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# Analysis of interaction between nutritional and developmental instability in mediaeval population in Wrocław

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Abstract: In this paper, we test the hypothesis that indices of nutritional stress from enamel hypoplasia increase the incidence of indicators of developmental instability in fluctuating asymmetry, even in high social status individuals. The studied material consisted of a medieval sample of 58 skulls from the Wrocław area. Radiographs were taken in postero-anterior (P-A) and base projections. Images were scanned and calibrated by means of MicroStation 95 Academic Edition software, and measurements of the skull images were used to estimate fluctuating asymmetry. The presence of hypoplasia and caries was assessed using standard anthropological methods and all data was statistically analysed. The highest levels of fluctuating asymmetry were observed in the skull base region. Hypoplasia was observed in 40% and caries in 55.5%. Differences were noted in the level of fluctuating asymmetry in relationship to the presence or absence of hypoplasia, where a higher level predisposes individuals to enamel hypoplasia and a decline in buffering capacity, regardless of their socio-economic status.

Key words: fluctuating asymmetry, directional asymmetry, anti-symmetry, enamel hypoplasia, caries, developmental instability

## Introduction

The presence of fluctuating asymmetry (FA) has been used as an indicator of developmental stability since 1962, (Van Valen 1962). Fluctuating asymmetry denotes small accidents during development; sometimes called "noise"

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(Rasmuson 2002; Polak 2003; Żądzińska 2003, 2004). A high level of fluctuating asymmetry is associated with many different kinds of environmental stress experienced during pregnancy. These include pollution, malnutrition, alcohol abuse and smoking (Gawlikowska-Sroka 2006; Gawlikowska et al. 2007a, 2007b, 2010; Ozener 2010a, 2010b, 2011; Polak 2003; Rasmuson 2002 and Żądzińska 2003, 2004). While this is a non-specific index of stress existing during pregnancy and leading to asymmetry of bilateral traits in the adult female body, dental enamel hypoplasia is a non-specific index of stress acting during childhood. Hypoplasia has been used in many anthropological studies as an indirect measure of childhood nutritional status (Armelagos and Rose 1980 a, 1980b; Goodman and Rose 1991, Goodman et al. 1980a, b; Goodman et al. 1984; Hillson 1996; Krenz 1994; Krenz and Piontek 1996; Krenz-Niedbała 2000; Hoove et al. 2005; Hoover and Matsumura 2008, Krenz-Niedbała and Kozłowski 2011 and Tomczyk et al. 2012). It has been used for this purpose mainly because of the association between the peak of hypoplasia incidence and weaning (Hoover and Matsumura 2008). The nutritional status in later phases of life can be assessed by the presence of dental decay. A high level of caries indicates a high percentage of carbohydrates in the diet, and in historic populations this has been associated with high socio-economic status (Hillson 1996; Kwiatkowska 2005; Larsen 1998; Staniowski et al. 2011), but only a few reports have analyzed the correlation between these factors. Although previous international studies have indicated a weak correlation between increased odontometric fluctuating asymmetry and hypoplasia presence (Corrucini et al. 2005; Hoover et al. 2005; Hoover and Matsumura 2008), nothing has currently been established in this regard from Polish excavated material. The international researchers suggest that individuals with greater fluctuating asymmetry are less developmentally stable, less buffered and thus more prone to additional stress.

This paper tests the hypothesis that the indices of nutritional stress seen in enamel hypoplasia increase in a coincidence of indicators of developmental instability expressed by fluctuating asymmetry. This relationship exists despite high social status.

