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## Early life histories of Great Moravian children – carbon and nitrogen isotopic analysis of dentine serial sections from the Early Medieval population of Mikulčice (9th-10th centuries AD, Czechia)

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**Abstract:** In order to compare the early life experiences of different population subgroups from the Early Medieval centre of Mikulčice, carbon and nitrogen isotopic values were measured in dentine serial sections from the first permanent molar of 78 individuals. Age-at-death, sex (estimated in subadults with the help of proteomics) and socio-economic status were considered as explicative variables. Average values of both nitrogen and carbon maximal isotopic offset within the isotopic profile were higher than the recommended range for weaning under healthy circumstances:  $3.1 \pm 0.8\text{‰}$  for  $\Delta^{15}\text{N}_{\text{max}}$  and  $1.6 \pm 0.8\text{‰}$  for  $\Delta^{13}\text{C}_{\text{max}}$ . Individuals who died during the first decade of life showed earlier ages at the final smoothing of the nitrogen isotopic curve (suggesting complete weaning) than older individuals. Most individuals ( $n = 43$ ) showed positive covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values during the period of breastfeeding. The average  $\delta^{15}\text{N}$  values from the post-weaning period were similar to those of bone, while post-weaning  $\delta^{13}\text{C}$  values were significantly higher.

Though an increased  $\Delta^{15}\text{N}_{\text{max}}$  suggests a common presence of physiological stress, the intra-population comparison of early life experiences does not suggest that individuals who died during their first decade experienced greater levels of environmental stress during infancy.

The predominance of positive covariance between carbon and nitrogen isotopic values during the breastfeeding period, together with an increased  $\Delta^{13}\text{C}_{\text{max}}$  and increased post-weaning  $\delta^{13}\text{C}$ , suggest

that millet was either a part of a special diet preferred during lactation or was introduced as a first dietary supplement.

Keywords: Breastfeeding, physiological stress, diet, stable isotopes, Middle Ages

## 1. Introduction

Since the pioneering work by Fogel et al. (1989) described the relationship between the hair isotopic values of mothers and their breastfed babies, the reconstruction of breastfeeding and weaning behaviour has naturally attracted the attention of bioarchaeologists. The duration of breastfeeding, as well as the timing of the introduction of complementary food, clearly affect the health and physical well-being of the child both in the short term (Lamberti et al. 2011, Shamir 2016, Wilson et al. 2006) and the long term (Berti et al. 2017, Demmelmair et al. 2006, Kendall et al. 2021, Lamberti et al. 2011, McDade 2005, Palou and Picó 2009). Moreover, as a bio-socio-cultural phenomenon, infant and young child feeding practices are influenced by a number of cultural, religious, economic and also environmental factors (Fildes 2017, Quinlan 2007, Thorvaldsen 2008, Tomori et al. 2017, Yovsi and Keller 2003).

Last but not least, the duration of breastfeeding has a considerable impact on women's health and fertility (Bentley et al. 2001, Jay 2009). For all these reasons, information on this aspect of childcare helps substantially to understand the population dynamics and living conditions of past populations.

For more than a decade, researchers generally followed a cross-sectional approach, analysing carbon and nitrogen isotopes in the bone collagen of infants and young children of various ages, and comparing their values to female population averages (e.g. Fogel et al. 1989, Katzenberg et al. 1996, Mays 2010, Pearson et al. 2010, Prowse et al. 2008, Schurr 1997), often attempting to link observed isotopic patterns with the mortality and morbidity profiles of subadults.

Subsequently, however, some hidden pitfalls of this approach were addressed, such as the omission of the intra-population dietary variation in lactating mothers or potential mortality biases in weaning interpretations in terms of the osteological paradox (Fuller et al. 2003, Wood et al. 1992). A solution to these problematic issues was seen in the enforcement of an intra-individual approach, recovering dietary information from different periods of an individual's life. Samples were taken from different mineralized tissues (Herrscher 2013, Howcroft et al. 2012, Kaupová et al. 2014), from different parts of bone (Waters-Rist et al. 2011), and finally from serial sections of dental tissues, with the number of serial samples increasing along with development in mass spectrometry reducing the sample size (Beaumont et al. 2013, Eerkens et al. 2011, Fuller et al. 2003, Howcroft et al. 2012).

Gradually, scientists' attention moved to dentine tissue, which, due to the absence of turnover, retains the isotopic signal from the period of tooth development throughout life (Balasse et al. 2001, Richards et al. 2002), and thus allows the inclusion of adult individuals into studies while avoiding the risk of mortality biases. Sampling horizontal sections of dentine results in an isotopic profile covering the entire period of the tooth development (Beaumont et al. 2013). However, rather than resolving the issue of breastfeeding, the more detailed sampling methodology revealed that the dentine isotopic record results from a complex interaction of a number of dietary and non-dietary factors, among which physiological stress plays a key role (Beaumont et al. 2013, 2018, Craig-Atkins et al. 2018, King et al. 2018).

## Potential sources of isotopic variation in dentine isotopic values

### Breastfeeding

The isotopic effect of breastfeeding is a reflection of a phenomenon called the “trophic level effect” (Ambrose and Norr 1993, DeNiro and Epstein 1978, 1981, Schoeninger and DeNiro 1984), whereby heavier isotopes are discriminated against at each level of the food chain, resulting in isotopic enrichment of the consumers' tissues above those of their prey. In bone collagen, enrichment by 3-5 ‰ was observed with each trophic level for  $\delta^{15}\text{N}$  values (direct estimates for human give slightly higher estimates of 5.5-6 ‰, O’Connell et al. 2012) and by 1 ‰ for  $\delta^{13}\text{C}$  values. However, focusing on breastfeeding, direct observation of mother-infant pairs reported somewhat lower shifts in  $\delta^{15}\text{N}$  values associated with breastfeeding – between 2-3 ‰ (Fuller et al. 2006, Herrscher et al. 2017).

Prior to birth, the foetus is an integral part of the maternal organism (Fogel et al. 1989). Although a small offset has been observed in the tissues formed in-utero in mother-offspring pairs (de Luca et al. 2012, but see Herrscher et al. 2017), much larger deviations occur once breastfeeding starts. Due to the trophic level effect, breastfed infants exhibit an elevation of both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values above maternal values. During weaning, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values of the newly formed tissues drops continuously, along with the decreasing dietary proportion of breast milk (Fogel et al. 1989, Fuller et al. 2006).

### Nutritional or physiological stress

As demonstrated by a number of controlled-feeding experiments on different animal species (for review see e.g. Reitsema 2013), as well as by direct observations of humans with eating disorders, pregnancy complications, and a number of serious diseases (Fuller et al. 2005, Mekota et al. 2006, Tea et al. 2021), changes in nitrogen balance under physiological stress are responsible for notable isotopic shifts. To compensate for protein insufficiency under conditions of negative nitrogen balance, body tissues are catabolized and recycled. Thus, the fractionating processes of transamination and deamination are repeated, resulting in enrichment in the  $^{15}\text{N}$  of the newly formed tissues (Reitsema 2013). The impact of decreased protein bioavailability on carbon isotopic values is, however, less unequivocal. In some studies, the above-described shifts in  $\delta^{15}\text{N}$  values have been found to be accompanied by decreased  $\delta^{13}\text{C}$  values, this results from the combination of the altered ratio between routed dietary and endogenously synthesized amino acids and of the mobilization of fat stores, which are isotopically depleted (Beaumont et al. 2018, Mekota et al. 2006, Neuberger et al. 2013, Schwarcz 2002). Based on these findings, a typical isotopic “stress pattern” has been described, which is commonly used especially in studies of dentine isotopic profiles, stressing the opposing covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values during the stress episode (Beaumont et al. 2018, Beaumont and Montgomery 2016, Craig-Atkins et al. 2018).

An increasing number of studies (Canterbury et al. 2020, D’Ortenzio et al. 2015, Drtikolová Kaupová et al. 2021, Fuller et al. 2005, Katzenberg and Lovell 1999), however, have found no change in  $\delta^{13}\text{C}$  values during the stress episodes affecting  $\delta^{15}\text{N}$  values. There are even studies (Drtikolová Kaupová et al. in press, Neuberger et al. 2013) reporting positive covariance between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values during such stress episodes.

