

Correlation between childhood episodes of stress and long bone-ratios in samples of medieval skeletons – using linear enamel hypoplasia as proxy

Cecilie Cordua Mattsson¹ 

¹ ADBOU, Institute of Forensic Medicine, University of Southern Denmark, Campusvej 55, DK 5230 Odense M, Denmark.

Citation:

Mattsson, C. (2021). Correlation between childhood episodes of stress and long bone-ratios in samples of medieval skeletons – using linear enamel hypoplasia as proxy, *Human Biology and Public Health* 3. <https://doi.org/10.52905/hbph2021.3.23>.

Received: 2021-10-28
Accepted: 2022-03-31
Published: 2022-06-16

Copyright:

This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Conflict of Interest:

There are no conflicts of interest.

Correspondence to:

Cecilie Cordua Mattsson
email: ceciliemattsson@outlook.com

Keywords:

Early childhood stress, linear enamel hypoplasia, catch-up growth, anthropometrics, body proportions, Danish medieval cemeteries

Abstract

Background Linear enamel hypoplasia (LEH) in the canines is used as an indicator of ill health in early childhood. LEH is assumed to be an indicator of catch-up growth in archeological material. Previous research indicated that certain body proportions were altered due to catch-up growth during childhood.

Objectives The aim of this study was to assess whether catch-up growth during childhood affects the long bone ratios of the arms and legs of adults.

Sample and Methods Positive or negative scores for LEH in the four canines and anthropometric measurements of humerus, radius, femur, and tibia were obtained for 67 skeletons from two Danish medieval cemeteries in Nordby (Jutland) and Refshale (Lolland). Age and sex was estimated to see if any uneven distribution was present. The data was processed using the St. Nicolas house analysis, t-tests, univariate ANOVA and Kaplan-Meier survival analyses.

Results The St. Nicolas house analysis showed no correlation between hypoplasia score and anthropometric measurements. No statistically significant difference was found between the long bone ratios of the arms and legs in adults with and without LEH.

Conclusion Contrary to earlier research on body proportions in Medieval Denmark this study showed no significant difference in the long bone ratios of arms and legs associated with LEH. It did, however, find an alteration of femur and tibia length associated with hypoplasia. The findings indicate that males and females react differently to physiological stress in childhood.

Take home message for students Linear enamel hypoplasia is associated with an alteration of femur and tibia length of males and females from medieval Danish cemeteries, but not with the ratio of the long bones. The findings indicate that males and females react differently to physiological stress in childhood.

Introduction

Conventionally, human growth is seen as a target-seeking process (Tanner 1986). But recent research suggests that human height to a large extent depends on the living conditions and a person's social, economic, political and emotional (SEPE) environment (Bogin 2021; Hermanussen and Scheffler 2016) height tends to cluster (Aßmann and Hermanussen 2013b; Hermanussen and Scheffler 2016; Jørkov 2015). When a child reaches adolescence and experiences the adolescent growth spurt, he/she tends to follow a distal-to-proximal growth pattern; first the feet, hands, and the distal parts of the lower and upper extremities starts to grow, thereafter the proximal parts of the extremities and last, the central parts of the skeleton (Hermanussen 2013). Humans grow for a limited time and reach final height in early adulthood, which means that adjustments to body height are limited to this period of life (Hermanussen and Scheffler 2016). Different factors can influence an individual's growth. If the child experiences physiological stress such as malnutrition or severe illness it may stop to grow. Although the causes of growth failure can be many, the response to overcome is one: the child resumes growing and experiences catch-up growth (Tanner 1986). Catch-up growth is characterized by height velocity above the limits of normal for age for at least 1 year after a transient period of growth inhibition (Hermanussen 2013; Wit and Boersma 2002; Wit et al. 2013). In archeological material, linear enamel hypoplasia (LEH) is a sign of physiological stress during childhood. LEH is defined as horizontal lines of deficiencies of the thickness of the enamel in the labial or buccal surface of the tooth crown (Boldsen 2007; Marchewka et al. 2014). In paleopathology, LEH is generally used as an unspecific

indicator of physiological stress during childhood. It is used as an indicator of growth stop in early childhood.

The physiological mechanisms controlling catch-up growth are unknown. Based on Boldsen (1998) who stated "... secondly, in regaining size after such episodes – through catch up growth – certain body proportions were altered" an idea evolved regarding a possible pattern of catch-up growth in historic samples: Catch-up growth will lead to simultaneous growth of the whole body so that the three ratios between the proximal and distal arm- and leg-bones (humerus/radius and femur/tibia) and upper and lower extremities (arms/legs) will decrease.