## Material and methods

The study material comprised 58 male and female skulls excavated from Wrocław municipal series cemeteries, as follows; (1) the collection from St. Elisabeth's church was 45 skulls dated to the 13$^{th}$ to 14$^{th}$ century, (2) the collection from Ołbin's cementery numbered 10 skulls dated to the 14$^{th}$ to 15$^{th}$ century and (3) the collection from St. Giles's church was 3 skulls dated to the 12$^{th}$ to 13$^{th}$ century. The historical documents and archaeological research show that these cemeteries were located in affluent areas of the Wrocław and consequently the subjects had enjoyed high social status (Kwiatkowska 2005, Dąbrowski and Gronkiewicz 1996, 2009). Gender was assessed from skull morphology applying accepted anthropological standards (Acsádi and Nemeskéri 1970; Buikstra and Ubelaker 1994; Piontek 1999), and age was determined by obliteration of cranial sutures (Buikstra and Ubelaker 1994) and wear of the tooth crowns, as in Miles (1963). All skulls were well preserved without pathological lesions and they were described either as *adultus* (22–35 years) or *maturus* (36–55 years).

The second phase of the study comprised X-ray photographs taken in posterior-anterior (P-A) and basal projections (Daniel 2011; Marchiori 1999, Poyton 1989; Zaborowski and Piontek 1977). These images were scanned and calibrated by MicroStation 95 Academic Edition software. The required geometry was obtained by transformation using the Helmert first-order polynomial followed by vectorization of the axes and area boundaries. Software tools for measuring vector elements were applied to measure distances between the chosen bilateral points of both sides in relation to the mid-line and to a second corresponding point located on the midline. The measurements in P-A projection were as follows:

*v-eu* = vertex – euryon,
*eu-ml* = euryon – midline,
*v-fmt* = vertex – frontomalare temporale,
*fmt-ml* = frontomalare temporale – midline,
*fmt-pr* = frontomalare temporale – prosthion,
*n-apt* = nasion – aperthion,
*apt-ml* = aperthion – midline,
*n-mf* = nasion – maxillofrontale,
*mf-ml* = maxillofrontale – midline,
*mf-ek* = maxillofrontale – ectoconchion,
*spa-sbk* = supraconchion – subconchion,
*area orb.*-area of orbit

Measurements in basal projection were:
*op-eu* = opisthion – euryon,
*eu-ml* = euryon – midline,
*ast-sphba* = asterion – sphenobasion,
*sphba-spal* = sphenobasion – palatinolaterale,
*spal-ml* = palatinolaterale – midline,
*spal-ol* = palatinolaterale – orale,
*fol-ml* = foraminolaterale – midline,
½ for. mag.–1/2 of area of *foramen magnum*,
*ba-f.ov* = basion – foramen ovale,
*ba-f.s* = basion – foramen spinosum,
*f.ov-ml* = foramen ovale – midline,
*f.s.-ml* = foramen spinosum – midline,
*a. f.ov.*=area of foramen ovale,
*a. f.s.*=area of foramen spinosum

All types of hypoplasia were identified macroscopically on present teeth using the Developmental Defects of Enamel Index developed by Federation Dentaire Internationale (FDI 1982). The incidence of lesions and the age of the subjects at the onset of the lesion were assessed by comparing measurements with Swärdstedt's modified Massler mineralization diagram for permanent human enamel (Goodman et al. 1980a, 1980b). Lesions due to caries in the teeth were examined by a probe using accepted standard procedure (Whittaker and Molleson 1986; Kerr et al. 1990). Every measurement was taken twice by the same investigator to determine the technical error of measurement (TEM) and to assess the reliability index (R). Measurement error was assessed using the mixed two-way ANOVA, and the normal distribution (P – L) of traits was ascertained by the Kolmogorov-Smirnov *d* test and the Shapiro-Wilk *W* test. The skew and curtosis of the distributions were analyzed. Directional asymmetry and anti-symmetry were excluded by studying the distribution of differences. Fluctuating asymmetry was studied by FA1 and FA2 indices determined for individual samples (Palmer and Strobeck 2003; Żądzińska 2003, 2004 and Gawlikowska et al. 2007a, b):

$$FA1 = Mean\left[\frac{|P-L|}{(P+L)0.5}\right]$$

$$FA2 = Mean\left|ln\left(\frac{P}{L}\right)\right|$$

and population:

$$FA1 = \frac{Mean\,|P–L|}{Mean\,[(P+L)0.5]}$$

$$FA2 = ln\,[Mean(P/L)]$$

The significance of differences in FA levels between genders was assessed using the Kruskal-Wallis test, and the significance of differences in the incidence of hypoplasia between genders was determined by the Chi$^2$ test. The association of hypoplasia with FA of the traits was analyzed by the Mann-Whitney U test and the Student's T test for unpaired samples. The gender dependent volatility of decay was evaluated using multivariate analysis of variance (MANOVA). All data was statistically analysed by Statistica 8.0 for Windows. Value levels < 0.05 were determined statistically significant.

