defined by Thomas (2003), but the consistent scoring of AS combined with the appearance of the survivorship patterns suggests that this scoring method is capturing meaningful trends (Boldsen, 2007; Thomas, 2003). It is also consistent with Fitzgerald and Saunder’s (2005) assertions that any accentuation in striae of Retzius may be biologically meaningful from a stress standpoint. While variable expression of AS may be biologically meaningful per se, it is also important to note the challenges involved in classifying enamel defects by level of expression. These difficulties are fundamentally linked to the complex etiological background in defect formation. While striae of Retzius are recognized as histological structures that have a regular and individually-specific time periodicity (Antoine, 2000; Antoine et al., 2009; Bromage et al., 2012; Lacruz et al., 2012; Reid and Ferrell, 2006; Rineses, 1986; Smith, 2006), and while AS (Goodman and Weise, 1992) and the associated surface changes (such as dental enamel hypoplasia) have been directly linked to the occurrence of ‘stress’ early in life (Goodman et al., 1991; Mellanby, 1934, 1930, 1929; Schwartz et al., 2006; Sukling, 1980; Sukling et al., 1986, 1983; Sukling and Pearce, 1984; Sukling and Purdell-Lewis, 1982), the identification of AS is highly problematic (FitzGerald and Saunders, 2005; Goodman and Rose, 1990; Thomas, 2003). The appearance of these structures varies across the length of the crown (Hillson, 1996; Hillson and Bond, 1997) and depending on the location of the histological section in relationship to the tip of the dentin horn (Antoine, 2000; Antoine et al., 2009).

These complications mean that AS expression will depend upon factors that are independent of individual frailty (Wood et al., 1992) and stress parameters (timing, duration, severity). However, since part of this variability is dictated by the location of the defect and since location is related to developmental age (Hillson, 2014, 2005, 1996; Hillson and Bond, 1997; Reid and Dean, 2006, 2000; Thomas, 2003), an interpretation of AS patterns that explores individual frailty will necessarily be confounded by the fact that individual frailty may change in association with development through childhood (and by extension frailty may be variable across crown development). The use of a definition, such as striae extension across 75% of the distance from the dentino-enamel junction to the crown surface (FitzGerald and Saunders, 2005; Goodman and Rose, 1990) is therefore problematic, as it does not fully acknowledge the variability of expression and the complex etiology in relation to frailty that influences defect expression. Fitzgerald and Saunders (2005) noted these complexities and emphasized that they too, used a loose definition for AS – only roughly linked to a 75% rule. Furthermore, the use of the AS frequency distributions to define stress thresholds might have obscured certain relationships. Ideally, biologically meaningful thresholds should be implemented. However, the identification of such thresholds is dependent upon improved knowledge of how stress stimuli interact with individual stress responses and consequent defect formation, as well as upon the need for greater insight into the interaction between individual frailty and stress response. In other words, we still do not fully understand how the frequency, duration, and timing of stress stimuli interact with individual frailty to form a complex array of enamel microstructures (FitzGerald and Saunders, 2005). The fact that we are likely dealing with a sliding threshold that changes in all of these interactions over the course of growth and development, which is variable both in developmental timing and overall experience between males and females, further makes this a daunting task. In spite of these challenges, the current results point to complex sex-specific and developmental patterns and these developmental findings echo those Thomas (2003) found for the Tirup sample.

Finally, the estimation of adult age at death is a complex and highly problematic procedure, and the limitations of the techniques need to be recognized. The difficulties involved in adult age at death estimation have been extensively examined (for recent reviews see Milner and Boldsen, 2012a, 2012b). These stem from the fundamental nature of the adult aging process, which is further complicated by by-products of the age estimation techniques (Bocquet-Appel and Masset, 1982), and by our inability to know the age distribution of the original population in archaeological samples (Angel, 1969; Hoppa and Vaupel, 2002; Johansson and Horowitz, 1986; Milner et al., 1989; Paine, 1989; Sattenspiel and Harpending, 1983; Wood et al., 1992). Ideally, then, caution is necessary when applying strict age estimation techniques that have been developed using a different population to the one being studied (Hoppa and Vaupel, 2002).

The current investigation used a multi-variable approach to adult age estimation, combining traditional methods with a system of experience-based inference (Milner and Boldsen, 2012a, 2012b; Weise et al., 2012, 2009). The practice employed in this study, in which age ranges for each individual in the sample are estimated and then the midpoint of these ranges derived for the statistical analyses, is common practice in bioarchaeological research. A central issue with the use of midpoint age estimates is that an individual placed in the middle of the estimated age range may have had an actual age towards the upper or lower end of the range. However, if both high accuracy in the age range and a high level of precision is obtained, the midpoint may closely correlate with actual age (Milner and Boldsen, 2012a). This is a shortcoming that cannot be eliminated with traditional age estimation techniques, and transition analysis has been demonstrated to exhibit significant bias largely due to the nature of the features being scored (Milner and Boldsen, 2012b). However, early results from experience-based inference techniques have been encouraging (Weise et al., 2012, 2009). Furthermore, as noted above, a familiarity with population-specific age-related changes will help to contextualize and refine individual age at death estimates.

Aside from the broad issues with age estimation, there were implicit limitations in capturing the older age cohort. While the age estimation techniques used in this study was able to estimate age at death for individuals over 50 years of age, the requirements of this study meant that many aged individuals were eliminated due to excess dental attrition. The ability of this investigation to capture some in this older cohort was critical, but the small sample size also places limitations on conclusions. Further investigation with larger sample sizes and improved statistical techniques for deriving point age at death estimates would permit greater resolution.

5. Conclusion

The consideration of AS in relationship to later life experiences provides a mechanism for studying life course patterns in past human populations. This approach is longitudinal in nature, looking at a variable over the life course of individuals. As such, archaeological populations provide data that are challenging to achieve in modern clinical studies (Armelagos et al., 2009). Approaching the life course through the study of human remains overcomes the inherent ambiguity of dental defects (Neiburger, 1990), since regardless of etiology, defects indicate that a particular individual sustained a level of systemic stress (Goodman and Rose, 1990). It is likely that we will never be able to determine etiology, since the range of possible stressors that can elicit the same response is extensive. We can gain further insight, however, into the biological relationships between stress and age at death. Perhaps the greatest value to be gained from the study of nonspecific stress indicators such as AS lies in their very ability to capture a specific record of stress experiences, regardless of cause. The results from this study emphasize the importance of a nuanced approach.