## Other factors

The next point potentially deconstructing the paradigm of the "typical isotopic stress pattern" is that there are a number of other factors of both dietary and physiological origin, concerning mother and/or child, which may evoke isotopic shifts emulating or overriding isotopic reflection of both breastfeeding and stress. The use of distinct weaning foods is quite common throughout the world (e.g. Sellen 2001), as are specific dietary rules imposed on pregnant or lactating women (Baumslag 1987). Alternatively, annual or seasonal variation in the isotopic composition of the mothers' diets, and therefore of breastmilk, can never be excluded. This argument is especially important in all the contexts with documented consumption of C4 plants and/or both marine and freshwater products (King et al. 2018). Further, although there is limited information on the isotopic composition of breastmilk, it seems that there are isotopic shifts in breastmilk values over the course of breastfeeding independent of maternal diet or health (Herrscher et al. 2017), probably linked with changing macronutrient composition over the course of lactation (Czosnykowska-Łukacka et al. 2018). The practice of wet-nursing, well-known in continental Europe since Antiquity (Fildes 2017), can also affect the isotopic reflection of breastfeeding, when the isotopic values of the wet-nurse are different from those of the mother (Herrscher 2004).

For all the reasons mentioned above, it is evident that the reduction of isotopic variation in infants and young children to a simple typology of isotopic curves reflecting "weaning" or "physiological stress" (e.g. Craig-Atkins et al. 2018) is not viable. In this study, we thus attempt to avoid this descriptive approach and rather analyse the interplay between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotope values in early life without such categorization, to compare the isotopic values and their shifts between biologically and socio-culturally defined groups and to highlight the multiple potential interpretations of incremental profile shapes.

For that reason, we have explored the early life experiences of a sufficiently numerous ( $n = 78$ ) population sample from the Great Moravian settlement agglomeration of Mikulčice, defining a number of simple questions: first, do all the individuals exhibit a isotopic peak in the early life period attributable to breastfeeding? Second, due to the well-described impact of early life experience on health and physical well-being in a long-term perspective (Demmelmair et al. 2006, McDade 2005, Palou and Picó 2009), we searched for the potential differences in isotopic profiles between individuals who died during M1 formation, those who died after M1 formation but before adulthood (i.e. during the second decade of the life) and finally individuals who survived to adulthood. To check for different parental investment in infants of different sexes, which are common in populations with high gender inequality (Eerkens and Bartelink 2013, Jayachandran and Kuziemko 2011), we explored the potential differences in isotopic profile between males and females. And finally, we explored the whether the level of parental effort and/or environmental risk differed between members of Great Moravian elites vs. common folk.

## 2. Materials and methods

Great Moravia (9th to beginning of the 10th centuries AD) was the first Slavic proto-state structure in Central Europe (Figure 1). Along with rapid political consolidation and the introduction of the first proto-urban centers, Christianisation probably extended into Moravia at the beginning of the 9th century (Herold 2012, Kalhous 2020, Macháček 2013). The skeletal material analysed in this study

comes from the settlement agglomeration at Mikulčice (Czechia, N 48°48'15.9", E 17°05'08.5", Kuna et al. 2018), which is believed to be one of the prominent power centres of the Great Moravian Empire (Poláček 2018). It attained a degree of urbanization unprecedented in the region, with a high concentration of ecclesiastical buildings. The settlement complex consisted of a fortified acropolis or “castle” and a bailey, surrounded by unfortified suburbs. Ongoing archaeological research begun in the 1950s has uncovered more 2500 graves both in the suburbs and at the acropolis, including presumably dynastic graves in the interior of the main churches as well as a number of richly equipped graves, suggesting the presence of the true elites of Great Moravian society (Poláček 2008). The adult and peripherally the subadult diets of the Mikulčice population have previously been explored (Halffman and Velemínský 2015, Jílková et al. 2019, Kaupová et al. 2018), providing a good framework for comparison with current results and documenting the notable input of millet in the Great Moravian diet.

The dataset included 46 adults and 32 subadults, of which 25 died during M1 formation, while 7 died after the completion of the M1 root. There were 41 males and 37 females. In terms of socio-economic status, 38 individuals were classified as elite, the rest (n = 40) as non-elite. The age-at death distribution of the adult dataset was affected by dental wear, so individuals younger than 40 years were strongly prevalent. In adults, sex estimation was based primarily on the morphology (Brůžek 2002, Phenice 1969) and metrics of the innominate bones (Brůžek et al. 2017, Murail et al. 2005). Where these were absent or poorly present, an evaluation of the morphological traits of the skull was used (Walker 2008). Age-at death estimation was based on the evaluation of senescent changes of the auricular surface (Schmitt 2005), pubic symphysis (Schmitt 2008) and the acetabulum (Calce 2012). In young adults, indicators of skeletal maturation of iliac crests and clavicles were used (Scheuer et al. 2008). For subadults, sex was estimated with the help of sex chromosome-linked isoforms of the peptide amelogenin from human tooth enamel. The modification of the method by Stewart et al. (2017) was used, employing a minimally destructive acid etching procedure and subsequent nano liquid chromatography tandem mass spectrometry. Further details of the method and the sex estimates for the subadults can be found in the Online Resource 1. The reliable sex estimation in the subadults, still not common in bio-archaeological practice, greatly increases the testimonial power of the subadult skeletons and helps to avoid the interpretative biases resulting from unknown sex ratios in particular age classes or socio-economic groups. Age-at-death for subadults was assessed using dental development (Smith 1991).

The character of grave goods was used for the categorization of socio-economic status. Elites were considered to be individuals from well-equipped graves containing gold, luxury jewellery and textiles, belt strap-ends or cutlery, gilded buttons, metal weapons, spurs, or iron coffin accessories. Individuals from graves with objects of daily use, such as knives, ceramics, glass buttons or beads, simple jewellery or from graves lacking any grave goods, were considered to be non-elite.

The criterion for inclusion into the dataset was the preservation of at least one first permanent molar (M1) and a low degree of dental wear, admitting max. stage E according to Lovejoy (1985). Samples for stable isotope analysis were taken preferentially from the lower M1, but in cases of absence, damage (e.g. by dental caries) or notable dental wear, the upper M1 was sampled. After removing adhered dirt, the M1 was halved along the mesio-distal (lower M1) or vestibulo-palatinal (upper M1) axis, with half of the tooth preserved for further analyses. Enamel was removed from one half of the M1 using dental burs and saws. Collagen was extracted using Method 2 described by Beaumont et al. (2013) in the modification by van der Haas et al. (2018). The demineralized dentine was sectioned into 10 horizontal sections (in the case of fully formed teeth) reflecting dental developmental stages (Smith 1991). As far as possible the calculations of the approximate ages for each section considered the distinct rate of

dentine secretion at various stages of tooth development, being calculated separately for each particular tooth segment (Czermak et al. 2020): the crown (Cri-Crc, divided into 5 slices), the superior half of the root (Crc-R1/2, divided into 2 slices), the inferior half of the root (R1/2-A1/2, divided into 2 slices) and the closing apex (A1/2-Ac, one slice). According to the Smith (1991) developmental scheme, the M1 crown forms approximately from birth, with the age at crown completion being  $2.2 \pm 0.5$  years. The fully formed tooth crowns were cut in 5 horizontal increments, each representing 1/5th of 2.2 years. Analogously, calculations were made for the M1 root.

All the sample preparations were carried out at the Department of Anthropology, National Museum, Prague, CZ. EA-IMRS (Elemental Analysis – Isotope Ratio Mass Spectrometry) was performed at Iso-Analytical, Crewe, UK. Stable carbon and nitrogen isotopic compositions were calibrated relative to the VPDB and AIR scales using IAEA-CH-6 and IAEA-N-1 inter-laboratory comparison standards. Measurement uncertainty was monitored using in-house standards: IA-R068 (soy protein,  $\delta^{13}\text{C}_{\text{VPDB}} = -25.22$  ‰,  $\delta^{15}\text{N}_{\text{AIR}} = 0.99$  ‰), IA-R038 (L-alanine,  $\delta^{13}\text{C}_{\text{VPDB}} = -24.99$  ‰,  $\delta^{15}\text{N}_{\text{AIR}} = -0.65$  ‰), IA-R069 (tuna protein,  $\delta^{13}\text{C}_{\text{VPDB}} = -18.88$  ‰,  $\delta^{15}\text{N}_{\text{AIR}} = 11.60$  ‰) and a mixture of IAEA-C7 (oxalic acid,  $\delta^{13}\text{C}_{\text{VPDB}} = -14.48$  ‰) and IA-R046 (ammonium sulphate,  $\delta^{15}\text{N}_{\text{AIR}} = 22.04$  ‰). Precision was determined to be  $\pm 0.14$  ‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values based on repeated measurements of calibration standards, check standards, and sample replicates. Accuracy or systematic error was determined to be  $\pm 0.07$  for  $\delta^{13}\text{C}$  and  $\pm 0.11$  for  $\delta^{15}\text{N}$  values based on the difference between the observed and known  $\delta$  values of the check standards and the long-term standard deviations of these check standards. The total analytical uncertainty as defined by Szpak et al. (2017) was estimated to be  $\pm 0.16$  ‰ for  $\delta^{13}\text{C}$  values and  $\pm 0.18$  for  $\delta^{15}\text{N}$  values.