The overall hypothesis for this study was developed: Adults scored positive for hypoplasia will have smaller bone-ratios than adults scored negative for hypoplasia.

In contrast to Boldsen (1998) who studied vertebrae, this study was based on anthropometric measurements of humerus, radius, femur, and tibia, and the ratio between the proximal and distal arm bones (humerus/radius), the ratio between the proximal and distal leg bones (femur/tibia), and the ratio between the arms and legs ((arm ratio)/(leg ratio)).

Sample and Methods

Sample

The skeleton material for this study was excavated from the two Danish medieval cemeteries Nordby in Jutland and Refshale on Lolland. The Nordby cemetery was excavated in 1996 and is assumed to have been in use in The Middle Ages from approx. AD 1050 to 1250. From the Nordby cemetery a total of 235 skeletons were excavated (Lynnerup et al. 2000). There have

been multiple excavations of the Refshale cemetery, the first in 1969 and the last in 1994, where a total of 218 skeletons were excavated. The cemetery of Refshale is estimated to have been in use from around AD 1100 until mid-14th century (Lolland-Falster Museum 2020). Due to the clay soil combined with the fact that the excavation has taken place in cultivated land many of the bones from Refshale were broken.

Only skeletons of people who survived into adulthood were analyzed. A skeleton is classified as an adult if all epiphyses of the long bones had fused prior to death.

Methods

The teeth scored positive for LEH were classified as having a visible line seen with the naked eye and/or felt by carefully scratching the labial surface of the canine enamel. To insure no false negative score of LEH the teeth were only scored with “0” or “1” if more than half of the tooth crown was visible, otherwise the score was “/” indicating that the relevant skeletal parts were not preserved. Enamel of the canines mineralizes around the age 2 to 5 years (Boldsen 2007), and thus, defined the age period of investigation for childhood stress of the individuals of this study.

Maximum length of humerus (1, page 199), radius (1, page 201), femur (1, page 217), and tibia (1a, page 220) were measured according to (Knussmann 1988), and registered in millimeters. If bones were broken postmortem, in not more than two pieces and could be fitted at the location of the break, the maximum length was measured. If bones were broken in more than two pieces or partially missing the score “/” was registered.

Age at death was estimated visually by “standard method” with different age markers in the cranium, pelvis, and postcranial skeleton (Milner and Boldsen 2012).

An overall assessment of the age-related changes of the long bones including the proximal femur was made (Tarp 2017). Sex was estimated visually by markers in the cranium, pelvis and postcranial skeleton (White and Folkens 2005). Sex was scored from 1 to 7, with 1 and 2 for male, 6 and 7 for female, and “4” for undeterminable (Table 1).

All skeletons were registered for sex, for age at death, and for LEH in the four canines. Maximum length of left and right humerus, radius, femur, and tibia were measured in millimeters. The material consisted of 67 individuals providing reliable information of the individual and at least the measurement of humerus and radius or femur and tibia.

Table 1 Sex estimation scores used for sex determination of the sample.

Score	Description:
/	Sex cannot be estimated – the relevant skeletal parts are not preserved
1	Clear male morphology
2	Predominantly male morphology
3	Slight male morphology
4	Sex indeterminable/children
5	Slight female morphology
6	Predominantly female morphology
7	Clear female morphology

Statistics

To get a first impression of data St. Nicolas House Analysis (SNHA) (Hermanussen et al. 2021) was used. The dataset was analyzed using the Shapiro-Wilk normality test to check for normal distribution, subsequently the Welch two sample t-test was used to test the hypothesis. A univariate ANOVA was used to compare the means

of the long bones and their ratios in correlation to sex and hypoplasia. A Kaplan-Meier survival analysis was applied to test for mortality differences between the sexes. Most of the statistical analyses were performed using the statistical program R studio version 1.2.5033 for Mac. For SNHA the R package “asg” (Groth et al. 2019; Hermanussen et al. 2021) was used. Waterfall chart was made in Microsoft Excel version 16.53 for Mac. The crosstabulation of sex^xhypoplasia, Kaplan-Meier survival analysis, and univariate analysis of variance was performed in IBM SPSS Statistics version 28.0.0.0 for Mac.

Results

The material consisted of 67 individuals (37 females, 29 males, one indeterminable). Nearly three quarters (71.6%, 48 individuals) of the individuals in the sample were scored positive (1) for LEH in the canines, whereas only 19 individuals were scored negative (0).

