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NUTRITIONAL IMPLICATIONS IN 19th CENTURY PORTUGAL: A LEH STUDY

ABSTRACT: Linear enamel hypoplasia (LEH) has been frequently used to infer general health conditions in past populations. This study documents LEH presence to infer and compare the quality of life between temporally similar rural and urban populations from late 19th – early 20th century Portugal. Data were collected from the Coimbra identified collection and the new Lisbon collection. Prevalence of LEH was documented by counting the frequency of LEH in each tooth class. Measurements of each hypoplasia were taken to calculate the percentage of enamel affected as a total hypoplastic area variable for each tooth. The frequency comparison for central incisors (p-value = 0.001) suggests there is a significant difference between the groups. The sample comparison using total hypoplastic area (p-value = <0.0001) also indicates there is a significant difference between the two populations. For both frequency and hypoplastic area methods, there were no significant sex differences found within and between each sample. However, age differences were detected for the central incisor and canine in both groups. MANCOVA was used to assess the differences in both methods used and found significant population differences between Lisbon and Coimbra (Wilk's Lambda = 0.0047, 0.0023), respectively. These results support previous studies that the total hypoplastic area method may not be a more sensitive parameter than frequency alone. Concomitantly, a bias was found in the distribution of LEH within each tooth that may indicate the width differences may be due to the developmental nature of the enamel matrix and not indicative of the distribution of stress episodes. The results of this study illustrate the need to detect more sensitive parameters employed in future LEH studies.

KEY WORDS: Enamel hypoplasia – Health – Population density

INTRODUCTION

Skeletal data can reflect socioeconomic effects within a population through the appearance of biologically and culturally induced traits. LEH has been frequently used to infer health and nutrition within and between populations (Cucina 2002, King *et al*. 2005, Larsen 2010, Lewis 2002, Malville 1997, Paluebeckaite *et al*. 2002). LEH is a form of enamel hypoplasia associated with developmental disturbances (FDI 1982, Goodman, Rose 1990). It is a quantitative furrow-type defect that can be identified macroscopically as an external defect

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of surface enamel and is defined as a disturbance in the hard tissue that occurs during mineralization (FDI 1982, Hillson 1996, Kreshover 1940, 1960). Typically, the developmental disturbances are environmental in nature and not related to similar genetic defects (King *et al*. 2005); thus, they have been instrumental in studies reconstructing the diets of past peoples (Goodman, Armelagos 1985).

LEH results from general systemic perturbations during enamel secretion; thus, it can be used as a non-specific indicator of stress (Suckling 1989, Suckling, Thurley 1984). Enamel secretion is performed by ameloblasts, which are epithelium cells derived from inner enamel epithelium found at the dentin enamel junction (DEJ). Ameloblasts lay enamel in a ring-like formation around the DEJ and once established cannot be remodeled (Hillson, Bond 1997, Sarnat 1940, Sarnat, Schour 1941). These characteristics of LEH suggest that physiological stressors weaken ameloblast secretion resulting in the decrease of enamel thickness, which becomes a permanent defect on the tooth surface (Goodman, Rose 1990, Hillson 1996). These defects are typically identified as horizontal lines on the enamel surface with a gradually sloping or trough-like appearance (King *et al*. 2002).

While LEH is not indicative of a specific stressor, it varies in frequency and pattern providing an overall view of health within and between populations (Cucina 2002, King *et al*. 2002). Use of the dentition is also advantageous because of its increased survivorship in the archaeological record and it has been shown to be more resistant to stress relative to other skeletal features (Cardoso 2007, Goodman, Rose 1990, Larsen 2010). As a result, LEH can provide a more conservative estimate of population health than skeletal indicators (Cardoso 2007). Another relevant characteristic of enamel is its inability to remodel after deposition causing LEH to be permanently visible on the tooth surface unless lost to attrition (Goodman, Rose 1990). Their visibility in the archaeological record has allowed for their utility in understanding stress and disease in past populations (Cardoso 2007, Conceição, Cardoso 2011).

Many different approaches have been used in understanding the relationship between LEH prevalence and human lifeways. These include determining frequency of hypoplastic events, temporal intervals between events, earliest age of occurrence, and periodicity of events. These parameters have been used to understand relationships within and between populations, such as possible relationships between enamel defects and age-at-death (King *et al*. 2005). In addition, these data can be used to understand sex and age divisions with regards to differential access to resources and social roles. The ability to measure differences in the incidence of LEH can allow for inferences of nutritional stress within a population, particularly in reference to sex and age divisions. LEH frequency also indicates the disease susceptibility, or morbidity of a population when combined with information about population densities and subsistence practices as the prevalence of LEH will vary according to environmental and cultural factors (Cucina 2002).

While many studies have used LEH frequency to assess population differences, other studies have incorporated an analysis of sex differences within populations (Goodman, Rose 1990, Larson 2010). These studies are problematic because of the inherent differences in enamel development between males and females (Hillson 1996). Theoretically, it is assumed that males are more susceptible to physiological stress because they have a reduced metabolic buffer response relative to females (Guatelli-Steinberg, Lukacs 1999). However, the osteological paradox suggests that this may not be reflected by an increase in LEH frequency for males as the formation of LEH may be related to an individual's survival post-insult (Wood *et al*. 1992).

Most studies involving frequency of LEH have been used to explore differences between and within populations. Other methods have arisen to investigate the complexity of LEH patterning. The most common macroscopic method involves measuring the width of individual LEH as a proxy for duration of physiological perturbation. Blakely *et al*. (1994) described two different types of LEH: 1) relatively wide/deep defects known as major growth arrests and 2) narrow/shallow lesions. These apparent differences in defect duration initially suggested an alternative methodology involving width analysis could be more revealing (Corruccini *et al*. 1985).