## Results

Directional asymmetry in the examined material was established in 5 traits: *spal-ml, ba-f.o, f.o.-ml, fmt-pr, n-apt,* so these were excluded from analysis. The highest levels of FA1 index of fluctuating asymmetry in both genders were found for non-metric traits of the foramen spinosum and foramen ovale on the skull base (Berry and Berry, 1967) and also for the *mf-lp* dimension in the orbital region (Table 1 and Table 2). Meanwhile, the lowest asymmetry levels were noted in the cranial vault dimensions. Although FA 1 was more frequently higher in males than females, the differences were non-significant. The FA2 index had the highest and lowest levels in the same skull regions as

Table 1. Descriptive statistics for FA1 asymmetry index, Males

| Trait | N | Mean | Median | Min | Max | SD | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|---|
| op-eu | 33 | 0.041 | 0.035 | 0.003 | 0.107 | 0.030 | 0.712 | –0.494 |
| eu-ml | 33 | 0.059 | 0.048 | 0.001 | 0.222 | 0.046 | 1.461 | 3.204 |
| ast-spba | 33 | 0.046 | 0.041 | 0.002 | 0.165 | 0.032 | 1.761 | 4.896 |
| spba-spal | 32 | 0.038 | 0.028 | 0 | 0.156 | 0.036 | 1.857 | 4.117 |
| spal-ol | 32 | 0.032 | 0.025 | 0.001 | 0.089 | 0.025 | 0.723 | –0.519 |
| fol-ml | 31 | 0.071 | 0.055 | 0 | 0.223 | 0.054 | 0.917 | 0.378 |
| 1/2for.mag | 31 | 0.079 | 0.051 | 0.001 | 0.265 | 0.069 | 0.935 | 0.077 |
| ba-f.s | 28 | 0.041 | 0.033 | 0 | 0.099 | 0.028 | 0.628 | –0.421 |
| ml-f.s | 28 | 0.052 | 0.046 | 0 | 0.142 | 0.042 | 0.789 | –0.292 |
| area f.o | 29 | 0.275 | 0.239 | 0.033 | 0.902 | 0.212 | 1.585 | 2.695 |
| area f.s | 28 | 0.312 | 0.256 | 0 | 0.927 | 0.235 | 0.973 | 0.854 |
| v-eu | 32 | 0.046 | 0.032 | 0 | 0.159 | 0.039 | 1.200 | 0.947 |
| eu-ml | 32 | 0.057 | 0.059 | 0.006 | 0.171 | 0.041 | 0.828 | 0.542 |
| v-fmt | 33 | 0.076 | 0.026 | 0 | 1.604 | 0.276 | 5.661 | 32.332 |
| fmt-ml | 33 | 0.032 | 0.0217 | 0.002 | 0.108 | 0.028 | 1.067 | 0.444 |
| apt-ml | 32 | 0.125 | 0.118 | 0.006 | 0.344 | 0.084 | 0.657 | 0.102 |
| n-mf | 33 | 0.116 | 0.102 | 0.006 | 0.304 | 0.075 | 0.752 | 0.152 |
| mf-ml | 33 | 0.171 | 0.155 | 0 | 0.461 | 0.104 | 0.653 | 0.605 |
| mf-ek | 32 | 0.039 | 0.024 | 0.003 | 0.187 | 0.039 | 2.048 | 5.346 |
| spa-sbk | 32 | 0.028 | 0.020 | 0.002 | 0.089 | 0.021 | 1.182 | 1.221 |