To describe the carbon and nitrogen isotopic profiles with respect to dietary and physiological changes, several parameters were chosen: for  $\delta^{15}\text{N}$  values, we noted the presence of the initial peak in isotopic values as primary evidence for breastfeeding. We calculated the maximal isotopic offset ( $\Delta^{15}\text{N}_{\text{max}}$ ) to describe the height of this peak and noted the ages at i) the first notable decrease from the peak value and ii) the smoothing of the isotopic profile to describe its extension (Figure 2A). In both cases  $0.4$  ‰ (a double of the analytical error at two standard deviations) was considered to be a significant isotopic change. For  $\delta^{13}\text{C}$  values, we also calculated the maximal isotopic offset ( $\Delta^{13}\text{C}_{\text{max}}$ ). We then evaluated the shape of the carbon isotopic profile in relation to that of nitrogen during the initial peak described above. Four basic types of carbon isotopic profiles were defined: i: positive covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, ii: negative covariance when  $\delta^{13}\text{C}$  values are low during the time of peaking  $\delta^{15}\text{N}$  and subsequently increase along with decreasing  $\delta^{15}\text{N}$  values, iii: Flat carbon profile, and finally iv: positive covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in most of the slices, but with an initial decrease in  $\delta^{13}\text{C}$  values preceding the shift in  $\delta^{15}\text{N}$  values (Figure 2B).

To describe the isotopic values after the period of breastfeeding, we calculated the average post-weaning isotopic value (i.e. the average value from all the slices developed after smoothing the nitrogen isotopic curve as described above). This was to describe childhood diet in the period following weaning, and to compare it with previously published dietary info from later life periods (Jílková et al. 2019, Kaupová et al. 2018). For each individual, we also searched for two potential patterns described in previous isotopic studies of dentine incremental profiles: i) the presence of the so-called 'post-weaning dip' in nitrogen isotopic values, which is usually linked to the special character of the post-weaning diet, with low input from animal products (Tsutaya and Yoneda 2015), and ii) cases of notable shifts in both carbon and nitrogen isotopic values showing a pattern of a negative covariance, usually attributed to biological stress (Beaumont and Montgomery 2016).

Statistical analyses were examined in R software version 3.3.3 (R Core Team 2017). The effect of age, sex, and socio-economic status on the values of nitrogen and carbon post-weaning averages, as well as

age at first decrease and age at final smoothing, were modelled using linear models and tested with ANOVA. The values of age at first decrease and age at final smoothing, which were classified into 3 and 6 levels respectively (i.e. an ordinal scale), were treated as numerical variables in the analyses, because there were relatively high numbers of levels with only a few observations per level.

As maximal offset of nitrogen, maximal offset of carbon, age of weaning, and age at first decrease correlated with each other (with a maximal correlation coefficient of 0.31 between the the maximal offset of nitrogen and maximal offset of carbon), the joint effect of age, sex, survival, and socio-economic status was modeled with RDA analysis using the library "vegan" (Oksanen et al. 2015). The effect of the predictors was tested with an ANOVA-like permutation test with the function "anova.cca". The incidence of distinct carbon isotopic profiles in particular age-groups was compared by Fisher exact test.

### 3. Results

Complete isotopic results are given in full in the Online Resource 2. Several increments (N = 6) did not yield enough collagen for analysis, this concerns exclusively apical sections including only a small portion of dentine from dentine horns. All the analysed samples met the criteria for good collagen preservation. Individuals analysed in this study exhibit highly varied  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  profiles, which are shown for each individual in the Online Resource 2.

#### Nitrogen isotopic profiles

Almost all the individuals show some sort of decrease in  $\delta^{15}\text{N}$  values during the earliest life period, which could be viewed as a reflection of breastfeeding and subsequent weaning. However, notable variation exists in both the timing and magnitude of these shifts. Based on the study by Fuller et al. (2006), an isotopic offset of 2-3 ‰ could be seen as primary evidence of weaning from full breastfeeding under healthy circumstances. Shifts beyond these margins (in both directions) suggest the combined impact of the trophic level effect and biological stress, and/or the other factors listed above. In this study, the average  $\Delta^{15}\text{N}_{\text{max}}$  was slightly higher than the recommended range ( $3.1 \pm 0.8$  ‰). In 37 (of the 78) individuals,  $\Delta^{15}\text{N}_{\text{max}}$  was above 3 ‰, with a maximum of 5.1 ‰. In nine individuals,  $\Delta^{15}\text{N}_{\text{max}}$  was below 2 ‰ with six of them not showing a typical isotopic curve shape attributable to weaning: five individuals, (Graves 112-VI, 462, 673, 1058, 1171) show very low  $\delta^{15}\text{N}$  in the earliest dentine slice following by a delayed peak in  $\delta^{15}\text{N}$  (Figure 3). No. 207 shows a specific nitrogen isotopic profile with low  $\delta^{15}\text{N}$  in the earliest dentine slice followed by the two subsequent peaks in  $\delta^{15}\text{N}$  of smaller extent (Figure 3).

When comparing the  $\Delta^{15}\text{N}$  between the defined population subgroups, there were no statistically significant differences between age classes, sexes or socio-economic groups (Table 1, Figure 4). In the case of age, the result was close to the 0.05 % level of significance, with non-survivors showing surprisingly lower nitrogen isotopic offsets than older individuals.

Concerning the start and end points of the above described isotopic shift attributable to weaning, in most of the individuals (n = 37)  $\delta^{15}\text{N}$  values decreased significantly in the second slice, representing isotopic values at the age of 5-11 months. In 30 individuals the first decrease was observed in the third slice (representing isotopic values from approx. 11-16 months), while in five individuals,  $\delta^{15}\text{N}$  values did not decrease before 16-21 months (4th slice). In detailed comparison, females showed the first

isotopic decrease at a later age than males (Table 1). Age-at-death and socio-economic status had no statistically significant relationship to this parameter (Figure 4).

**Table 1.** Relationship between defined parameters of both carbon and nitrogen isotopic curve and the explicative variables (p – values<sup>a</sup>)

	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$	
	$\Delta^{15}\text{N}_{\text{max}}$	Age at first decrease	Age at final smoothing	Post-weaning average	Type of the profile	Post-weaning average
Age	0.064	0.897	0.007	0.222	0.008	0.096
Sex	0.835	0.008	0.411	0.038	x	0.097
Socio-economic status	0.617	0.550	0.030	<0.001	x	0.058

<sup>a</sup> results significant at 0.05 level are in bold

The age at the final smoothing of the isotopic curve showed much higher variation, with the youngest individuals (n = 7) being 11-16 months old (slice 3). In the majority of individuals the final smoothing was relatively uniformly dispersed between the age classes of 16-21 months (slice 4, n = 20), 21 months to 2.2 years (slice 5, n = 25) and 2.2-3.7 years (slice 6, n = 18). Finally, in two individuals the final smoothing occurred as late as at 3.7-5.3 years. Surprisingly, individuals, who died during the first decade of life showed an earlier age at final smoothing than older individuals (Table 1). Also, in elites, the final smoothing occurred on average earlier than in non-elites. The sex of the individual had no important impact at this parameter (Figure 4).

According to the RDA analysis, which included all four factors (maximal nitrogen and carbon offsets, age at first decrease, age at final smoothing), the RDA1 axis shows that adults and males display a higher maximal offset, but non-survivors a higher age at first decrease. RDA1 however explains only 7 % of the variability and this result is not statistically significant (ANOVA-like permutation test, F = 1.45, p = 0.848).

Carbon isotopic profile and the covariance between carbon and nitrogen isotopic values

The maximal carbon isotopic offset ( $\Delta^{13}\text{C}_{\text{max}}$ ) ranged between 0.3 and 3.6 ‰, with an average value of  $1.6 \pm 0.8$  ‰, which is substantially higher than the trophic level effect of exclusive breastfeeding (Fuller et al. 2006).