To account for these apparent distribution differences, Blakey (1981) developed a width methodology that involves measurements of the incisal and cervical aspects of LEH to estimate duration of LEH episode. This was based on the assumption that the width of LEH is indicative of an increased metabolic insult (Blakely, Armelagos 1985). However, this method involved the use of one tooth at a time and produces several variables per individual, which can overestimate the length of time an individual suffered insult. An alternative method was developed by Ensor and Irish (1995), termed the hypoplastic area method. This method involved the creation of a single variable term per individual (individual hypoplastic area) and the ability to separate a hypoplastic area variable per tooth type chosen (total hypoplastic area) (Ensor, Irish 1995).

Studies have incorporated the use of both methods, particularly by the authors who developed these methods (Blakely 1981, 1984, Blakely, Armelagos 1985, Goodman *et al*. 1980, Goodman, Armelagos 1985, Goodman, Rose 1990, Ensor, Irish 1995). However, these methods have experienced some criticism of late. Hillson and Bond (1997) found that a progressive decrease in perikymata spacing occurs from cervical to occlusal ends on the crown sides. This spacing decrease inherently affects the prominence and width of a LEH defect, which implies that width measurements may not be a useful indicator of duration, as they pertain to metabolic insult (Hillson, Bond 1997, Witzel *et al*. 2008). Hubbard *et al*. (2009) also found that due to the differences in perikymata spacing across the tooth crown, width may only be a useful indicator under restrictive conditions. They state that width measurements of LEH may only be useful to rank populations in terms of a relative average of stress duration rather than an isolated measure of health (Hubbard *et al*. 2009). In addition, variation in the rate of enamel formation may obscure LEH present macroscopically, particularly in the cervical regions (Hassett 2012, 2014).

Other studies indicate a biased distribution of LEH within the tooth crown itself. Goodman and Armelagos (1985) found a higher prevalence of LEH in the middle third of each tooth crown and the lowest in the occlusal third. Hillson and Bond (1997) suggest this biased distribution is based on the varying ages of ameloblast cells responsible for enamel secretion within each tooth third. Microscopic methodologies have been developed to address the issues with defect formation and biased distribution (Hillson 1996). However, the definition of LEH changes when identified microscopically to a greater than expected spacing between pairs of perikymata (King *et al*. 2002). An advantage of microscopic methods is the ability to disregard the assumption that the tooth crown maintains a uniform rate of growth and the ability to account for cuspal enamel development (King *et al*. 2005, 2002, Reid, Dean 2006). King *et al*. (2005) found that utilizing a microscopic method accounting for perikymata spacing allows for a more sensitive parameter of total percentage of enamel affected. This parameter measures the total enamel surface that contains increased perikymata spacing that implies a hypoplastic defect (King *et al*. 2005). Cunha *et al*. (2004) also used a methodology that incorporates perikymata spacing to address physiological stress in Sima de los Huesos Middle Pleistocene hominins. Their study found a low prevalence of LEH suggesting a low level of developmental stress. These methods have the potential to advance our knowledge of health in past populations by accounting for cuspal enamel growth and the non-uniformity of crown development. However, further investigation of microscopic methods has suggested some difficulty in interpreting the results as multiple methods have been utilized in the study of LEH (Hassett 2012, 2014).

The differing methodologies presented here are based on alternative assumptions about the formation of LEH and the microscopic approach appears to rely on the least number of fundamental assumptions. However, this approach is likely more subjective in its reliance on observing abnormal areas of perikymata spacing that may not be visible on the enamel surface (Hillson 2005, King *et al*. 2002, 2005). One solution has been proposed involving the quantification of LEH defects across perikymata spacing (Hassett 2012). The frequency, macroscopic methods may provide more conservative estimates due to the knowledge that these defects create a visible, macroscopic defect and currently there is no consensus on the minimum expression of an LEH (Hillson 2005). However, the known variation in enamel formation may explain the differences in distribution of LEH within the tooth (Witzel *et al*. 2008).

This study documents LEH presence to infer and compare the quality of life between temporally similar rural and urban populations from Portugal. LEH can vary in frequency, duration, and age of occurrence within and between populations allowing for comparison, in some cases utilizing the different manifestations of LEH rather than solely its presence (Steckel 2003). This should be emphasized as studies have shown both rural and urban European populations experienced stressful development during the 19th century; although, their responses have differed across populations for severity and age of occurrence illustrating differences in overall health between both types of populations (Cucina 2002, Lewis 2002). Interestingly, the Iberian Peninsula has been found to deviate from the traditional 19th century European findings. Ubelaker *et al*. (2009) found only 2.3% of individuals affected by LEH in a 19th century Spanish population, while King *et al*. (2005) found 100% of individuals affected by LEH in two 18th–19th century English populations. However, the differences in methodological approaches may account for some of the differences between these two assessments, which have been noted as a difficulty when interpreting multiple studies of LEH (Hassett 2012, 2014).

Historical context

In terms of industrialization, Portugal is distinct from its other surrounding countries. The first wave of industrialization in Portugal followed their independence from the Spanish crown in 1642 and their inability to trade in salt, corn, and fish that formerly brought prosperity to Portugal (Birmingham 2003). Industrialization attempted to make use of the country's raw material resources and slave labor force. However, the Brazilian gold rush in 1697 stifled the need for production innovation and increased their colony holdings while rendering industrialization obsolete (Disney 2009).