Table 2. Descriptive statistics for FA1 asymmetry index, Females

| Trait | N | Mean | Median | Min | Max | SD | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|---|
| op-eu | 24 | 0.032 | 0.026 | 0.004 | 0.110 | 0.026 | 1.335 | 2.015 |
| eu-ml | 24 | 0.041 | 0.030 | 0 | 0.174 | 0.041 | 1.607 | 3.485 |
| ast-spba | 23 | 0.035 | 0.024 | 0.002 | 0.109 | 0.031 | 1.224 | 0.652 |
| spba-spal | 24 | 0.065 | 0.061 | 0 | 0.183 | 0.046 | 0.702 | 0.397 |
| spal-ol | 25 | 0.029 | 0.026 | 0 | 0.107 | 0.024 | 1.529 | 3.109 |
| fol-ml | 21 | 0.093 | 0.074 | 0 | 0.352 | 0.087 | 1.377 | 2.372 |
| 1/2for.mag | 21 | 0.117 | 0.084 | 0.011 | 0.323 | 0.087 | 0.788 | –0.166 |
| ba-f.s | 21 | 0.043 | 0.035 | 0.006 | 0.147 | 0.034 | 1.563 | 3.155 |
| f.s.-ml | 21 | 0.056 | 0.066 | 0.002 | 0.173 | 0.042 | 0.898 | 1.512 |
| area f.o | 24 | 0.309 | 0.236 | 0.034 | 1.471 | 0.306 | 2.606 | 8.709 |
| area f.s | 21 | 0.300 | 0.322 | 0 | 0.614 | 0.1904 | –0.104 | –0.901 |
| v-eu | 25 | 0.044 | 0.037 | 0.007 | 0.133 | 0.035 | 0.984 | 0.177 |
| eu-ml | 25 | 0.061 | 0.052 | 0.015 | 0.131 | 0.039 | 0.460 | –1.221 |
| v-fmt | 25 | 0.028 | 0.020 | 0 | 0.086 | 0.025 | 1.024 | 0.069 |
| fmt-ml | 25 | 0.028 | 0.017 | 0 | 0.129 | 0.029 | 2.365 | 5.848 |
| apt-ml | 24 | 0.118 | 0.078 | 0 | 0.353 | 0.101 | 1.253 | 0.462 |
| n-mf | 25 | 0.109 | 0.088 | 0.010 | 0.274 | 0.074 | 0.829 | –0.241 |
| mf-ml | 25 | 0.129 | 0.104 | 0 | 0.405 | 0.099 | 1.378 | 1.552 |
| mf-ek | 23 | 0.046 | 0.042 | 0.005 | 0.118 | 0.028 | 0.862 | 0.328 |
| spa-sbk | 25 | 0.046 | 0.041 | 0.003 | 0,122 | 0.036 | 0.623 | –0.669 |