Concerning the shape of the carbon isotopic profile and the relationship to nitrogen isotopic values, most of individuals (n = 43) follow the pattern of positive covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values at least in the initial portion of the isotopic profile, i.e. in the period of supposed breastfeeding and weaning (Profile i). Instances where carbon and nitrogen isotope values negatively covary during this period, with decreasing  $\delta^{15}\text{N}$  values along with increasing  $\delta^{13}\text{C}$  values, are relatively scarce (n = 7, Profile ii). A flat carbon profile (Profile iii) occurs in nine cases. Lastly, profile iv, where carbon and nitrogen isotopic values covary positively for most of the time, but the decrease in  $\delta^{13}\text{C}$  values precedes the decrease in  $\delta^{15}\text{N}$  values, occurs in eleven cases. There are eight cases (graves nos. 112/VI,

207, 182, 462, 625, 673, 727, 1171) in which the carbon isotopic profile does not correspond with any of the types described above. In 112/VI and 673 carbon isotopic values covary positively with  $\delta^{15}\text{N}$ , but do not follow the typical weaning scenario (see Figure 3). In 462 and 1171 atypical carbon profiles occur along with atypical nitrogen profiles, but neither positive nor negative covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values is present. In 182, 625 and 727, unexpected shifts in  $\delta^{13}\text{C}$  values occur along the nitrogen isotopic peak typical for breastfeeding. Finally, in individual 207 carbon isotopic values covary negatively with nitrogen isotopic values during both episodes of increased  $\delta^{15}\text{N}$  values (Figure 3).

The type of the carbon isotopic curve differed significantly between individuals, who died before M1 completion and others, with a flat carbon isotopic profile being more common in non-survivors (seven of the nine cases). It was not possible to assess the potential relation to sex or socio-economic status or to include this variable in the multifactorial analysis due to the dominance of profile I, and the subsequently low number of cases in some categories.

#### Post-weaning isotopic values

The average nitrogen isotopic values from all the slices developed after the smoothing of the nitrogen isotopic curve ranged between 9.1 and 12.5‰ with a mean of  $11.2 \pm 0.8$  ‰. The average carbon isotopic values ranged between  $-19.1$  and  $-16.3$  ‰ with a mean of  $-17.6 \pm 0.6$  ‰. Seven individuals died too early to identify the point of the final smoothing of the nitrogen isotopic curve and thus were not included in this analysis. Among adults ( $n = 44$ ) we were able to compare these "post-weaning averages" with previously published adult isotopic values from bone collagen (Jílková et al. 2019, Kaupová et al. 2018). Childhood nitrogen isotopic values were similar to those from adulthood (mean =  $11.0 \pm 0.9$  ‰,  $p = 0.297$ ), while carbon isotopic values in dentine samples were significantly higher than those from bone (mean =  $-18.0 \pm 0.5$  ‰,  $p < 0.001$ , Figure 5). In the case of nitrogen, post-weaning averages differed significantly between socio-economic classes (Table 1, Figure 5), with elites showing higher  $\delta^{15}\text{N}$  values (mean =  $11.5 \pm 0.6$  ‰) than non-elites (mean =  $10.9 \pm 0.8$  ‰). Sex too appeared to have a significant impact on  $\delta^{15}\text{N}$  with males (mean =  $11.4 \pm 0.7$  ‰) showing higher  $\delta^{15}\text{N}$  values than females (mean =  $11.0 \pm 0.9$  ‰). Multifactorial analysis, however, suggests that this probably results from the distinct distribution of both sexes into socio-economic classes (ANOVA,  $p = 0.001$  for socio-economic status and 0.139 for sex). Age-at-death had no significant impact on post-weaning  $\delta^{15}\text{N}$ . None of the studied factors show a significant impact on post-weaning carbon isotopic values (Table 1).

Episodes of negative covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values during the post-weaning period were observed in twelve cases, including two of the evaluated 18 non-survivors. As stated above, the seven individuals who died too early to identify the exact end point of the isotopic shift associated with weaning were not included in these counts.

Fisher exact test showed no significant difference in the incidence of negative covariance between those who died during M1 formation and others ( $p = 0.490$ ). The number of cases with opposing covariance was too low to effectuate any deeper statistical comparison concerning differences between sexes or socio-economic classes.

In a number of individuals, however, we observed a mild increase in  $\delta^{15}\text{N}$  in later childhood following the period of depletion in  $\delta^{15}\text{N}$  during the post-weaning period. The presence of this event, known as the "post-weaning dip" (Tsutaya and Yoneda 2015) was evaluated only in individuals who died after M1 completion, thus presenting a complete isotopic profile. A post-weaning dip was observed in 25 of the 46 individuals. There were no differences in the incidence of post-weaning dip between sexes (Fisher exact test,  $p = 0.758$ ) or between socio-economic groups ( $p = 0.773$ ).

#### 4. Discussions

Nitrogen isotopic values during the period of supposed breastfeeding

In this study, we have considered three potential indicators of physiological stress. Firstly, a peak in nitrogen isotopic values during the period of infancy higher than 3 ‰ (Fuller et al. 2006) may suggest physiological stress during full breastfeeding or around the introduction of the first dietary supplements (which form a relatively small proportion of the diet and are thus still isotopically invisible). This pattern was present in 37 individuals (47 %). This percentage is relatively high, taking into account the known information on the background levels of stress acting on the Mikulčice subadult population. In general, the expansion of Great Moravia corresponded to a favourable era of mild and stable weather, which, together with other favourable natural conditions in South Moravia, enabled high productivity on cultivated land and thus enabled strong population growth (Hladík 2020). On the other hand, Mikulčice's inhabitants surely had to face the negative aspects of urbanization, such as poor sanitation, parasitic infestations, an elevated risk of infection, or dependency on food supplies from the hinterland (Walter and DeWitte 2017). However, the degree of urbanization never exceeded a relatively extensive, proto-urban formation. Thus, though life in a newly-established centre clearly brought problems and challenges previously unknown in a rural and tribal community, the level of stress most probably did not reach that observed in the purely urban formations of the High Middle Ages. This is supported by the results of osteological analysis, according to which the prevalence of non-specific stress indicators in Mikulčice infants and young children was comparable to those from the rural hinterland (Kaupová et al. 2014).

Traditionally, the first introduction of dietary supplementation is viewed as a highly risky period in respect to infection, as the child's immune system encounters a range of new pathogens (Lamberti et al. 2011). It has however been proven that the immunological buffering of breastfeeding still acts during complementary feeding (Kendall et al. 2021).

Moreover, isotopic data from later childhood suggest that the observed pattern could be augmented by the character of post-weaning food. As a "post-weaning dip" in nitrogen isotopic values was observed in more than half of the individuals, the lower proportion of animal products consumed during early childhood may boost the  $\Delta^{15}\text{N}_{\text{max}}$  of these individuals above the 3 ‰ margin.

Secondly, the delayed starting point of the isotopic decrease in  $\delta^{15}\text{N}$  values could suggest biological stress during the early phase of weaning. This parameter, however, has a less precisely defined range than the previous one. According to current medical recommendations, an insufficiency of breastmilk to meet an infant's dietary needs in terms of both micronutrients (e.g. iron) and calories may threaten after just six months of age (Fewtrell et al. 2007, Pérez-Escamilla et al. 2019). Although the portions of supplementary foods could be negligible at the beginning, it is hardly believable that a child could prosper under a regime of exclusive breastfeeding after 1 year of age. In our sample, such a delayed start of the decline in nitrogen isotopic values, with the first decrease observed at 1.3-1.8 years, was present in five individuals (6 %).

Finally, physiological stress in the later phases of weaning would be expressed by a slower decline and the delayed final smoothing of the nitrogen isotopic profile. However, due to the extreme variation reported in the duration of breastfeeding, which could be over 6 years (Dettwyler 2004, Fildes 2017,

Piovanetti 2001), it is not possible to identify a point on the nitrogen isotopic curve (before the final smoothing) beyond which isotopic enrichment cannot be due to partial breastfeeding.

Looking at the data from a dietary point of view, the first decrease in  $\delta^{15}\text{N}$  values was observed in most of the individuals at approx. 5-10 months. This suggests a practice of weaning roughly in accordance with current medical recommendations (WHO 2009), implying exclusive breastfeeding for 6 months. Occasionally, infants could have received a notable amount of dietary supplements even earlier, as suggested by the presence of individuals with maximal nitrogen isotopic offset lower than 2 ‰ ( $n = 3$ ), in these cases, however, other explanations such as change in maternal diet or the use of a wet nurse, cannot be excluded. Due to the competing impact of physiological stress, the detection of the latest age for the first dietary supplements is impossible, and won't be attempted in this paper.

The earliest observed age-at-smoothing of the nitrogen isotopic curve suggests that the first children could well have been fully weaned before 11 months, which is much earlier than the current medical recommendation of partial breastfeeding for 2 years (WHO 2009). This was observed in seven individuals, which is a minor but not negligible proportion of the population. Even if our estimates on age-at-complete weaning are considered to be rather maximalistic (as the presence of biological stress could mimic longer breastfeeding), all except two individuals appeared to have been weaned before 2.2 years. This appears to be relatively early in the perspective of historical populations (Dettwyler 2004, Fildes 2017, Thorvaldsen 2008), but it must be kept in mind that minor supplements of breast milk below 10% of the dietary input would be unobservable isotopically (King et al. 2018). It should also be mentioned here that, due to the chosen sampling strategy, the sixth slice corresponds to a relatively long period between 2.2 and 3.75 years. This could further help to blur the potential minor consumption of breastmilk for some time after 2.2 years.