During the 19th century, there was a dearth of men as a result of emigration to the colonies; thus, women had taken a primary role in land plots for intensive agriculture (Anderson 2000, Birmingham 2003). Women had become heads of households and led the Portuguese rebellion in order to register land ownership. The end of this rebellion also shifted the economic importance to Lisbon with the corresponding growth of industry with the adoption of free trade for Portuguese shipping (Reis 2001). However, agriculture continued to dominate the economy until 1890 with 61% of the labor force devoted to its production. Industry began to grow in Lisbon once again in 1890. Industrialization grew more slowly this time as the domestic market was heavily protected by the mainland population and low productivity was typical due to the tradition of small familial or traditional sectors that comprised most of industry (Cardoso 2007, Giner 1982, Reis 2001). However, Lisbon continued increasing its industrial production resulting in an emigration from other regions to increase the labor force (Cardoso 2007, Reis 2001).

As a result of increased population density, this migration increased the disease loads of many of the city inhabitants where water was often not potable and overcrowding was typical (Cardoso 2007). Another result of this urbanization were class changes occurring due to economic development, as the middle class was subsumed into the elite classes and the working class devolved into a "peasantry" (Cardoso 2007).

Coimbra experienced many of the same issues, but was more impacted by inadequate nutrition (Santos 1995). This is largely due to slight differences in diet between towns such as Lisbon and Coimbra. Lisbon, a coastal city, primarily relied on marine sources of meat and ate a wide variety of imported cereals such as wheat, barley, maize, and rye (Cardoso 2007). In contrast, towns such as Coimbra relied on a more domestic variety of meat from cattle and sheep. They also subsisted on a maize diet due to wheat restrictions (Santos 1995). This created a nutritional disparity between the larger port cities and inland towns.

Disparities such as these can be seen in the skeletal record. The effects of urbanization have been seen in both the Lisbon and Coimbra populations from this time period with an increase in the mortality rates and a decreased stature development (Cardoso 2009, Cardoso 2007, Santos 1995). LEH should further illuminate these differences from a comparative framework to discern patterns related to disease load and nutritional intake. It is hypothesized that 19th century Portuguese rural and urban populations will show similar frequencies of hypoplastic events due to dependence on maize and wheat subsistence (Wasterlain *et al*. 2009). However, differential diets should also be reflected with Lisbon exhibiting a relatively healthy diet as the rural population was more dependent on maize and domestic animal consumption while the urban population was more dependent on wheat imports and marine diets due to location. Maize has been shown to be of low nutritional benefit (Katz *et al*. 1974). However, the increased population density of Lisbon would have increased sedentism and exposure to disease. Depending on the severity of disease exposure, these populations are hypothesized to exhibit similar frequencies, but larger duration within the Lisbon sample related to disease exposure.

MATERIALS AND METHODS

Three identified Portuguese samples were used in this study: the Lisbon Collection, the Coimbra Identified Skeletal Collection, and the Coimbra Medical School Skull Collection. Both the Lisbon and Coimbra collections contain skeletal material collected from local cemeteries and Portuguese medical schools (Cunha, Wasterlain 2007). Individuals in all three collections have known sex, age-at-death, occupation, date-of-death, cause-of-death, and city of residence. Data were collected from both adults and juveniles in this study to compare mortality and morbidity estimates. The sample composition is presented in *Table 1*.

Individuals chosen from the samples were selected by level of attrition and secondary dentition represented. The tooth types included were the maxillary central incisor, maxillary canine, and the maxillary second molar. These teeth were selected for their length of developmental timing to acquire a holistic representation of metabolic insult throughout enamel development. In addition, these teeth were more commonly articulated with the individual than mandibular dentition. When neither the left nor right maxillary central incisor was available, the maxillary lateral incisor was substituted. Only individuals who possessed these tooth types with mild attrition were selected for study. Juveniles were selected based on progression of dental development with only secondary dentition considered. Age-at-death ranges for individuals are also listed in *Table 1*.

TABLE 1. Sample composition.

Group	N	Age Ranges	Collection
Coimbra, Portugal			Coimbra Identified Skeletal Collection (CISC)
Total (N)	32		
Male (N)	6	18–75	
Female (N)	23	22–45	
Subadults (N)	13	9–17	
Coimbra, Portugal			Medical School Skull Collection
Total (N)	91		
Male (N)	55	18–59	
Female (N)	36	18–55	
Subadults (N)	6	11–17	
Lisbon, Portugal			Lisbon Collection
Total (N)	111		
Male (N)	49	19–43	
Female (N)	62	18–45	
Subadults (N)	22	10–17	

At least one of the three tooth classes examined was lost postmortem in the majority of individuals for all three collections. In addition, most individuals above 60 were missing one or more of the tooth classes antemortem or had too great attrition for comprehensive assessment. This was most prevalent in the Coimbra Medical School Skull Collection (see *Figure 1*). The age-at-death distributions of the collections and those available for study are present in *Figures 1 and 2*. In order to directly compare the samples, similar age-at-death distributions were selected as LEH is associated with increased mortality risk (Goodman, Rose 1990). Crown height was measured for individuals exhibiting no attrition from each sample in order to determine percentage of enamel affected.

From the two Coimbra samples, only those individuals known to have died in or around the vicinity of Coimbra were selected for study in order to attain

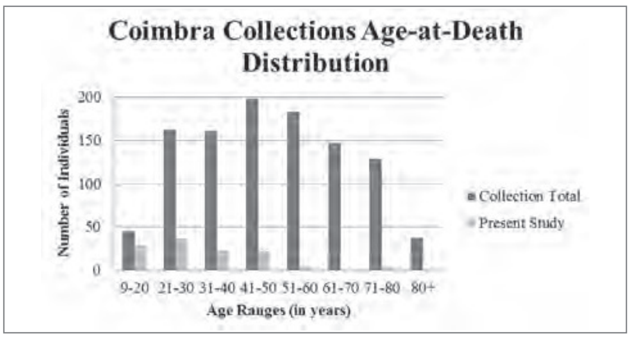

FIGURE 1. Age-at-death distribution of this study compared to total individuals in the Coimbra collections.

