



Tracing early life histories from Roman times to the Medieval era: weaning practices and physiological stress

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Received: 27 June 2023 / Accepted: 20 October 2023 / Published online: 22 November 2023
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Abstract

In humans, breastfeeding and weaning depend on the infant's needs and physiology but are also influenced by environmental and cultural factors. While infant feeding strategies vary across different regions and historical eras, the associated transition from breastmilk to solid foods is universally thought to be stressful. However, still little is known about infant feeding practices and possibly associated stress in former times. This also applies to the period of transition from classical antiquity to medieval times, which shaped modern Western civilization. To enhance the understanding of childhood nutrition and stress during this period, we first analyzed stable carbon and nitrogen isotopes in serial dentine samples from the first molars of 38 individuals buried in the region once known as the Roman frontier province of *Raetia secunda*, now encompassing Southern Bavaria. In addition, we investigated the presence of linear enamel hypoplasia (LEH), known to be a marker of unspecific physiological stress, within their dentition. We used this data to create isotope profiles that display dietary changes in comparison with the occurrence of LEH. We found highly variable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and different shapes of isotope profiles which indicate different nutrition of breastfeeding individuals, complementary foods and post-weaning diets, and individual weaning patterns. For most individuals, the weaning process was completed between the ages of two and three. Interestingly, some females of non-local origin show longer weaning periods, likely displaying the influence of different cultural practices in other communities. We also found that LEH most frequently occurred in the post-weaning phase, which supports the assumption that children were at increased risk once breastfeeding had ceased completely. Furthermore, a change in the post-weaning diet in the seventh century coincided with an increased prevalence of LEH, indicating that the foods chosen or available during this time affected the susceptibility of children to stress. In conclusion, our study unveiled diverse infant feeding strategies practiced across various communities, both in different historical eras and geographical locations.

Keywords Early childhood · Serial dentine isotope analysis · Weaning process · Post-weaning diet · Post-weaning stress · Linear enamel hypoplasia (LEH)

Introduction

Early childhood is a critical stage in human life. During these initial years, the development and growth of children are significantly influenced by their living conditions, particularly their diet and exposure to stress (e.g., diseases), two factors that are closely intertwined (Caulfield et al. 2006).

Typically, breastmilk serves as the primary source of nutrition for infants. It offers a secure and hygienic supply of essential macro- and micronutrients such as proteins, fats, carbohydrates, vitamins, and minerals (e.g., Ballard and Morrow 2013; Walker 2010). Moreover, it contains substances that help to protect an infant against infection and inflammation (e.g., Walker 2010), while also contributing to

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the development of the infant's immune system (e.g., Lönnerdal 2000; McDade 2003). However, breastmilk alone cannot meet the requirements for optimal development of an infant after approximately six months (e.g., Kramer and Kakuma 2004; Jay 2009; Pérez-Escamilla et al. 2019), necessitating the inclusion of complementary foods.

Weaning is the process that starts with the gradual or immediate introduction of non-breastmilk liquids and solid foods, replacing breastmilk, which can result in nutritional stress and raise the risk of exposure to pathogens from contaminated food (e.g., Humphrey et al. 2008, Black et al. 2008). The tradeoff between these risks and the potential impediment to development associated with prolonged exclusive breastfeeding is often termed the “weanlings dilemma” (e.g., Rowland et al. 1978; Katzenberg et al. 1996). Many early bioarcheological studies have inferred that “weaning stress” represents a significant health risk or even threat to survival to infants (e.g., Katzenberg et al. 1996; Dittmann and Grupe 2000).