Initially, the SNHA was applied to get an idea of what to expect from the analysis. The SNHA plot, Figure 1, is built based on the Spearman correlation matrix, Figure 2, with $\alpha = 0.05$ and a threshold = 0.001. The SNHA plot shows a positive correlation between the hypoplasia mean scores (hpm) of the four canines and the scores for the upper (hpu) and lower (hpl) canines. The hpm variable is computed with the mean value of the four hypoplasia scores. The hpm variable can therefore also be seen as a kind of probability for childhood stress. The hpu and hpl are variables computed of the upper and lower canines scores – if the left-side score is available this is used, otherwise the right-side score is used. The association chains between hpm, hpu, and hpl indicate that there is

no direct correlation between the scores of the upper and lower canines. Furthermore, there is no correlation either between the LEH scores and the anthropometric measurements nor the sex of the individuals, not even with an $\alpha = 0.2$. As expected, the SNHA shows a correlation between the mean values of the anthropometric measurements.

Figure 2, described how well the variables correlate to each other. The correlation values for the hpm and the hpu and hpl are 0.9 and 0.89, respectively. The correlation between the hpm and the mean length of the long bones are all under 0.15 which explains the pattern of SNHA in Figure 1. As expected, the correlation matrix, Figure 2, shows a high correlation (0.89-0.94) between the mean lengths of the long bones. Out of the 48 individuals scored positive for LEH, 26 were females and 22 were males. In addition, 11 females and 7 males scored negative for LEH. There were no significant ($p = 0.782$) sex difference between hypoplasia scores. The Kaplan-Meier survival analysis showed no significant ($p = 0.392$) difference in mean age at death in adults scored positive or negative for LEH. There is only a tendency showing lower mean age at death (= 34.99 years) in those scored positive for LEH than in those scores negative (= 38.42 years).

Even though the original dataset contained 67 individuals, only 15 individuals were tested by the Welch two-sample t-test due to missing values. To compute the arm-mean-ratio (armMR) and leg-mean-ratio (legMR) variables, the anthropometric measurements of both sides of humerus/radius and femur/tibia were needed. Further, to compute the arm/leg ratio variable, values for both armMR and legMR had to be present.

To secure an unambiguous LEH score the “hypoplasia-mean” (hpm) score should be either “0” or “1”.

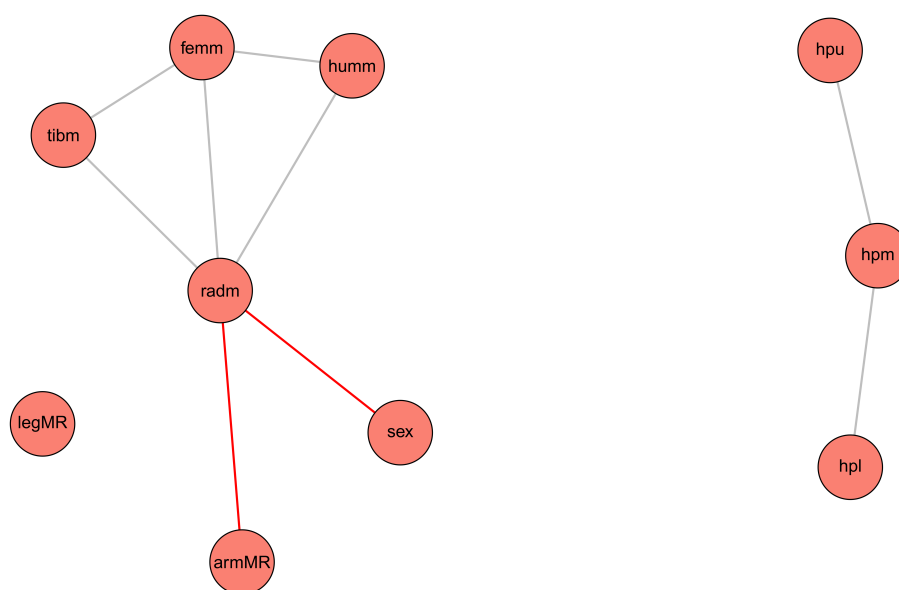


Figure 1 SNHA for the bone measurements, hypoplasia scores and sex. Alpha = 0.05 and threshold = 0.001. Red lines = negative association, grey lines = positive association. hpl = hypoplasia lower, hpm = hypoplasia mean, hpu = hypoplasia upper, armMR = arm mean ratio, humm = humerus mean, radm = radius mean, sex = sex, femm = femur mean, tibm = tibia mean, legMR = leg mean ratio.