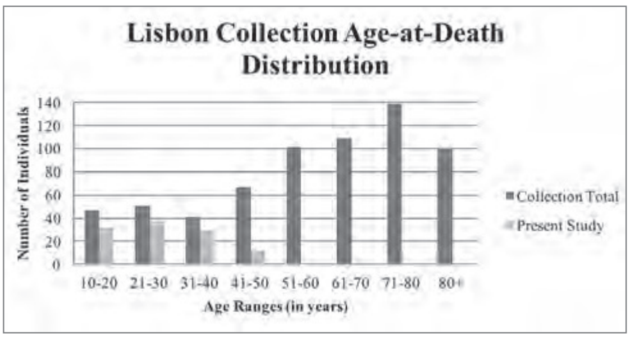

FIGURE 2. Age-at-death distribution of this study compared to total individuals in the Lisbon collection.

homogeneity of the sample. These individuals also represent low socioeconomic groups from central Coimbra (Santos 1995). From the occupation data, this collection appears to represent individuals from a low socioeconomic status engaged in an agrarian lifestyle. The composition of the Lisbon sample represents a low to middle socioeconomic strata of an urban population (Cardoso 2006). The number of individuals available for study was also decreased by the restriction of individuals who died in the vicinities of Lisbon and Coimbra.

Data Collection

In this study, each cranium from the Coimbra and Lisbon collections were examined for LEH in their maxillary dentition using a DeskBrite® 200 5X magnifying lamp to better visualize perturbations in the enamel. Enamel defects were identified on the criteria set by the DDE Index (1982). An example is shown in *Figure 3*. When present, the frequency of LEH for each tooth class (central incisor, canine, and second molar) was recorded. LEH were correlated across multiple tooth classes to ensure etiology from developmental perturbations and not originating from localized trauma to the dentition. This was accomplished by determining age of occurrence for each defect recorded using the developmental timing charts developed by Reid and Dean (2006). Defects that overlapped in developmental timing were considered as one developmental perturbation event.

For completeness, the width of each LEH was measured using Mituotyo® Caliper 573–721 Digital Pointed Jaw calipers following the Ensor and Irish (1995) method. The LEH width was recorded along with the distance between the vertical midpoint of the LEH and the cemento-enamel junction (CEJ) to reference where each LEH was distributed among each tooth. Total crown height was also measured from a subsample of individuals to calculate the total hypoplastic area. The subsample size is provided in *Table 2*.

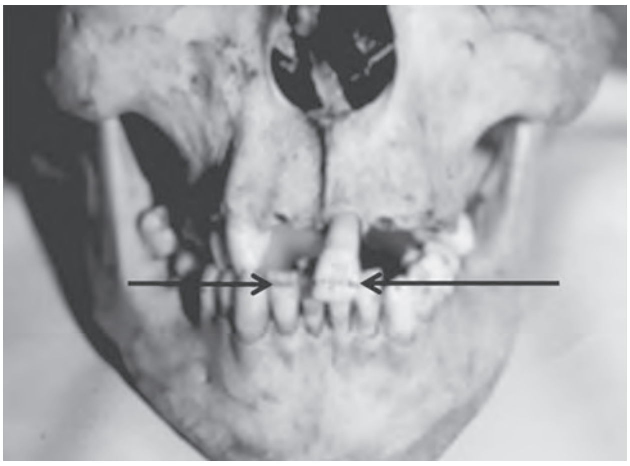

FIGURE 3. Linear enamel hypoplasia defects indicated by arrows. Courtesy of Ann H. Ross.

TABLE 2. Sample size and age range for overall crown height.

Group	N	Age Range
Coimbra, Portugal		
Females (N)	12	19–44
Males (N)	13	16–47
Lisbon, Portugal		
Females (N)	11	13–24
Males (N)	10	16–21

The measurement recorded from the midpoint of each LEH to the CEJ was used to provide the distribution of LEH within each tooth third (occlusal, middle, and cervical) for each population. The overall tooth crown height was divided into thirds to provide the most accurate demarcation of each tooth third. Then, the measurement from the LEH to the CEJ was placed within the appropriate third. These were counted and divided by the total number of individuals within that population exhibiting LEH.

Total hypoplastic area (THA) was calculated for each tooth by dividing the width of the each LEH by the average total crown height for the tooth class. The equation is as follows:

$$THA = (\Sigma\ width_i) / \text{tooth crown height} \quad (1)$$

where i = all hypoplasia widths per individual. Hypoplasias found to be 0.5mm or less (e.g. acute

TABLE 3. Variables collected in this study.

Variables
Population
Sex
Age
C. Incisor Frequency
C. Incisor THA
Canine Frequency
Canine THA
2nd Molar Frequency
2nd Molar THA

hypoplasias) were given an area of 0.1 mm according to the Ensor and Irish (1995) method and those larger than 0.5 mm were calculated by dividing the width by total crown height. These values were added for each individual if more than one LEH occurred within a tooth class to comprise the THA score. Thus, each individual had two variables per tooth class for each method used and are listed in *Table 3*.