However, Kendall et al. (2021) criticize that a lot of these studies underrate the complexity of breastfeeding and the processes involved. They argue that the period of greatest risk to children does not occur during weaning but after complete discontinuation of breastfeeding. Fernández-Crespo et al. (2022) have referred to this as the “post-weanling’s conundrum”: a phase of heightened risk characterized by increasing dietary needs and exposure to pathogens with a simultaneous loss of nutritional and antimicrobial support provided by breastmilk while the child’s immune system is not yet fully developed. Additionally, they point out that food choices are critical to health in the post-weaning phase, but that even continued breastfeeding may not fully protect against health risks stemming from contaminated, unsuitable, or quantitatively insufficient foods during the weaning phase.

In any case, the practices of infant feeding and supposedly associated stress are influenced by various cultural, socio-economic, and environmental factors (e.g., Fildes 1986; Quandt 1995; Sellen 2009; Britton et al. 2015; Eerkens et al. 2017), including dietary traditions, resource availability, or infant sex and health (Bereczkei 2001, Fernández-Crespo et al. 2018, Tsutaya 2017, Chinique de Armas and Roksandic 2018) which can result in different patterns of weaning and stress across different temporal and geographical contexts.

In this study, we investigate dietary changes and physiological stress experienced during the first ten years of life in a group of 38 individuals buried in Late Roman and Early Medieval times within the former Roman province of *Raetia secunda* (*Raetia II*). Although these individuals were buried in the same region, some of them spent their childhood in different places, supposedly adhering to various cultural backgrounds and distinct dietary habits (Velte et al. 2023). We characterize infant feeding strategies

through serial stable isotope analysis and assess their correlation with the formation of linear enamel hypoplasia (LEH) as an indicator of stress. This not only provides information on different early life histories of people living in Bavaria between the fourth and seventh centuries but also contributes to the ongoing discussion of “weaning stress” and its timing.

Reconstruction of early childhood diet with serial isotope analysis

Analysis of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes from preserved human tissues offers a unique opportunity to study breastfeeding and weaning practices in past populations (e.g., Fogel et al. 1989, Fuller et al. 2006, Lössch 2009, Eerkens et al. 2011, Bourbou et al. 2019, Siebke et al. 2019, Ganiatsou et al. 2023). A newborn who is exclusively breastfed after birth exhibits an increase in $\delta^{15}\text{N}$ by approximately one trophic level of around 3.0‰, compared to the breastfeeding female (e.g., Fogel et al. 1989, Millard 2000, Fuller et al. 2006, Herrscher et al. 2017) (Fig. 1). Some studies also noted a rise of approximately one trophic level of around 1.0‰ in $\delta^{13}\text{C}$ (e.g., Richards et al. 2002; Fuller et al. 2003, 2006) for exclusively breastfed infants, while others found slightly smaller increases of around 0.5‰ (Herrscher et al. 2017) or no increase at all (Fogel et al. 1989). Nonetheless, increasing consumption of complementary foods leads to a gradual decrease in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ down to the level of the breastfeeding female, i.e., until weaning is completed (e.g., Fogel et al. 1989, Fuller et al. 2006, Fernández-Crespo et al. 2018) (Fig. 1). However, this pattern can deviate when the infant’s diet significantly differs from that of the breastfeeding female. Still, individual variations in physiological and metabolic processes can also affect $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the body tissues (e.g., Neuberger et al. 2013, Fuller et al. 2004, 2005, Crowder et al. 2019) of both the breastfeeding female and the infant.

Employing serial isotope analysis of tooth dentine allows us to monitor the course of isotopic values throughout the period of dentine formation within an individual (e.g., Eerkens et al. 2011; Beaumont et al. 2013; Czermak et al. 2020). The development of first molars starts around birth and continues throughout the initial 10 years of life, providing information about the phase of exclusive breastfeeding, the complementary feeding (weaning) phase, and the post-weaning phase. Isotope data derived from specific dentine sections can be assigned to their approximate formation ages to create isotope profiles (e.g., Czermak et al. 2020). Consequently, this approach enables us to estimate the age at which supplementary foods were introduced and when weaning was completed.