Due to missing values, the original sample of 67 individuals reduced to 15 individuals (Figure 3). The Welch two sample t-test supported the result of the SNHA, there was no statistically significant interaction between the hpm and the arm/leg ratio ($p = 0.62$) There was also no significant association between hpm and the proximal and distal part of the extremities (humerus/radius ratio, $p = 0.40$; femur/tibia ratio $p = 0.61$).

The results of the univariate ANOVA, Table 2, showed no difference in length of humerus, radius, or the ratios of the arm, leg, and arm/leg in males and females associated with hypoplasia. But there was a significant difference between male and female lengths of femur ($\sigma = 0.015$) and tibia ($\sigma = 0.007$) when associated with hypoplasia.

The profile plot of femur, Figure 4, illustrates that males and females reacted differently to physiological stress in childhood. Males with hypoplasia had shorter femora than males without hypoplasia, whereas females with hypoplasia had longer femora than females without hypoplasia.

The differences of mean femur length between males and females were smaller in those with hypoplasia than in the males and females without hypoplasia. The difference of mean length of the femur was larger in males than in females.

The profile plot of tibia, Figure 5, illustrates the same tendency as Figure 4. Males with hypoplasia had shorter tibia than males without hypoplasia, whereas females with hypoplasia had longer tibia than females without hypoplasia. The difference of mean tibia length between male and female were

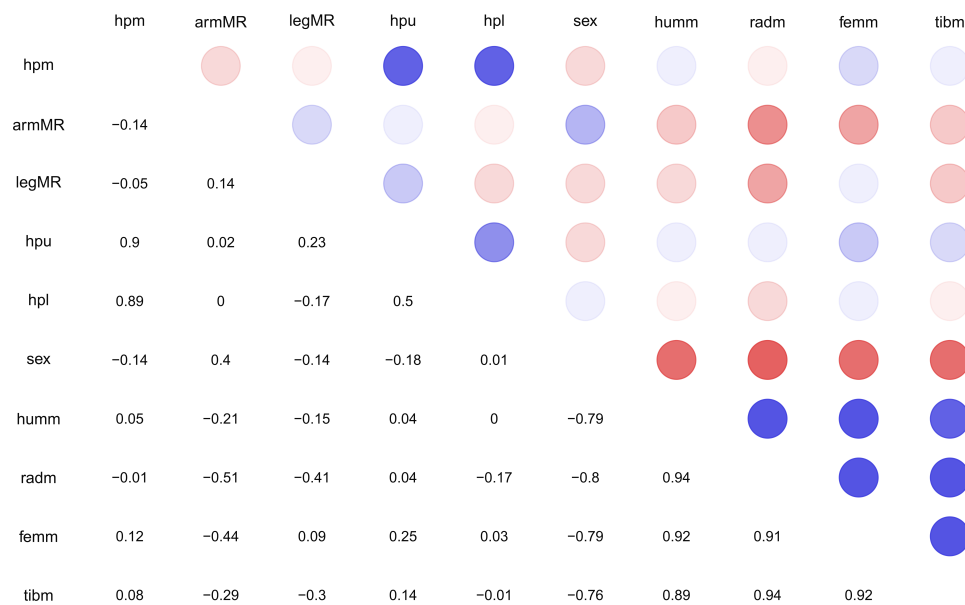


Figure 2 Spearman correlation matrix for pairwise correlations between the measurements of the bones and scores of hypoplasia. Blue color circles indicate positive and red colors indicate negative correlations. hpl = hypoplasia lower, hpm = hypoplasia mean, hpu = hypoplasia upper, armMR = arm mean ratio, humm = humerus mean, radm = radius mean, sex = sex, femm = femur mean, tibm = tibia mean, legMR = leg mean ratio.

smaller in those with hypoplasia than in the males and females without hypoplasia. Furthermore, the difference of mean length of tibia was larger in males than in females.

Discussion

There is little knowledge about the effect of catch-up growth on body proportion and on the ratios of long bones. This study uses an archeological collection from ADBOU, Denmark, to test the association of enamel hypoplasia scores as an indicator of early life growth inhibition and long bone ratios. We used skeletal material of 15 individuals. Though small, the study may be considered

Table 2 Results of univariate analysis of variance showing the levels of significance of the 7 variables. Hp = hypoplasia.