Statistical Analyses

A one-way analysis of variance (ANOVA) was performed to test the null hypothesis that there is no significant mean variation according to frequency and THA for each tooth class across the populations and among sex and age groups. The ANOVA was also used to ascertain if there was an interaction between age and sex that could affect the variation seen between populations. The model for this test is:

$$y_{ijk} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijk} \qquad (2)$$

where y_{ijk} is the response for the tooth frequency/THA for both age and sex of an individual, μ is the overall population mean, τ_i is the effect due to sex, β_j is the effect due to age, $\tau\beta_{ij}$ is the two-way interaction between age and sex, and ε_{ijk} is the random error associated with all the factors combined (Ott, Longnecker 2010).

Because the interaction between age and sex was not significant both were used as covariates for one-way analysis of covariance (ANCOVA) to test the null hypothesis that there is no significant mean variation according to frequency and THA for each tooth class while controlling for age and sex. The model used to test this hypothesis is:

$$y_{ij} = \beta_o + \tau_i + \beta_1 x_{ij} + \beta_2 x_{ij} + \varepsilon_{ij} \qquad (3)$$

where y_{ij} is the response for the tooth frequency/THA for each individual, β_o is the intercept, τ_i is the effect due to population, $\beta_1 x_{ij}$ is the regression slope on the effect of age as a covariate, $\beta_2 x_{ij}$ is the regression slope on the effect of sex as a covariate, and ε_{ij} are the random errors (Ott, Longnecker 2010). ANCOVA was also used to compare the tooth variables using a contrast for the age of occurrence groupings.

A one-way multivariate analysis of covariance (MANCOVA) was performed to test the null hypothesis that there is no significant mean variation using all variables combined across the two populations. MANCOVA allows for all of the tooth variables to be placed in one model as the response variables and then be measured across a dependent variable in matrix form. Thus, it follows the model above for ANCOVA; however, it combines the response variables and model parameters in matrix form (Johnson, Wichern 2007). This test controls for the variation seen in the other variables when testing each tooth variable. These statistics were performed using SAS 9.2 for Windows (SAS Institute 2011).

TABLE 4. Overall percentage of LEH in Coimbra sample.

Variable	N	Percentage
Central Incisor	123	73%
Canine	123	74%
Second Molar	123	16%

TABLE 5. Overall percentage of LEH in Lisbon sample.

Variable	N	Percentage
Central Incisor	110	55%
Canine	110	62%
Second Molar	110	12%

RESULTS

Percentages for frequency of LEH in both populations are presented in *Tables 4–5*. Graphical distributions are presented in *Figures 4–5*. The Coimbra sample shows a generally higher frequency in comparison to the Lisbon sample. Males also exhibit a generally higher frequency in both samples relative to females for both central incisor and canine. The central incisor exhibited the largest disparity in frequency with 73% of individuals from Coimbra exhibiting at least one LEH and 55% of Lisbon individuals exhibiting at least one LEH. However, the canine had the highest percentage of individuals with at least one LEH for both populations. This is likely due to the canine's increased timing for enamel deposition (Goodman, Rose 1990, Reid, Dean 2006). The second molar exhibited a lower frequency for both populations with 16% of Coimbra affected and 12% of Lisbon affected. This is likely due to the lower susceptibility of molars to developmental disturbance because of their crown geometry (Hillson, Bond 1997). The percentage of sex frequencies is also presented in *Tables 6–7* for Coimbra and *Tables 8–9* for Lisbon.

The distribution of LEH by tooth thirds is shown in *Figures 6–7*. These diagrams visually represent the distribution of LEH by tooth third to demonstrate the bias in their development towards the middle third. *Figure 6* represents the distribution for the Coimbra population.

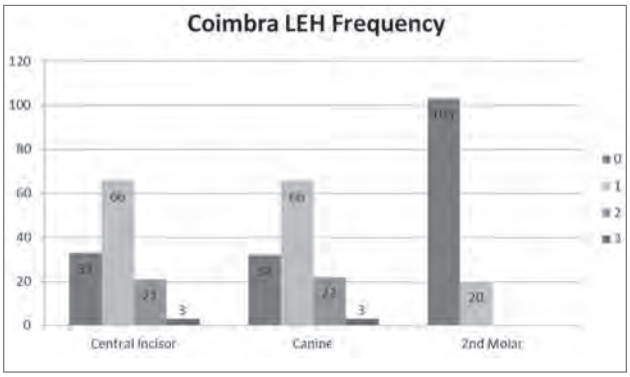

FIGURE 4. LEH frequency for Coimbra sample. Key indicates number of LEH present (0 = 0 LEH; 1 = 1 LEH; 2 = 2 LEH; 3 = 3 LEH).

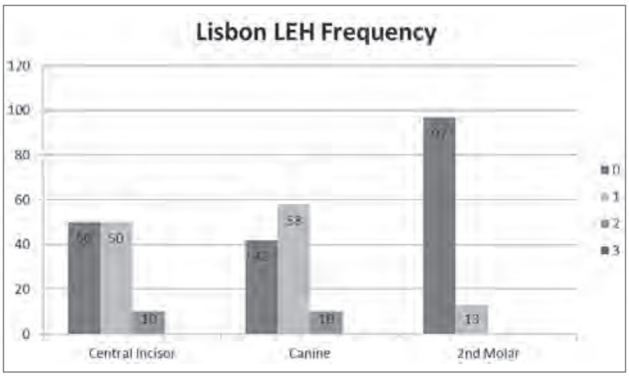

FIGURE 5. LEH frequency for Lisbon sample. Key indicates number of LEH present (0 = 0 LEH; 1 = 1 LEH; 2 = 2 LEH; 3 = 3 LEH).

Figure 7 represents the distribution for the Lisbon population.