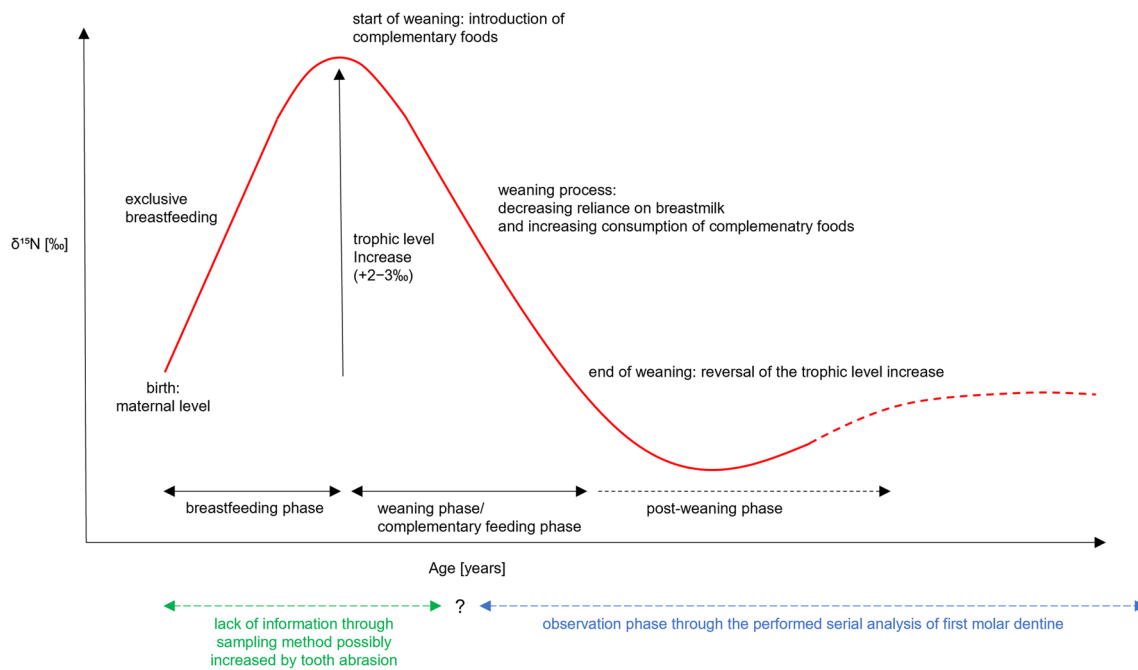


Fig. 1 Schematic course of an infant's $\delta^{15}\text{N}$ values during the phases of exclusive breastfeeding, weaning, and post-weaning diet.

Linear enamel hypoplasia as an indicator of physical stress

Linear enamel hypoplasia (LEH) results from a disruption in the enamel secretion by ameloblasts (e.g., King et al. 2002) during tooth development. Thus, LEH presumably indicate unspecific physiological stress during childhood (e.g., Goodman and Rose 1990, Guatelli-Steinberg and Lukacs 1999, King et al. 2005, Hillson 2008). Since tooth enamel is not remodeled, it is possible to estimate the approximate age at which any observed defect was formed.

Over the last decades, several studies have implied a connection between weaning and the occurrence of LEH. While many of them have remained rather vague about during which phase “weaning stress” occurred (e.g., Goodman et al. 1984, Corruccini et al. 1985, Berbesque and Hoover 2018), some have observed an increase in the number of LEH towards or shortly after the end of complementary feeding (e.g., Moggi-Cecchi et al. 1994, Crowder et al. 2019, Orellana-González et al. 2020).