Sig.	Humerus	Radius	Femur	Tibia	Arm ratio	Leg ratio	Arm/leg ratio
Sex	<0.001	<0.001	<0.001	<0.001	0.168	0.901	0.171
Hp	0.117	0.121	0.163	0.543	0.658	0.452	0.447
Sex*Hp	0.172	0.153	0.015	0.007	0.360	0.583	0.390

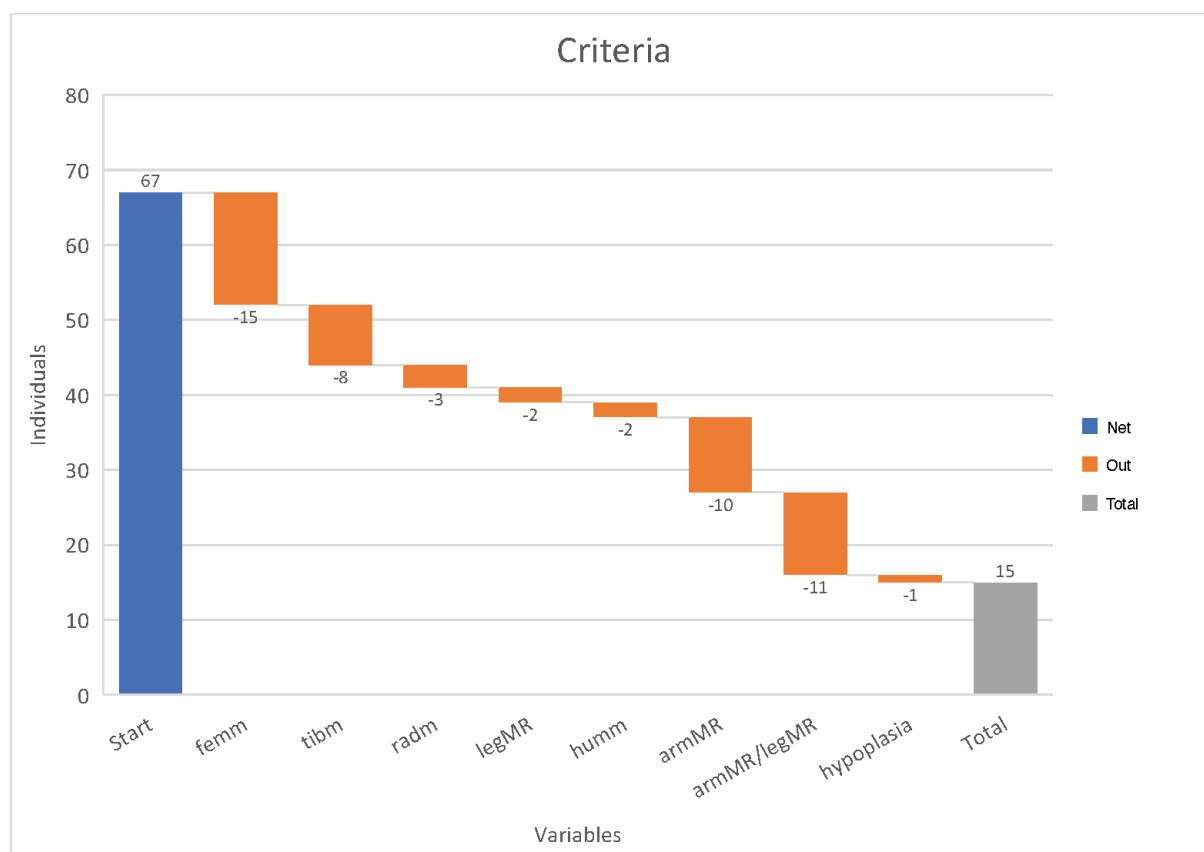


Figure 3 Waterfall chart showing the different variables made for the analysis and how many individuals were discarded due to missing measurements. Start = number of individuals registered, femm = mean value femur, tibm = mean value tibia, radm = mean value radius, legMR = leg mean ratio (femm/tibm), humm = mean value humerus, armMR = arm mean ratio (humm/radm), armMR/legMR = arm mean ratio/leg mean ratio, hypoplasia = unambiguous hypoplasia score.

a pilot study, and may be seen as an introduction to this field of research.

The analyses showed no significant differences in the ratios of either the length of the proximal and the distal arm bones (humerus/radius, $p = 0.40$) or the ratios of the proximal and the distal leg bones (femur/tibia, $p = 0.61$) in association with enamel hypoplasia scores. We studied adults. Adults are survivors which means they had approx. 10 to 12 years of growth to catch-up and compensate for any early childhood growth impairment.