ANOVA tests were performed to analyze the sex and age differences for each tooth between the populations. It was also used to measure a statistical interaction between age and sex for each variable. No interaction was detected for the tooth classes and methods as shown in *Table 10*. There were no significant sex differences in either population for any tooth class. The results are presented in *Tables 11–12*. Age-at-death distributions of frequency for both samples are shown in *Figures 8–9*. Both samples show a general trend with younger individuals having a greater percentage of increased LEH frequencies relative to older individuals. The Lisbon sample showed significant age differences in frequency of LEH for the central incisor. However, the Coimbra sample did not show significant age differences on the central incisor. These results are presented in *Tables 13–14*. All ANOVA results are reported for type III sums of squares.

An ANCOVA was used to analyze population differences among each tooth class for frequency, controlling for age and sex. There were significant population differences for the central incisor and the canine. The results are presented in *Table 15*. All ANCOVA results are reported for type III sums of squares.

TABLE 6. LEH frequencies for females in Coimbra sample.

Variable	N	Percentage
Central Incisor		
0	19	30.6%
1	31	50.0%
2	9	14.5%
3	3	4.8%
Canine		
0	20	32.3%
1	31	50.0%
2	9	14.5%
3	2	3.2%
Second Molar		
0	50	80.6%
1	12	19.4%

TABLE 7. LEH frequencies for males in Coimbra sample.

Variable	N	Percentage
Central Incisor		
0	14	23.0%
1	35	57.4%
2	12	19.7%
Canine		
0	12	19.7%
1	35	57.4%
2	13	21.3%
3	1	1.6%
Second Molar		
0	53	86.9%
1	8	13.1%

TABLE 8. LEH frequencies for females in Lisbon sample.

Variable	N	Percentage
Central Incisor		
0	22	44.9%
1	22	44.9%
2	5	10.2%
Canine		
0	18	36.7%
1	27	55.1%
2	4	8.2%
Second Molar		
0	42	85.7%
1	7	14.3%

TABLE 9. LEH frequencies for males in Lisbon sample.

Variable	N	Percentage
Central Incisor		
0	28	45.9%
1	28	45.9%
2	5	8.2%
Canine		
0	24	39.3%
1	31	50.8%
2	6	9.8%
Second Molar		
0	55	90.2%
1	6	9.8%

A MANCOVA was used to analyze the overall population differences for all tooth classes for frequency controlling for the effects of age and sex. The MANCOVA results suggest significant population differences (α=0.05). The results are presented in *Table 16*.

DISCUSSION

The LEH distributions between tooth thirds exhibits a definite bias towards the middle tooth third for each tooth used in this study for both the Lisbon and Coimbra populations (see *Figures 6–7*). The LEH distribution for

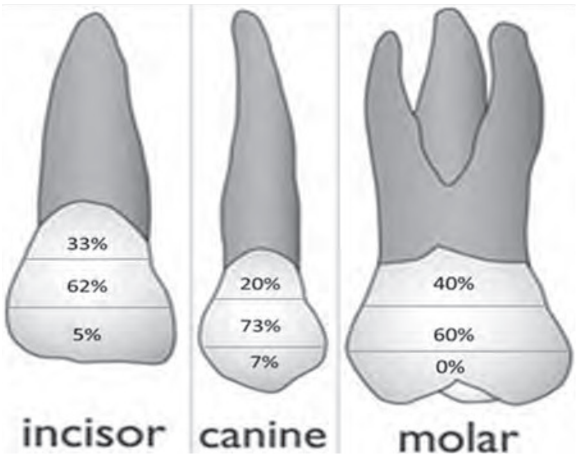

FIGURE 6. Percentage of LEH within each tooth third for the Coimbra sample.

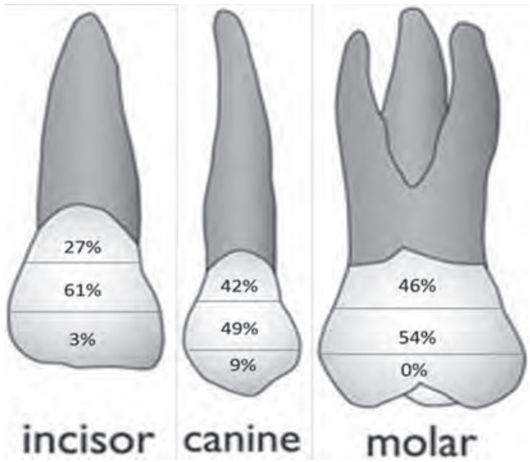

FIGURE 7. Percentage of LEH within each tooth third for the Lisbon sample.

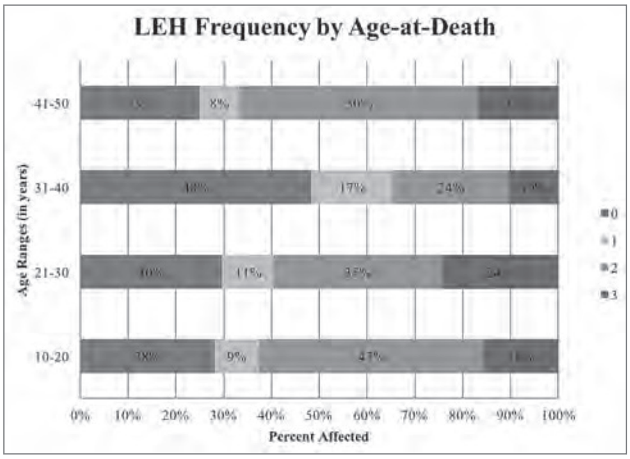

FIGURE 8. Age-at-death distribution showing percentage individuals affected by frequency in the Coimbra sample. Key indicates number of LEH present (0 = 0 LEH; 1 = 1 LEH; 2 = 2 LEH; 3 = 3 LEH).