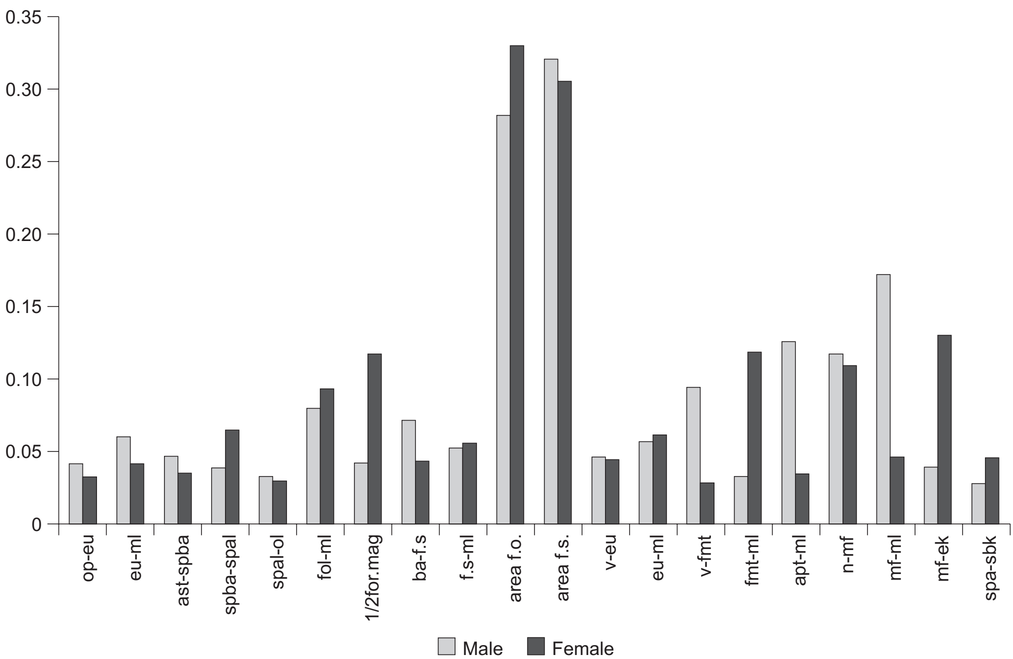


Fig. 1. Values of the asymmetry index FA1 and differences between males and females

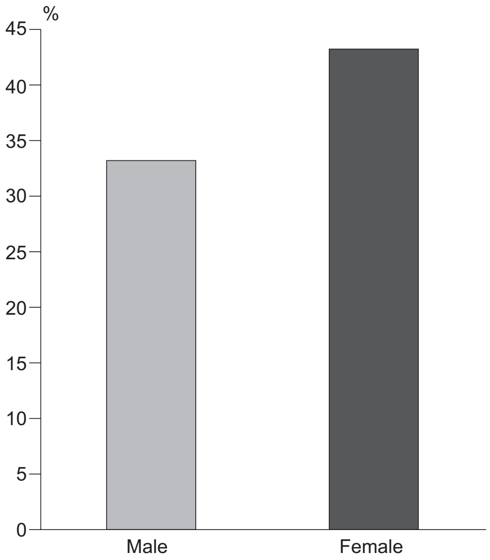


Fig. 2. Prevalence of hypoplasia

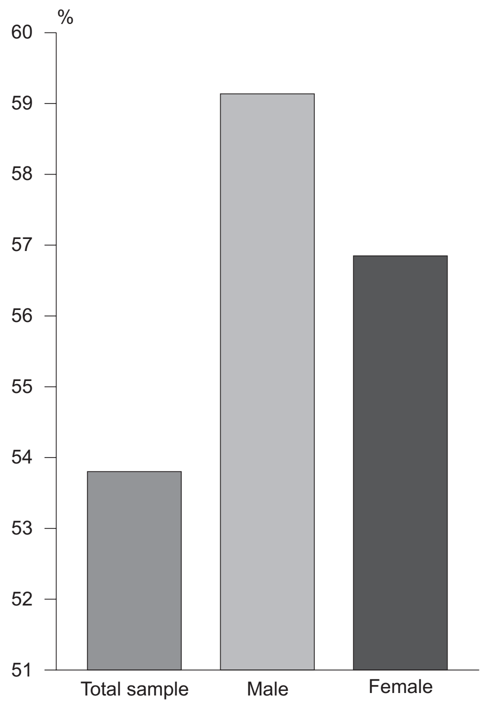


Fig. 3. Prevalence of carries

the FA1, but because the FA2 index values were only slightly higher than those for FA1, the difference was not statistically significant (Fig. 1). Again, both indices had higher levels in males, but the differences were not significant.

The incidence of hypoplasia was at a medium level, with lesions intensifying at 2.5–3.5 years of age, with insignificantly greater value in females (Fig. 2). The prevalence of dental caries in the examined material was 59.3% and these occurred more frequently in males (Fig. 3).

A higher level of fluctuating asymmetry correlated with the presence of hypoplasia, and this was observed at the level of traits and mean indices (Fig. 4).Statistically significant differences between hypoplasia presence and absence and the level of fluctuating asymmetry were established at $p<0.01$. The strongest correlation was for *ba-f.s.* and *eu-ml* measurements at $p<0.01$.