Yet we failed to detect any association between linear enamel hypoplasia (LEH) in the four canines and the bone ratio between arms and legs ((humerus/radius)/(femur/tibia)) after having grown up.

However, the effect of early growth impairment on later growth differed between the sexes. Males with hypoplasia had on average 22.02 mm shorter femora than males without hypoplasia, whereas females with hypoplasia on average had 6.17 mm longer femora than females without hypoplasia. The same pattern was seen for tibia indicating that males and females react differently to physiological stress in childhood (McLaughlin et al. 2021).

The present study is not in line with Boldsen (1998) who stated that early growth impairment followed by catch-up growth results in changes in body proportion. Yet, as we studied lengths and ratios of proximal and distal extremities instead of vertebrae, the present findings may not be taken as proof against the statement of

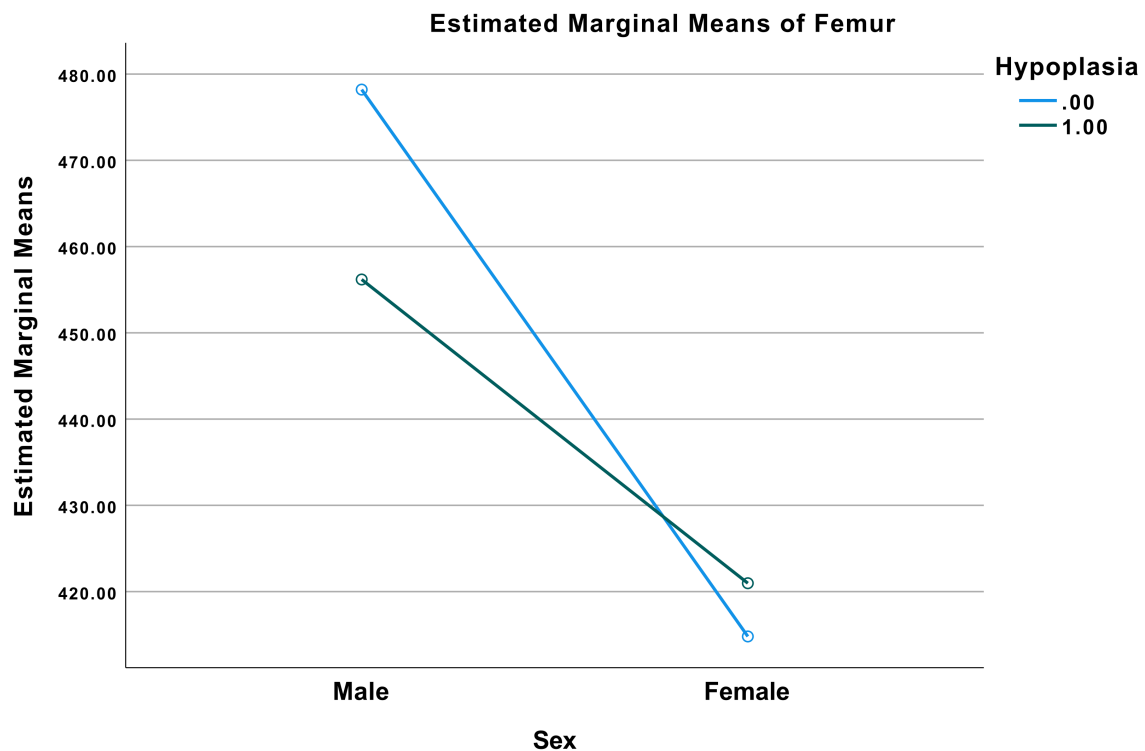


Figure 4 Profile plot from the univariate analysis of variance with dependent variable: Femur. Blue line (0) indicating no hypoplasia. Green line (1) indicating positive for hypoplasia. Values at (male, 0) = 478.20 mm, (male, 1) = 456.18 mm, (female, 0) = 414.80 mm, (female, 1) = 420.97 mm.

Boldsen. The present result may indicate that early illness or malnutrition differs in its effects on dental and skeletal growth (Boeker et al. 2022). In addition, survival bias, possibly even sex dependent survival bias, could have affected the outcome of this study. The children with LEH who did not survive to adulthood would probably have experienced more severe illness and/or malnutrition, and stopped growth early (Bhutta et al. 2017). Further research is needed in particular to study the association between the length of early growth arrests and the width of the enamel hypoplasia

The difference in age at death between those with (= 34.99 years) and those without LEH (= 38.42 years) though statistically not significant, suggests higher mortality among people who experienced physiological stress during childhood.