South Bavaria between Roman times and the Middle Ages

Southern Bavaria was initially integrated into the Roman Empire in the first century, forming a part of the province *Raetia*. After the province was subdivided in the late

fourth century, it became part of *Raetia II*. During the Roman Imperial period, the region experienced progressing urbanization and intensive trade led to the development of a healthy economy that presumably formed a thriving society in Southern Bavaria (e.g., Fehr 2010). However, this situation potentially changed as several crises began to accumulate, such as recurring attacks of German tribes from the mid-third century onwards and increasing supply shortages during the fourth century. The Roman administration and frontier defense for the entire pre-Alpine region eventually ended in 476 AD when the Germanic leader Odoacer replaced the last Western Roman Emperor. Little is known about the subsequent events in the region that was once *Raetia II*. But in the middle of the sixth century, Bavaria became a dukedom under Frankish rule, which lasted until 751 AD (e.g., summarized in Haas-Gebhard 2013).

In a previous study, we showed that part of the individuals buried in the former province of *Raetia II* around 500 AD spent their childhood in a region with differing strontium isotope signatures (Velte et al. 2023). Our findings also indicated that individuals exhibiting unusual dietary habits, e.g., frequent consumption of millet, a C4 plant that was rather uncommon in Bavaria during that time (e.g., Hakenbeck et al. 2010), likely have foreign origins. This was especially notable among some women with artificial cranium deformation (ACD) (Velte et al. 2023). Interestingly, Veeramah et al. (2018) demonstrated that most of these women show

a genetic similarity to people living in the South-East European region.

In this study, our aim is to explore whether the profound political, social, and economic changes that occurred during the transition from Antiquity to the Middle Ages, or potential cultural differences related to people's origins, had any influence on child feeding strategies.

Material

The human remains of the individuals studied ($n=38$) were excavated at four burial sites in Bavaria (Fig. 2). Most of these individuals ($n=32$) were uncovered at two cemeteries in Erding: Late Roman Erding-Kletthamer Feld (EKF, $n=4$) and Early Medieval Altenerding-Klettham (AED, $n=28$). The small cemetery of EKF (13 inhumations) dates from the second half of the fourth century AD until the first half of the fifth century AD (Sofeso et al. 2012). The burial site of AED came into use in the second half of the fifth century, and burials there continued well into the seventh century (Sage 1984, Losert and Pleterski 2003). It stands as one of

the biggest Early Medieval cemeteries excavated in Bavaria, comprising about 1450 graves. We selected 17 individuals dating around 500 AD, including two women with ACD and five other individuals of documented non-local origin (Veeramah et al. 2018; Velte et al. 2023), and eleven burials dating from the seventh century AD. In addition, we included individuals from two sites located less than 100 km away, namely, Straubing-Bajuwarenstraße (STB) and Burgweinting (BW), due to the presence of ACD in three cases and unusual dietary patterns likely linked to non-local origin (Codreanu-Windauer and Harbeck 2016, Trautmann et al. 2017, Veeramah et al. 2018, Velte et al. 2023). The cemetery STB was occupied from the mid-fifth until the mid-seventh century AD, containing over 800 graves (Geisler 1998). From this site, we included three individuals dating around 500 AD (Veeramah et al. 2018; Velte et al. 2023). Furthermore, we analyzed three individuals of a small grave group (15 inhumations named grave group A) from BW (BWA), dating between the late fifth to the early sixth century AD (Zintl 2012, Codreanu-Windauer and Schleuder 2013).

Many individuals included in this study had been previously investigated, providing valuable data on age at death