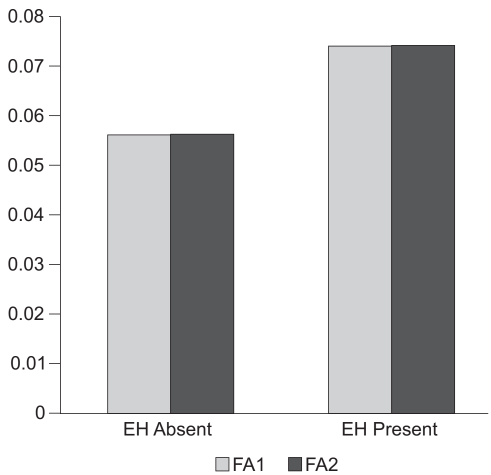


Fig. 4. Differences in mean values of asymmetry index FA1 and FA2 depending on presence or absence of hypoplasia

## Discussion

Fluctuating asymmetry (FA) is defined as the random difference between quantitative measures of a bilateral trait.(Polak 2003; Rasmuson 2002; Żądzińska 2003; 2004; Hoover et al. 2005 and Hoover and Matsumura 2008). The level of asymmetry depends not only on the type, duration and intensity of stressors acting during intra-uterine life, but also on the individual's ability to stabilise development in adverse conditions (Gawlikowska et al. 2007a, b; Gray and Marlowe 2002; Hershkovitz et al. 1990; 1992; Livshits and Kobyliansky 1991; Møller and Swaddle 1997 and Palmer and Strobeck 1992, 2003). Recent research has highlighted higher FA levels in patients suffering from Down's syndrome (Townsend and Dent 1983), aggression and schizophrenia (Burton et al. 2003; Saha et al. 2003; Dinwiddie 2005; Bates 2007; Fatjó-Vilas et al. 2008; and Shakibaei et al. 2011). FA analysis is useful in the examination of historic populations. For example, Doyle and Johnson (1977) studied fluctuating asymmetry of teeth in Eskimos from Alaska, American Indians from Arizona and current white males from Ohio and found the level lowest in modern white males. Perzigian (1977) studied differences in FA levels in three historic populations, with the highest levels noted in lowest socio-economic populations. Archaeological material of Indian Knoll pre-historic hunters provided the worst living conditions, with a large number of Harris lines, enamel hypoplasia and high mortality in this sample. This suggests a very stressful lifestyle.

These works corroborated the hypothesis that fluctuating asymmetry is an appropriate indicator for estimating intra- and inter-population developmental stability (Gilligan et al. 2000; Bailit et al. 1970; Doyle and Johnson 1977; Hershkovitz et al. 1993; Livshits et al. 1994; DeLeon 2007; Gawlikowska et al. 2007a, b and Özener 2010a, b, 2011).

While Hershkovitz, Ring and Kobyliansky's (1992) analysis of fluctuating asymmetry established highest levels in the skull's facial area, our observations were slightly different. Here, the lowest levels of fluctuating asymmetry were observed in the region of the skull vault similar to Hershkovitz, Ring and Kobyliansky (1992) findings, but our highest levels were noted in the region of the skull base. This same observation was noted in our previous examination of fluctuating asymmetry in the Gródek medieval population (Gawlikowska-Sroka 2006 and Gawlikowska et al. 2007a, b). Observed values were similar in both men and women, and the high level of fluctuating asymmetry in the cranial base may be due to cranial base composition. This has a large number of bony elements and each can be affected by harmful environmental factors (Gawlikowska et al. 2007a,b and Gawlikowska et al. 2010). In addition, it may signify insufficient nutrition during growth acting as a stress factor in accordance with Angel's (1982) observation that nutrition and health conditions influence skull-base development. In this regard, he reported that high skull-base fluctuating asymmetry levels are a sensitive measure of nutritional and disease-related stress during growth (Angel 1982). The FA index value was medium in medium-sized measurements but higher for longer and very short measurements.