Conclusion

Contrary to earlier research on body proportions in Medieval Denmark this study shows no significant difference in long bone ratios of the arms and legs associated with LEH. It did, however, find an alteration of femur and tibia length associated with hypoplasia. The findings indicate that males and females react differently to physiological stress in childhood.

Acknowledgements

I would like to thank my supervisor Jesper Boldsen and the department of ADBOU for the opportunity to collect data for this project and, furthermore, make it possible

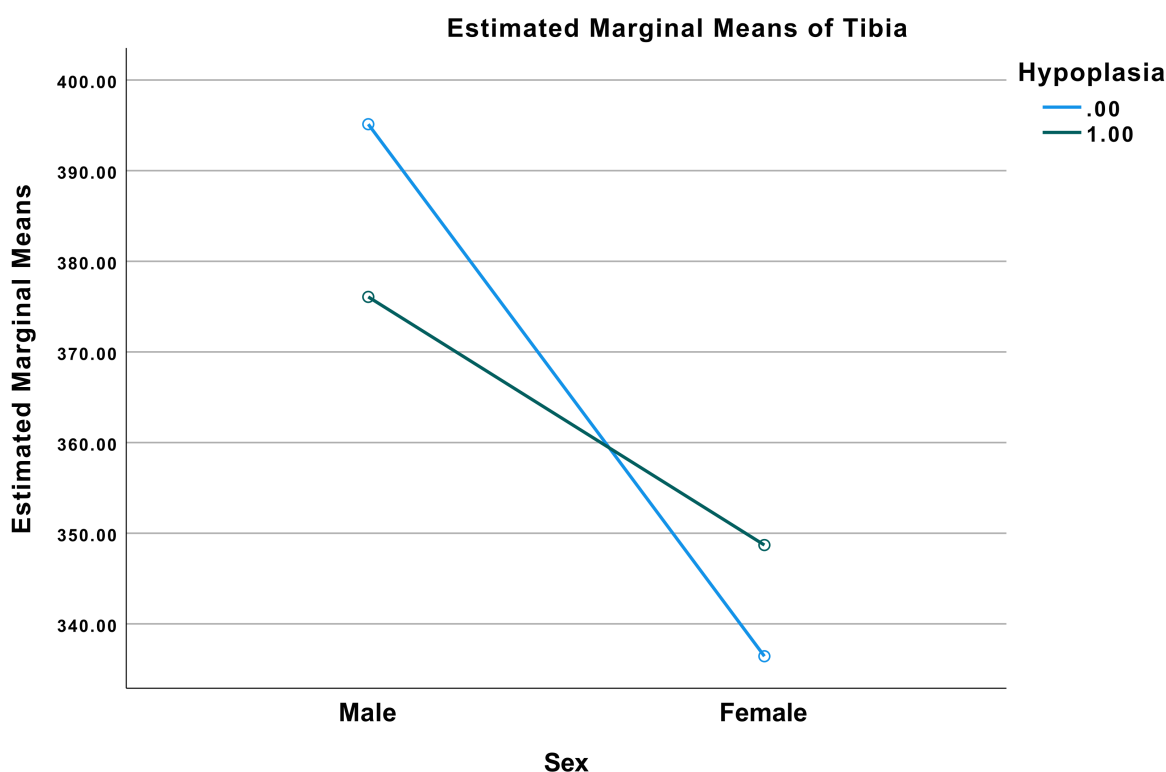


Figure 5 Profile plot from the univariate analysis of variance with dependent variable: Tibia. Blue line (0) indicating no hypoplasia. Green line (1) indicating positive for hypoplasia. Values at (Male, 0) = 395.13 mm, (male, 1) = 376.07 mm, (female, 0) = 336.43 mm, (female, 1) = 348.69 mm.

for me to go the International Summer School “Human Growth – Data Analyses and Statistics” in Potsdam/Gölpe from 19th to 24th of July 2021. Further, thanks to PD Dr. Christiane Scheffler, Prof. Dr. Michael Hermanussen, Dr. Detlef Groth, and the University of Potsdam for hosting the summer school, funded with KoUP.

References

Aßmann, C./Hermanussen, M. (2013a). Modeling determinants of growth: evidence for a community-based target in height? *Pediatric research* 74 (1), 88–95.

Bhutta, Z. A./Berkley, J. A./Bandsma, R. H. J./Kerac, M./Trehan, I./Briend, A. (2017). Severe childhood malnutrition. *Nature Reviews. Disease Primers* 3, 17067. <https://doi.org/10.1038/nrdp.2017.67>.