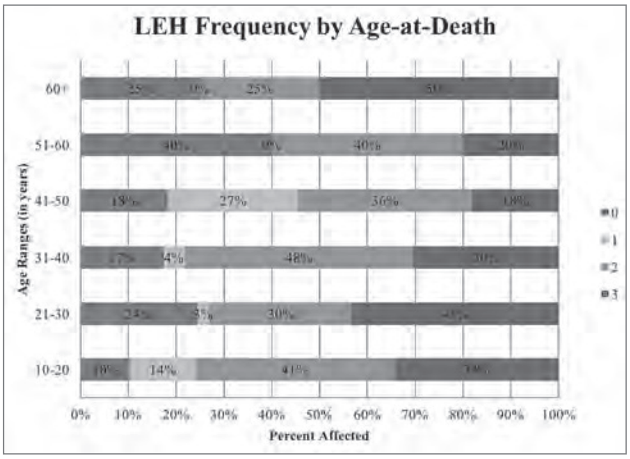

FIGURE 9. Age-at-death distribution showing percentage individuals affected by frequency in the Lisbon sample. Key indicates number of LEH present (0 = 0 LEH; 1 = 1 LEH; 2 = 2 LEH; 3 = 3 LEH).

TABLE 10. ANOVA results for age and sex interaction.

Tooth Variable	F value	df	p-value (α=0.05)
Central Incisor	0.08	1, 228	0.7714
Canine	1.15	1, 228	0.2857
Second Molar	1.81	1, 228	0.1801

TABLE 11. ANOVA results for sex differences in Coimbra for both frequency and THA. *Significance (α = 0.05).

Tooth Class-Frequency	F-statistic	df	p-value
Central Incisor			
Frequency	0.06	1, 121	0.8117
THA	0.26	1, 121	0.6084
Canine			
Frequency	1.50	1, 121	0.2227
THA	1.81	1, 121	0.1815
Second Molar			
Frequency	0.87	1, 121	0.3525
THA	0.45	1, 121	0.5018

the central incisor in the Coimbra population shows 62% of LEH found in the middle tooth third and 61% in the Lisbon population. The cervical third showed the next highest percentage of LEH for both populations and all tooth classes as well. However, the canine in the Lisbon population showed a much closer prevalence between the middle and cervical tooth thirds than any other tooth with the distribution being 49% and 42%, respectively. The middle tooth third ameloblasts have the fastest secretion rate and thus, may be more susceptible to metabolic disturbances (Hillson, Bond 1997). This distribution is also corroborated with Goodman and Armelagos (1985) and their study of the LEH distribution in the Dickson Mound populations were they found an increased distribution in the middle tooth third followed by the cervical tooth third.

In general, males from both populations exhibited a higher frequency of LEH than females. The same pattern is observed with the canine exhibiting the highest percentage for both males and females. This supports the assertion that the canine will be more sensitive because it has the longest developmental timing (Hillson 2005). The second molar shows the lowest population percentages for both sexes and populations consistent with decreased susceptibility of the molars and also that metabolic insult was rare later in the developmental period (Goodman, Rose 1990).

The pattern in both populations with males exhibiting the greater prevalence of LEH may be due to the hypothesis that females have greater developmental buffer mechanisms. The fundamental assumption that males are more susceptible to physiological stress because of innate female buffer systems has been supported elsewhere (Guatelli-Steinberg, Lukacs 1999). Males are expected to exhibit higher rates of LEH due to their increased susceptibility to metabolic insult and that is seen for both the Lisbon and Coimbra populations. This pattern is also mirrored in the Oloriz population with 4.5% of males exhibiting LEH in the maxillary dentition and 1.2% of females' affected (Ubelaker *et al*. 2009).

However, these sex differences were not significant within the Lisbon and Coimbra samples suggesting there may have not been differential treatment of the sexes during this time, which could be related to women taking more active political roles as they applied for land ownership and the emigration of males to colonial holdings at the end of the 17th century (Birmingham 2003). These results also suggest males may not have been more susceptible to metabolic insult contra the results from Guatelli-Steinberg, Lukacs (1999), which found males in stressed populations, were more afflicted than females.

TABLE 12. ANOVA results for sex differences in Lisbon for both frequency and THA. *Significance (α = 0.05).

Tooth Class-Frequency	F-statistic	df	p-value
Central Incisor			
Frequency	0.06	1, 108	0.8092
THA	0.06	1, 108	0.8092
Canine			
Frequency	0.01	1, 108	0.9382
THA	0.15	1, 108	0.7040
Second Molar			
Frequency	0.51	1, 108	0.4770
THA	0.51	1, 108	0.4770

TABLE 13. ANOVA results for age differences in Coimbra for frequency and THA. *Significance (α = 0.05).

Tooth Class-Frequency	F-statistic	df	p-value
Central Incisor			
Frequency	2.68	1, 121	0.1041
THA	4.70	1, 121	**0.0321***
Canine			
Frequency	0.53	1, 121	0.4667
THA	0.52	1, 121	0.4733
Second Molar			
Frequency	0.82	1, 121	0.3684
THA	1.97	1, 121	0.1632