Enamel hypoplasia is used as a non-specific indicator of morphological stress in current and historic populations. A wide variety of diseases and nutritional agents considered non-specific in nature can lead to hypoplasia. Metabolic disturbances affect a tooth only during its formation so the location of the defect on the tooth crown enables us to asseses an individual's age during defect formation (Krenz 1994; Krenz and Piontek 1996). The analysis of hypoplasia and other stress indictors such as cribra orbitalia and Harris lines provides information about living condition in past populations (Henneberg and Henneberg 1989; Goodman and Rose 1991, Haduch 2002; Krenz and Piontek 1996; Krenz-Niedbała 1999; Krenz-Niedbała 2000; Hoover et al. 2005; Hoover and Matsumura 2008; Krenz-Niedbała and Kozłowski 2011; Palubeckaité and Jankauskas 2001; Piontek 1992 and Tomczyk et al. 2012). Our research highlighted linear and pit hypoplasia in 36% of females and 44 % of males. Hypoplastic changes were observed on all types of teeth with the predominance on canines and premolars. This constituted a medium level of hypoplasia, similar to medieval series in other towns, while a higher level of approximately 60% was observed in medieval London and connected to population density and poor diet (Ogden et al. 2008)). While hypoplasia is certainly lower at 10% in modern European populations, in developing countries it often reaches levels similar to those in historic populations (Kozak and Krenz-Niedbała 2002; Krenz and Piontek 1996). Our research established that the majority of enamel defects occured between 2 and 3 years of age, and the peak is attributed to weaning stress. This is a similar result to other observations assessed by the Swärdstedt modification of Massler's chart (Krenz 1994; Krenz and Piontek 1996; Kozak and Krenz-Niedbała 2002; Goodman and Rose 1991, Hillson 1996). Meanwhile, defect peaks occured somewhat later in research based on Goodman and Song's method, with correction for hidden cuspal enamel (Krenz-Niedbała and Kozłowski 2011).

The relationship between oral health and social status has been analysed many times, and correlation is most clearly apparent in the transition from foraging to agriculture ( Krenz-Niedbała 2001; Tomczyk et al. 2012). The occurrence of caries here has been associated with a carbohydrate-rich diet and poor oral hygiene in high socio-economic status subjects. While this independently leads to oral bacterial colonization, hormonal influences may also be involved (Dąbrowski and Gronkiewicz 1996; Tomczyk et al. 2013) The most appropriate reconstruction of historic diets is via chemical analysis of strontium, barium, calcium and stable carbon isotope levels (Tomczyk et al. 2013). Kwiatkowska (2005) reported higher carie frequencies in populations from affluent areas of medieval Wroclaw (XII–XVth century) and the lowest in middle class residential areas. The combination of high carie frequency in the examined populations, archeological artefacts excavated with skeletons and the historic data on the town and its cemetery locations has confirmed that the subjects were in the high socio-economic class. In accordance with Hoover and Matsumura's 2008 results, we expected that individuals with hypoplasia would have greater levels of FA from synergistic relationships between stress and pathology. This hypothesis was confirmed in our research. The presence of developmental stress indirectly suggests lower stability in the assessed individuals, and a relationship also exists between foetal life stress and developmental stability.

## Conclusions

Differences in the level of fluctuating asymmetry in relation to the presence or absence of hypoplasia reflect intra-population differences in health status and developmental stability. A higher level of fluctuating asymmetry, as index of developmental noise, predisposes subjects to enamel hypoplasia and decreased buffering capacity. Here, this occurred despite the high socio-economic status determined by historical data, with a high number of caries most likely present. This study advances anthropological knowledge, especially in Poland where no analysis has been performed on comparison of skeletal material from different historical periods.

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### Author contribution

AG conceived and designed the paper, was a principal investigator for the research project, collected data of asymmetry, interpreted data, drafted and approved the final manuscript; PD collected material, took measurements, drafted manuscript; JS collected material, took measurements, drafted manuscript; TS, PD collected material, took measurements, drafted manuscript. All of authors read and approved the final manuscript.

### Conflict of interests

The authors declare that there is no conflict of interests.

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