Boeker, S./Hermanussen, M./Scheffler, C.(2021). Dental age is an independent marker of biological age. *Human Biology and Public Health. Human Biology and Public Health* 3.<https://doi.org/10.52905/hbph2021.3.24>.

Bogin, B. (2021). *Patterns of human growth*. Cambridge, United Kingdom/New York, NY/Port Melbourne, Australia/New Delhi, India/Singapore, Cambridge University Press.

Boldsen, J. L. (1998). Body proportions in a medieval village population: effects of early childhood episodes of ill health. *Annals of Human Biology* 25 (4), 309–317.

Boldsen, J. L. (2007). Early childhood stress and adult age mortality—a study of dental enamel hypoplasia in the medieval Danish village of Tirup. *American journal of physical anthropology* 132 (1), 59–66. <https://doi.org/10.1002/ajpa.20467>.

Groth, D./Scheffler, C./Hermanussen, M. (2019). Body height in stunted Indonesian children depends directly on parental education and not via a nutrition mediated pathway – Evidence from tracing association chains by St. Nicolas House Analysis. *Anthropologischer Anzeiger* 76 (5), 445–451. <https://doi.org/10.1127/anthranz/2019/1027>.

Hermanussen, M. (Ed.) (2013). *Auxology: studying human growth and development: 89 tables*. Stuttgart, Schweizerbart Science Publ.

Hermanussen, M./Aßmann, Ch./Groth, D. (2021). Chain Reversion for Detecting Associations in Interacting Variables—St. Nicolas House Analysis. *International Journal of Environmental Research and Public Health* 18 (4). <https://doi.org/10.3390/ijerph18041741>.

Hermanussen, M./Scheffler, Ch. (2016). Stature signals status: The association of stature, status and perceived dominance – a thought experiment. *Anthropologischer Anzeiger* 73 (4), 265–274. <https://doi.org/10.1127/anthranz/2016/0698>.

Jørvok, M. L. S. (2015). Stature in 19th and early 20th century Copenhagen. A comparative study based on skeletal remains. *Economics & Human Biology* 19, 13–26. <https://doi.org/10.1016/j.ehb.2015.07.002>.

Knussmann, R. (1988). *Anthropologie: Handbuch der vergleichenden Biologie des Menschen*. Fischer.

Lolland-Falster Museum (2020). Archive material 2020.

Lynnerup, N./Qvist, M./Homøe, P. (2000). Mellemøretændelse. *Hikuin* 27 (27), 287. Available online at <https://tidsskrift.dk/Hikuin/article/view/111692>.

Marchewka, J./Skrzat, J./Wróbel, A. (2014). Analysis of the enamel hypoplasia using micro-CT scanner versus classical method. *Anthropologischer Anzeiger* 71 (4), 391–402.

McLaughlin, C./Schutze, R./Henley, D./Pennell, C./Straker, L./Smith, A. (2021). Prenatal and childhood stress exposure and the sex specific response to psychosocial stress in adulthood. *Psychoneuroendocrinology* 125, 105109. <https://doi.org/10.1016/j.psyneuen.2020.105109>.

Milner, G. R./Boldsen, J. L. (2012). Transition analysis: a validation study with known-age modern American skeletons. *American journal of physical anthropology* 148 (1), 98–110. <https://doi.org/10.1002/ajpa.22047>.

Tanner, J. M. (1986). Growth as a Target-Seeking Function. In: F. Falkner/J. M. Tanner (Eds.). *Human Growth: A Comprehensive Treatise Volume 1 Developmental Biology Prenatal Growth*. Boston, MA, Springer US, 167–179.

Tarp, P. (2017). *Skeletal age estimation*. PhD. Odense, University of Southern Denmark.

White, T. D./Folkens, P. A. (2005). *The human bone manual*. Elsevier.

Wit, C. C. de/Sas, Theo C. J./Wit, J. M./Cutfield, W. S. (2013). Patterns of catch-up growth. *The Journal of pediatrics* 162 (2), 415–420. <https://doi.org/10.1016/j.jpeds.2012.10.014>.

Wit, J.-M./Boersma, B. (2002). Catch-up growth: definition, mechanisms, and models. *Journal of pediatric endocrinology & metabolism* : JPEM 15 Suppl 5, 1229–1241. Available online at <https://pubmed.ncbi.nlm.nih.gov/12510974/>.