ANOVA does show significant differences between juveniles and adults within the Lisbon and Coimbra populations, but not between populations. However, THA was significant for the central incisor only and juveniles showed no significant differences between the populations. These results show the increased susceptibility of the central incisor to metabolic insult, and also support studies (i.e., Hasset 2012, 2014) that indicate width is not always a more sensitive parameter when assessing mortality risk. Frequency appears to be equivalent, but the area affected of the insults was greater for adults in the Coimbra population. This is contra to most studies on morbidity and mortality of populations, which illustrate that metabolic insults in subadults should be higher than surviving adults in a population (Slaus 2000, Saunders 1992). However, Wood *et al*. (1992) suggest that these stress indicators, such as LEH, may be higher in surviving adults because they indicate survival of a stress episode during life. This may be the explanation for the increased prevalence of LEH in the adults for both the Lisbon and Coimbra populations. However, the requirement of minimal attrition likely biases the distribution of LEH within the samples (see *Figures 1 and 2*). This is particularly relevant when discussing mortality risk and prevalence of LEH as LEH presence has commonly been associated with decreased health (Goodman, Rose 1990, Goodman, Armelagos 1985). This is also seen in the frequency distributions within the populations. Figures 8 and 9 illustrate the general trend in both populations whereby the 9–20 and 21–30 age ranges have overall higher frequencies of LEH than the older age ranges (31–40 and 41–50 years). The 60 years and above group from the Coimbra sample shows the opposite pattern, but this is likely related to this age range only consisting of four individuals. In addition, most individuals with an age-at-death greater than 45 showed increased antemortem tooth loss that did not allow for LEH assessment in these older age groups.

TABLE 14. ANOVA results for age differences in Lisbon for both frequency and THA. *Significance (α = 0.05).

Tooth Class-Frequency	F-statistic	df	p-value
Central Incisor			
Frequency	7.05	1, 108	**0.0091***
THA	7.05	1, 108	**0.0091***
Canine			
Frequency	1.69	1, 108	0.1967
THA	0.28	1, 108	0.5975
Second Molar			
Frequency	0.19	1, 108	0.6613
THA	0.19	1, 108	0.6613

TABLE 15. ANCOVA results for population differences for frequency and THA.

Tooth Class-Frequency	F-statistic	df	p-value
Central Incisor			
Frequency	12.93	1, 229	**0.0004***
THA	14.19	1, 229	**0.0002***
Canine			
Frequency	8.54	1, 229	**0.0038***
THA	3.08	1, 229	0.0806
Second Molar			
Frequency	0.82	1, 229	0.3657
THA	1.20	1, 229	0.2752

When age and sex were controlled in the analysis, there were significant population differences between the Lisbon and Coimbra populations for the central incisor and the canine. However, the canine was only significant for frequency. The multivariate analysis also shows significant differences between the two populations for both frequency and total hypoplastic area. These results suggest the populations were impacted differently during development with Coimbra experiencing increased hardship possibly due to dietary difficulties.

TABLE 16. MANCOVA results for population differences using both methods.

Method Type	F-statistic	df	Wilk's Lambda (α=0.05)
Frequency	4.44	3, 229	**0.0047***
Total Hypoplastic Area (THA)	4.99	3, 229	**0.0023***

Overall, this study shows an increase in developmental stress for the Coimbra population with the central incisor and canine showing significant differences between both populations. However, the canine was only significant for frequency. These differences show that the Coimbra population has a significantly higher frequency of LEH prevalence for those teeth that are more susceptible to metabolic insult (e.g., central incisor and canine). The second molar showed no significant differences likely related to the decreased susceptibility of molars (Hillson 2005).

The population differences are likely related to their diet, which appears to indicate malnutrition and known unsanitary conditions (Santos 1995). The Lisbon populations had a more varied nutritional diet and did not rely on maize as a primary nutritional component

(Cardoso 2007). The analysis also indicates that width is not a more sensitive parameter for estimating LEH severity in populations. Significant results were found using only the frequency method. This may be related to both the biased distribution found in the tooth thirds where width may be more indicative of where the insult occurred during development rather than the etiology or duration of LEH.

CONCLUSION

The outcome of this study shows that the Coimbra population was more stressed during the period of dental development than individuals from the Lisbon population. These results suggest that malnutrition may play a greater role in the development of LEH than increased systemic disease as approximately 39% of both samples died from tuberculosis as the main cause of death and the remaining majority died of other infectious diseases, largely respiratory in origin, suggesting similar disease loads in the samples (Cunha 1995, Santos 1995, Cardoso 2007). The increased stress in the Coimbra population was likely related to both malnutrition and disease because they were living in unsanitary conditions and the population was more dependent on a maize diet (Santos 1995). Maize subsistence has been shown to decrease health in populations that use this as their primary source of caloric intake (Cohen, Armelagos 1984).

However, both populations only had at most approximately 70% prevalence of LEH. This is in contrast to 100% prevalence for individuals in 19th century England (King *et al*. 2005); indicating the Iberian Peninsula may have been more healthy than most of Europe during the transition to industrialization. This also supports the results from Ubelaker *et al*. (2009) that the 19th century Spanish populations were healthier than other European countries. However, the microscopic methods used by King *et al*. (2005), typically find more defects than the macroscopic methods used in this study and in Ubelaker *et al*. (2009) (Hassett 2012, 2014)

This study also shows that, macroscopically, there is a biased distribution in LEH prevalence with the middle third being the most susceptible in all tooth classes. This bias may affect the outcome of LEH studies that use duration because the more rapidly secreting cells in the middle tooth third may show a larger duration that may not be related to actual timing of metabolic insult.

These results were in contrast to the hypothesis proposed. The Coimbra population exhibited an increased frequency in LEH relative to the Lisbon population. However, the Lisbon population did not show greater prevalence of LEH related to increased population density. Typically, the more dense populations will exhibit increased evidence of physiological stress related to increased disease loads. However, the results presented in this study indicate malnutrition may be a more significant factor and the increased levels of malnutrition in Coimbra had a greater effect on physiological stress than increased disease load in Lisbon during the 19th century.

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