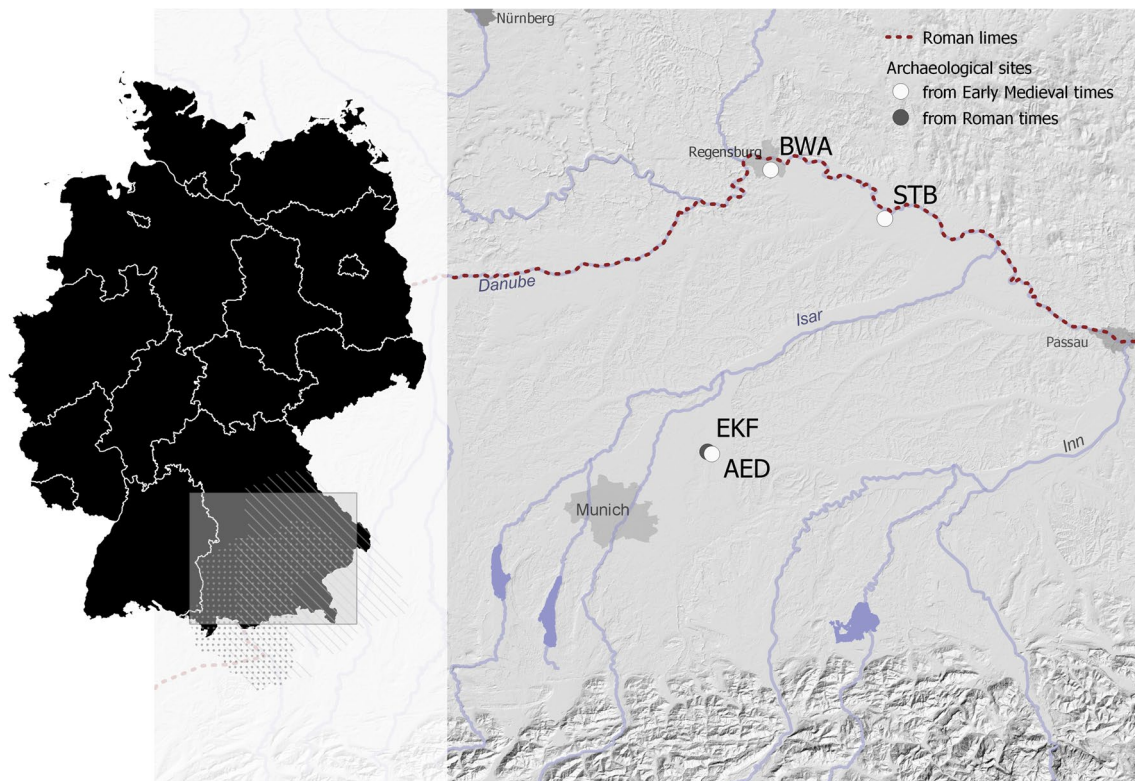


Fig. 2 (Left) Map of Germany with its federal states; the study area (shown in detail on the right) is backed by a gray box; dashed lines indicate the extent of the first duchy of Bavaria (after Weiß 2021) and dotted areas the presumed territory of Raetia II (after Haberstroh and Harbeck (2013)). (Right) Part of southern Bavaria with the geographical

positions of the archaeological sites included in this study (AED, Altenerding-Klettham; EKF, Erding-Kletthamer Feld; STB, Straubing-Bajuwarenstraße; BWA, Burgweinting Group A). Map data: EU-DEM © European Union, Copernicus Land Monitoring Service 2017, European Environment Agency (EEA).

and sex, the presence of LEH, the presence of ACD, strontium isotopes, genetic ancestry, dating and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios in bone, and bulk values of first molar dentine (Hakenbeck et al. 2010, Sofeso et al. 2012, Codreanu-Windauer and Schleuder 2013, Codreanu-Windauer and Harbeck 2016, Harbeck et al. 2016, Trautmann et al. 2017, Veeramah et al. 2018, Toncala et al. 2020, Neidich 2023; Velte et al. 2023). Detailed information on all data used and the corresponding literature are listed in the Supplementary (Table S2).

All individuals in the study were adults (> 20 years), except one juvenile individual (AED_1053: 13–20 years). The sample set comprises a slightly higher number of females ($n=21$) compared to males ($n=14$), with sex being indeterminable for three individuals (AED_100, AED_217, AED_724) due to poor preservation.

Based on the analysis of strontium, carbon, and nitrogen isotopes as well as genetic data, some individuals from around 500 AD in this study ($n=23$) can be reasonably assumed to be migrants ($n=13$, AED+STB+BWA), which includes five females with ACD (as discussed in Velte et al. 2023). Those individuals who showed no indication of foreign origin are considered part of the “local” population ($n=10$, AED), although it cannot be ruled out that unrecognized migrants are among them (also see Velte et al. 2023).