Finally, even with the incremental sampling methods, time averaging inevitably occurs as multiple incremental boundaries are crossed (Czermak et al. 2020). Also, a new dietary source may be introduced well in advance of a detectable isotopic shift. Further, there is some intra-individual variation in teeth development (e.g. Smith 1991). This means that the ages noted in this study as the periods of notable dietary/or physiological change are only rough estimates and should be used rather for comparative purposes.

At the individual level, six cases showed a nitrogen isotopic pattern not compatible with the breastfeeding and weaning scenario. All of these showed low nitrogen isotopic values in the first slice, which can be interpreted as a failure of breastfeeding. However, these originally low  $\delta^{15}\text{N}$  values are in all cases followed by one or two subsequent peaks in  $\delta^{15}\text{N}$  values extending between 0.6 and 3 ‰, which are difficult to interpret. Episodes of physiological stress had to be extremely common in the case of bottle-fed babies consuming non-sterilized food. Neither experimental studies focused on the effect of physiological stress (D'Ortenzio et al. 2015, Drtikolová Kaupová et al. 2021, Fuller et al. 2005, Katzenberg and Lovell 1999, Mekota et al. 2006) nor observations of subadult famine victims (Beaumont and Montgomery 2016), report isotopic shifts associated with malnutrition and/or disease higher than 2 ‰, but four of these six cases show a nitrogen isotopic peak ranging between 2-3 ‰ (see Figure 3, for example). Although an isotopic shift of this extent could well correspond to exclusive breastfeeding (Fuller et al. 2006), this explanation is highly improbable, as the first slice provides the dietary information from approx. 5 months of life. Though there could be variation in the growth pattern of the teeth (Smith 1991), it is extremely unlikely, that an infant could retain the ability to effectively suckle after several weeks (or more probably months) of artificial feeding. This suggests the role of some unknown factor (or a combination of several factors) affecting the isotopic values of these

children in early childhood. In one case only (No. 207, age-at-death = 8-10 years, Figure 3) the extent of the nitrogen isotopic shifts (together with the presence of negative covariance with carbon isotopic values) offers a typical image of repeated episodes of physiological stress (Beaumont and Montgomery 2016, Craig-Atkins et al. 2018), which however, was the child able to overcome. This, as well as the age of two of the afore-mentioned individuals (112-VI and 673), who survived till 40-50, and 20-30 years respectively, contradicts the general view that in the past there was no choice other than breastfeeding to ensure the survival of the child (Dettwyler 2004).

#### Intra-population comparison of early life $\delta^{15}\text{N}$

An intra-population comparison of early life experiences does not suggest that individuals who died during the first decade of life experienced a greater level of environmental stress during early childhood. None of the three parameters listed above was higher in non-survivors. In fact, the age at complete smoothing of the nitrogen isotopic curve was lower in non-survivors, as was the maximal nitrogen isotopic offset (though here, the difference was on the borderline of statistical significance). As lower  $\Delta^{15}\text{N}_{\text{max}}$  could result from an earlier start to the weaning process (before 5 months), the observed pattern may theoretically demonstrate lower parental investment (Quinlan 2007), impacting the life expectancy of a child.

The delayed onset of the nitrogen isotopic decrease observed in females in comparison to males is probably caused by the higher prevalence of individuals with a first decrease observed as late as in the fourth slice, with four of the five cases being female. As noted above, such a pattern is probably linked with physiological stress, but considering the low number of cases, it is unclear whether this can be seen as a reflection of a higher level of stress during the weaning process, imposed systematically on female children e.g. by the lower quality of complementary food.

The earlier occurrence of final smoothing observed in elites in comparison to non-elites has at least two equally valid explanations: the first relates to the higher incidence of stress during the weaning process in non-elites, the second to the earlier weaning of elite children. The latter may well be linked to the ongoing Christianization of the Great Moravian population. Although there is no direct written testimony from the Great Moravian context, certain rules concerning family life and childcare probably existed (Thorvaldsen 2008): as a unique example, the 9th century document "The Responses of Pope Nicholas I to the Questions of the Bulgars" recommends sexual abstinence during the entire period of breastfeeding (Bartoňková et al. 1971). As the pressure to abide by Christian rules was probably higher in elite groups, this could have led at least some elite women in Mikulčice to shorten the breastfeeding period. To estimate the strength of this argument, however, is beyond the scope of this paper.

In any case, as RDA analysis including all four explicative factors gave non-significant results, explaining only a small portion of the data variation, the results of uni-factorial analysis suggesting a relationship between some parameters and age-at-death, sex or social status must be considered with caution.

#### Carbon isotopic values during the period of supposed breastfeeding

The presence of negative covariance, usually regarded as an indication of physiological stress, in the initial part of the isotopic profile was relatively rare in this dataset, being observed in only seven cases. This however does not necessarily mean that the level of physiological stress during infancy and early childhood was low. As stated above, the results of experimental studies suggest that the isotopic changes in a carbon body pool in relation to physiological stress may vary (Canterbury et al. 2020, D'Ortenzio et al. 2015, Drtíková Kaupová et al. 2021, Fuller et al. 2005, Neuberger et al. 2013). This inconsistency can theoretically be caused by intra-individual differences in the amount of body fat and the phase of malnutrition. At first, a decrease in  $\delta^{13}\text{C}$  values may reflect the use of carbon from energy

sources to synthesize non-essential amino acids, but later body protein catabolism may predominate and cause an increase in  $\delta^{13}\text{C}$  values (Drtikolová Kaupová et al. in press, King et al. 2018, Salesse et al. 2019). Moreover, even if we accept negative covariance between carbon and nitrogen isotopic values as being the primary response of a subadult organism to biological stress, this concept could be valid only in a purely terrestrial C3 plant-based ecosystem, where isotopically distinct food groups such as fish (either freshwater or marine) or C4 plants were not accessible in substantial quantities. This was clearly not the case for the Great Moravian population (Halffman and Velemínský 2015, Jílková et al. 2019, Kaupová et al. 2018).

In fact, taking into account both the potential roles of stress and millet consumption, it is quite surprising that most of the individuals ( $n = 43$ ) show a positive covariance between carbon and nitrogen isotopic values, as can be observed during the process of breastfeeding and weaning under healthy circumstances and with a stable and isotopically analogous diet for both mother and child. This could mean that millet played a special role in the diet of infants or potentially also their breastfeeding mothers. As will be explained in detail below, carbon isotopic values from the post-weaning period suggest, that the childhood diet included more millet than that of adults. The persistence of the carbon isotopic peak in these circumstances is thus quite surprising. If an infant was being weaned onto resources with a higher millet proportion than those in the maternal diet, we would expect a rise in  $\delta^{13}\text{C}$  values, "cancelling out" the trophic level shift due to weaning. The result would be either a flat carbon isotopic profile (as observed in nine cases in this study) or the occurrence of negative covariance mimicking the stress pattern described above (King et al. 2018). As this is not true in a majority of the sample, it means that either millet was part of a special diet preferred for some reason by lactating women, or was introduced as the very first dietary supplement. Consumed in the form of porridge (Adamson 2004), millet has the ideal consistency for first dietary supplementation, it is also easily digestible, and in comparison to other cereals has a higher nutritional value with a high content of vitamins, minerals and antioxidants (Aurelia et al. 2020, Kulp 2000, Weber and Fuller 2008). While this information was not known to Great Moravian people, the consumption of millet could have reduced the risk of both malnutrition and disease in their children during the period of complementary feeding and – from the immunological point of view – the extremely risky time of the complete cessation of breastfeeding (Kendall et al. 2021). At the same time, due to a low protein content in comparison to breastmilk, millet-based dietary supplements have the potential to impact carbon isotopic values (as carbohydrates could be partially used to build the carbon skeletons of non-essential amino-acids), while breast milk remains the main source of nitrogen (Fuller et al. 2006). Another point suggesting a special role for millet impacting the isotopic values from infancy (either directly or indirectly via maternal diet) is the extent of maximal carbon isotopic offset occurring during the supposed breastfeeding period, which in the vast majority of individuals ( $n = 55$ ) surpassed the maximum of 1 ‰ which could be attributable to the trophic level effect of breastfeeding (Fuller et al. 2006). In 16 individuals  $\Delta^{13}\text{C}_{\text{max}}$  was even higher than 2 ‰.

In cases showing profile iv, where carbon and nitrogen covary positively but the decrease in  $\delta^{13}\text{C}$  precedes the decrease in  $\delta^{15}\text{N}$  (eleven cases), as well as in eight cases not corresponding with any of the outlined scenarios, a number of equally plausible explanations may be offered and thus, we will not attempt any interpretation here.

#### Incidence of distinct carbon isotopic profiles in particular age groups

The substantially higher prevalence of flat carbon isotopic profiles observed in those who died before M1 completion has no simple explanation. The absence of breastfeeding as a factor impacting further survival would be a valid cause (Craig Atkins et al. 2018), but all the non-survivors with a flat carbon isotopic profile show a nitrogen isotopic peak within or very close to the 2-3 ‰ range observed in fully

breastfed babies (Fuller et al. 2006). As discussed above, such a high nitrogen isotopic offset is unlikely to have been caused solely by physiological stress. A dietary explanation might be the weaning of the child onto resources with a higher millet proportion than in the maternal diet. However this, like other explanations arising from dietary factors, does not explain, why this scenario was more prevalent in non-survivors.

#### Carbon and nitrogen isotopic values in the post-weaning period

The intra-individual comparison of the post-weaning isotopic averages vs bone isotopic values confirms the earlier results of Jílková et al. (2019), reporting a higher importance of millet in childhood diet. While Jílková's team focused on the later period of childhood (approx. 8-10 years as a portion of the M2 root was sampled), this study documents the importance of millet in earlier phases of life. The span of the post-weaning period actually varies between 1.3-9.4 and 3.75-9.4 years, based at the age on the final smoothing of the isotopic curve.

On the other hand, the proportion of animal products consumed by individuals included in this study was stable throughout life, opposing the results from Jílková et al. (2019), who observed a lower proportion of animal products in the subadult diet of individuals buried around Mikulčice 6th church. It needs to be stated, however, that both studies focused on different population subgroups buried within the Mikulčice settlement agglomeration. While the 6th church is located in the suburb of Mikulčice, this study includes mainly individuals buried at the fortified Acropolis i.e. within a supposed residential area of the highest elites of Great Moravian society (Poláček 2008). Thus, the combined results of the two studies suggest that while the higher input of millet in a child's diet was a widespread practice among Mikulčice inhabitants, the access of children to animal products may have differed in particular parts of Mikulčice.

#### Intra-population variation in post-weaning isotopic values

The absence of any systemic differences in post-weaning averages between non-survivors vs individuals who died after M1 completion suggests that the quality of the post-weaning diet did not impact the chance of survival, and that there was no higher incidence of long-term physiological stress in non-survivors. However, it must be noted that severe and long-lasting physiological stress would have had to act to significantly impact the post-weaning values, averaging the isotopic signal from several years. The distinct nitrogen isotopic values of elite vs non-elite graves attest that the socio-economic differences in diet documented isotopically in later life periods (Jílková et al. 2019, Kaupová et al. 2018) were already present during childhood.

Exploring the latter part of the isotopic profile in greater detail, the episodes of negative covariance, generally viewed as evidence of physiological stress, had no higher prevalence in non-survivors. Cases of the so called "post-weaning dip", i.e. the notable increase in  $\delta^{15}\text{N}$  in later childhood, which is commonly observed in agricultural populations and is generally viewed as testimony to the lower input of animal products in the period immediately following weaning (Tsutaya and Yoneda 2015), were much more common than episodes of opposing covariance (in more than half of cases). While this could also be viewed as a reflection of anabolic activity linked to mid-growth spurt (Kendall et al. 2021), but the knowledge on the role of growth velocity is still limited, with current experimental studies not providing convincing evidence of the assertion of this factor on nitrogen isotopic values (Reitsema and Muir 2015, Waters-Rist and Katzenberg 2010). Further, the presence of the "post-weaning" dip appeared to be linked to the subsistence strategy (Tsutaya and Yoneda 2015). In a Romano-British population sample, for which we have written records on the perception of childhood, the timing of the dip coincides with the change in social status of children (Cocozza et al. 2021). Both of these findings further enhance the first explanation.

And finally, as emerges from the above discussion on the testimonial power of carbon isotopic values in respect to stress, it appears evident that neither can the presence of physiological stress be excluded in these individuals.

#### Limitations of the study

The interpretative difficulties due to equifinality (King et al. 2018), unavoidable by current methods, have been repeatedly stressed over the course of the discussion, so need no recapitulation here.

As a much more important point, we would like to stress the limited knowledge on the action of physiological stress in the subadult organism. Most of the experimental studies on physiological stress carried out on humans concern adult individuals only (D'Ortenzio et al. 2015, Drtikolová Kaupová et al. 2021, Fuller et al. 2005, Katzenberg and Lovell 1999, Mekota et al. 2006, Tea et al. 2021). The only exception is the work by Beaumont and Montgomery (2016), using incremental sampling of dentine tissue from developing teeth, this study is however based on an archaeological population, albeit from the well-documented context of a 19th century famine event. Moreover, it has to be kept in mind that the isotopic reflection of famine, where starvation is the primary cause of death (though it surely acted in synergy with a number of diseases, e.g. Solomons 2007), may well differ from the less extreme consequences, where the pathophysiological origin of tissue catabolism may prevail.

Moreover, the limited number of experimental studies based on subadult individuals from different animal species suggest that a meaningful level of nutritional stress need not necessarily induce an isotopic response in either  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  (Ambrose 2002, Kempster et al. 2007, Williams et al. 2007). This could be due to the potentially competing influence of negative and positive nitrogen balance (Waters-Rist and Katzenberg 2010). Also, a reduction in growth, rather than tissue catabolism, may be the primary response of subadults to dietary stress (Ambrose 2002). Thus, more experimental studies on subadult organisms are greatly needed to confirm that the pattern of negative covariance between nitrogen and carbon isotopic values is the real response of subadult body to physiological stress.

## 5. Conclusion

Although the current data – mainly maximal nitrogen isotopic offsets commonly increased over 3‰ – suggest that physiological stress might have been relatively common during early childhood, there are no indications that the occurrence of such physiological stress in this life phase affected life expectancy in the long term, or that the stress level in early life differed substantially between sexes or socio-economic classes.

The isotopic data also suggest that in the majority of the studied sample, a notable proportion of supplementary food was introduced between 5–10 months, i.e. roughly in the interval recommended by current medical advice. The first children in our dataset were fully weaned before 10 months of age. The latest age at the final smoothing of the nitrogen isotopic curve suggesting weaning was as late as 3.75–5.3 years, but this was observed in 2 individuals only. All the other individuals showed final smoothing of the nitrogen isotopic curve at 2.2–3.75 years. With respect to age-at-weaning, this has to be viewed rather as the upper range of the possible estimate, as the impact of physiological stress cannot be excluded in these seemingly late weaned children. Also, it has to be kept in mind that minor supplementation by breastmilk would not be observable by isotopic analysis. Six individuals do not show the nitrogen isotopic shift attributable to breastfeeding at all.

Carbon isotopic values from infancy and early childhood suggest that millet probably had an important role at the very beginning of the weaning process, and/or in the diet of lactating women. The isotopic data from later childhood, together with the data from a previous study (Jílková et al. 2019), suggest that a higher input of millet was characteristic of childhood diet during the first decade of life.

Nitrogen isotopic values from the post-weaning period suggest that though a small drop in the dietary proportion of animal products may occur in the period immediately following weaning, the average proportion of animal products consumed during childhood is comparable to that consumed in adulthood. The socio-economically motivated differences in dietary behaviour previously observed in adults (Kaupová et al. 2018) were already present during childhood.

In future, a comparison of the set of potential indicators of physiological stress listed above with other population samples with distinct documented levels of environmental stress will be carried out. Such comparisons may bring more knowledge about the level of environmental stress that the subadult population of Mikulčice had to face.

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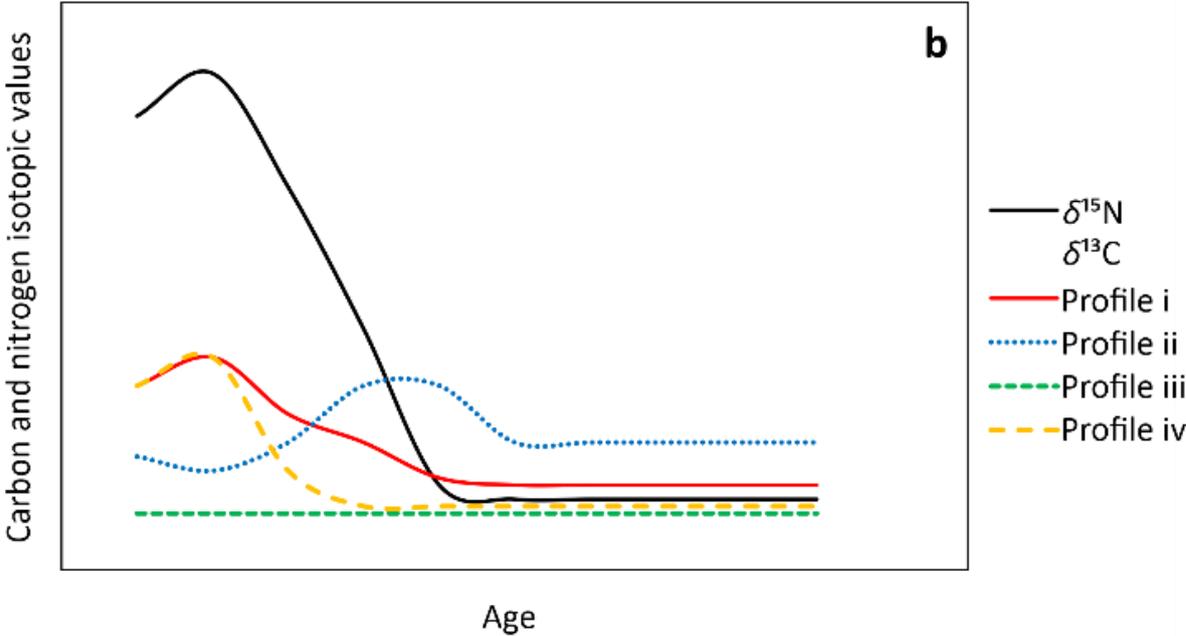
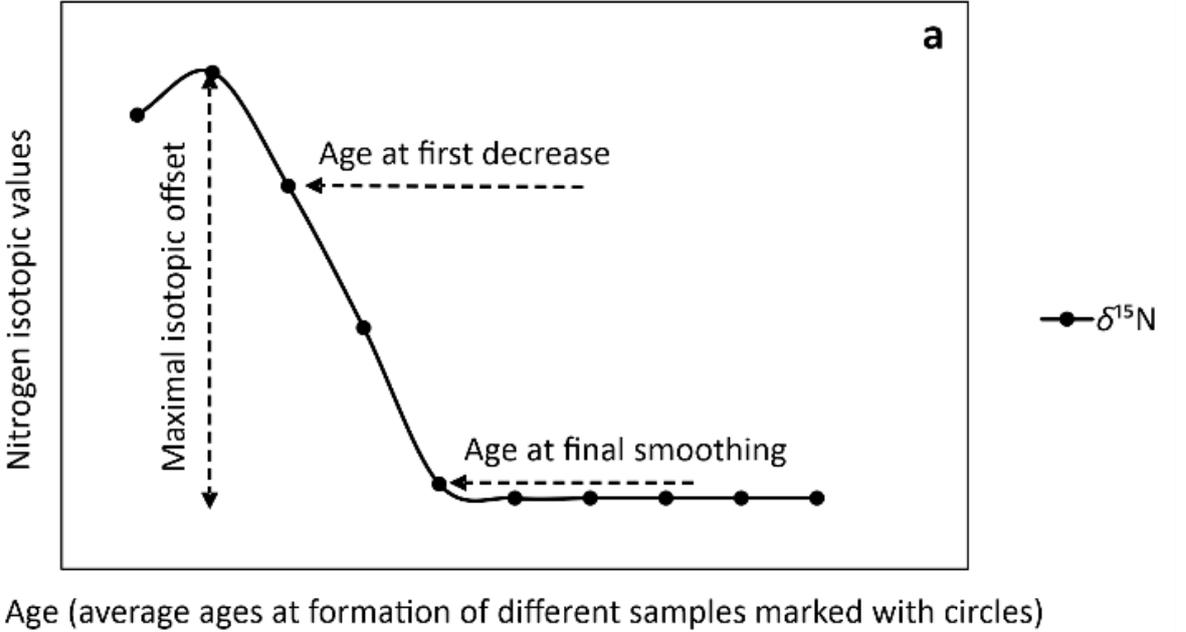
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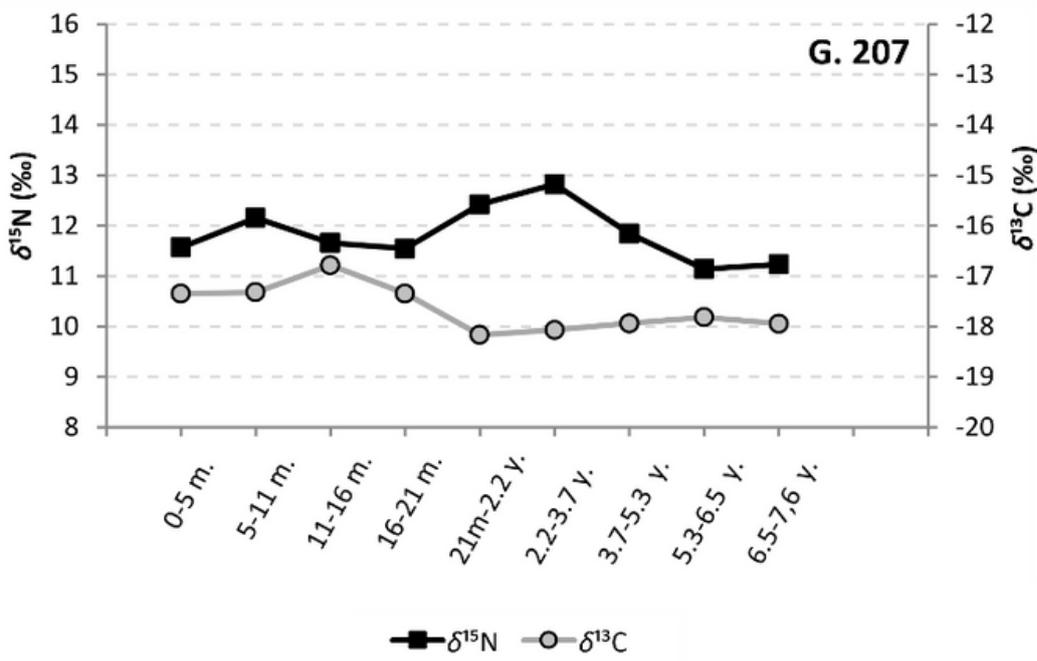
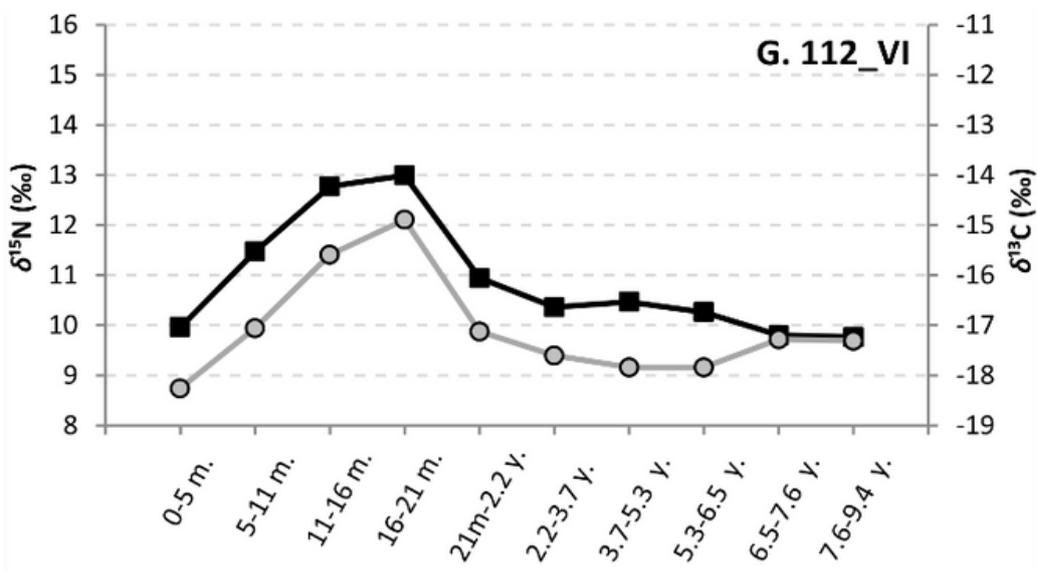
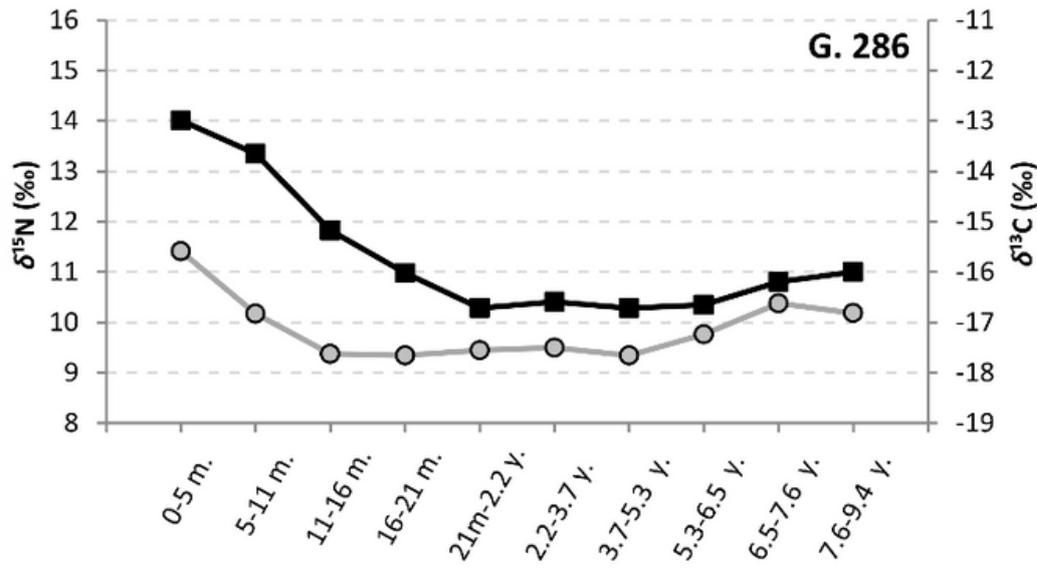
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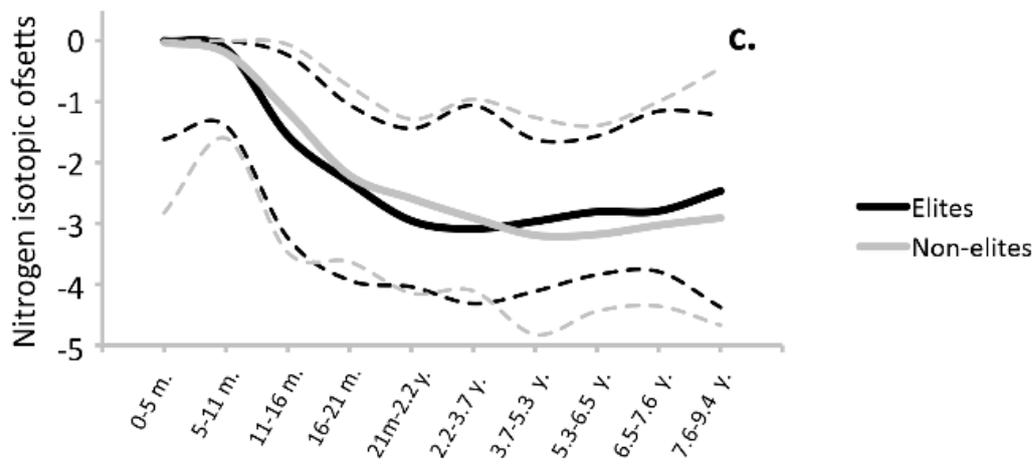
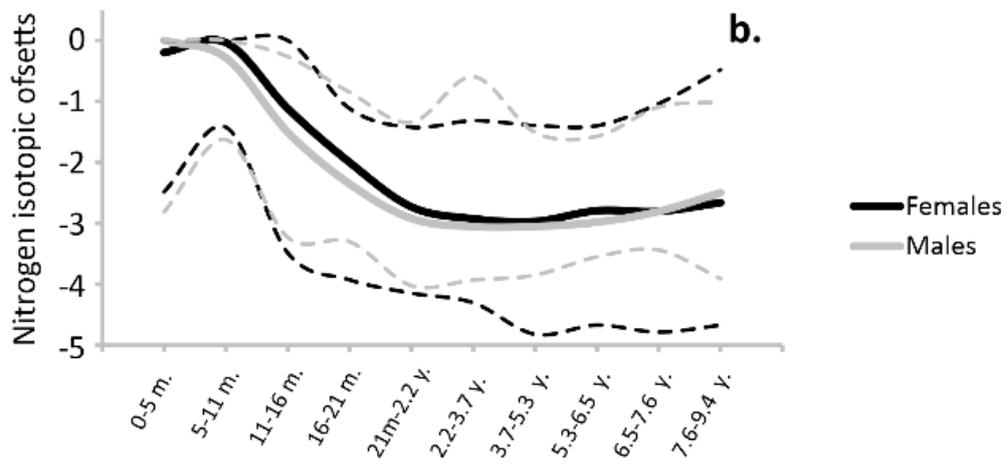
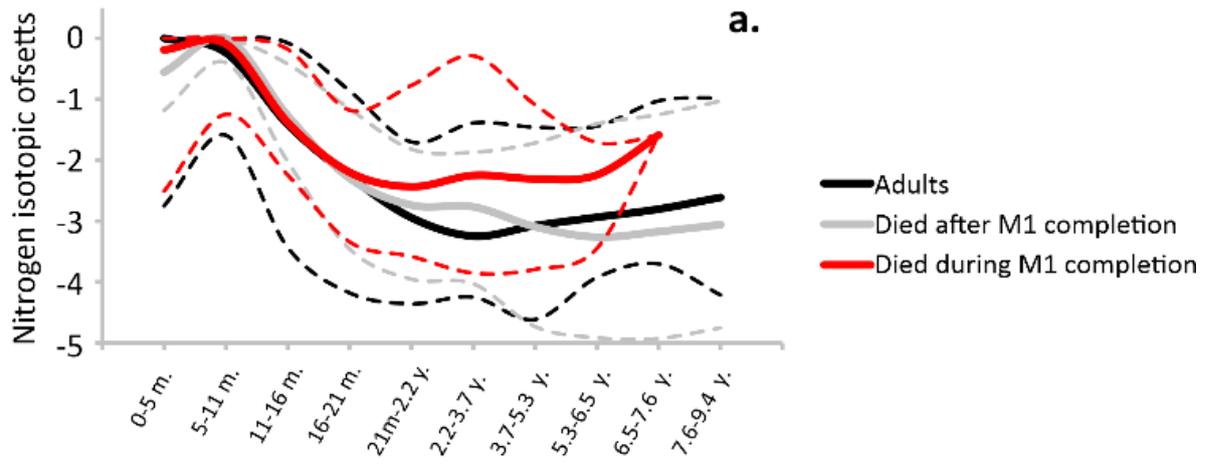
**Figure 2.** a: Theoretical weaning model with expected incremental nitrogen isotope data for an individual being weaned onto a diet similar to that of the mother. Note that 0.4 ‰ was considered a cut-off value of significant isotopic change when defining the points marked on the isotopic curve, b: four categories of incremental carbon isotope data in relation to nitrogen isotopic data present in the studied dataset: i: positive covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, ii: negative covariance when  $\delta^{13}\text{C}$  values are low during the time of peaking  $\delta^{15}\text{N}$  values and subsequently increase along with decreasing  $\delta^{15}\text{N}$  values. iii: flat carbon profile and finally iv: positive covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in most of the slices, but initial decrease in  $\delta^{13}\text{C}$  values preceding the shift in  $\delta^{15}\text{N}$  values



**Figure 3.** Examples of three individuals from the study cohort: Grave 286 shows a positive covariance between carbon and nitrogen isotopic values, typical for breastfeeding and subsequent weaning. Note the common patterns in the studied dataset: the presence of a small post-weaning dip in  $\delta^{15}\text{N}$  values and the  $\Delta^{13}\text{C}_{\text{max}}$  higher than the maximal trophic level effect of exclusive breastfeeding (Fuller et al. 2006), Grave 112\_VI shows an unexpected isotopic pattern with low nitrogen isotopic values in the first slice, suggesting a failure of breastfeeding with a subsequent rise in both carbon and nitrogen isotopic values of unknown origin, Grave 207 shows an isotopic pattern suggesting failure of breastfeeding with subsequent episodes of negative covariance between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values showing a typical picture of physiological stress as described by Beaumont and Montgomery (2016)



**Figure 4.** Typical nitrogen isotopic profiles for particular population groups (median values). To illustrate the shape of the isotopic curve while eliminating the confounding factor of underlying dietary variation among children and their lactating mothers, isotopic offsets from the highest  $\delta^{15}\text{N}$  value within the isotopic profile were used to create these graphs. a: divided according to age-at-death, b: divided according to sex, c: divided according to socio-economic status



**Figure 5.** Nitrogen and carbon post-weaning averages (defined as an average value from all the dentine slices formed after the termination of the initial decline in  $\delta^{15}\text{N}$ ): in comparison with bone isotopic values of the same individuals (A, B), compared between socio-economic classes (C, D)