For burials dating from the first half of the seventh century ($n=11$, AED), or between the second half of the fourth century and the first quarter of the fifth century ($n=4$, EKF), such a classification cannot be made, because the corresponding data are not available for all samples.

Methods

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios in collagen were analyzed from both bone and serial dentine samples. Bone samples from adults provide information about dietary patterns during a later life stage, due to tissue turnover, but a more precise timespan reflected by measured isotope values cannot be determined due to varying turnover rates (Hedges et al. 2007). In contrast, dentine is not remodeled once formed during early life, which allows us to associate measured values with the specific examination of chronological age (e.g., Czermak et al. 2020).

Bone collagen was extracted following the protocol described in Siebke et al. (2019) (also see Velte et al. 2023 and Supplementary S1.1). Measurements were carried out at the Isolab GmbH in Hanau using an Elementar Vario Cube EL connected to an Isoprime mass spectrometer. Accuracy and precision were verified through replicate analyses of laboratory standards (e.g., Collagen STD R (USGS 89), Collagen STD S, Collagen STD BRA), calibrated to international standards USGS40 and 41. The Isolab GmbH ensures an analytical precision of 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$.

Serial dentine sampling from the first molars and collagen extraction followed the procedure described in Velte et al. (2023) (for detailed method description see Supplementary S1.1). The samples were analyzed at the GeoCenter (Friedrich-Alexander University, Erlangen-Nürnberg), utilizing a Flash EA 2000 elemental analyzer connected to a ThermoFinnigan Delta V Plus mass spectrometer. Analytical precision ensured by the GeoCenter is 0.1‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ checked by replicate analyses of laboratory standards (e.g., Casein, Cyclo) calibrated to international standards USGS 40 and 41.

The results are reported in the conventional δ -notation in per mil (‰) relative to internationally accepted standards, VPDB for carbon, and AIR for nitrogen. The collagen quality of each sample was evaluated through the C/N atomic ratio, as well as carbon and nitrogen content.

Chronological age assignment of sampled dentine sections to illustrate changes in stable isotopes over time was made using a scheme developed in a prior study (Velte et al. 2023). This scheme is based on *The London Atlas of Tooth Development and Eruption* (AlQahtani et al. 2010) and takes potential wear into account for each tooth.

We defined weaning age as the approximate age at which either breastfeeding is finally terminated, reduced to a low amount, and/or when the isotope signal is masked by the consumption of higher protein sources. To estimate individual weaning age, a customized scheme based on the relative decrease in $\delta^{15}\text{N}$ values at the beginning of the isotope profiles is employed: Starting with the first dentine section, any dentine section showing a depletion in $\delta^{15}\text{N}$ by at least 0.2‰ (analytical error) compared to the previous section ($n_1, n_2, \dots, n_{\downarrow\text{end}}$) (Fig. 3A) is given a new value representing the relative decrease in $\delta^{15}\text{N}$ ($\downarrow\delta^{15}\text{N}_{\text{rel}}$). This value is calculated as the difference between the ratio of the last section in the series with continuously decreasing values ($n_{\downarrow\text{end}}$) and the ratio of the respective section ($\downarrow\delta^{15}\text{N}_{\text{rel}}(n_1, n_2, \dots, n_{\downarrow\text{end}}) = \delta^{15}\text{N}(n_{\downarrow\text{end}}) - \delta^{15}\text{N}(n_1, n_2, \dots, n_{\downarrow\text{end}})$). $\downarrow\delta^{15}\text{N}_{\text{rel}}$ values are then plotted against the approximate formation age of dentine sections (Fig. 3B). The intersection of the linear trend line for normal tooth development and the y-axis is determined as the weaning age (line function: $f(x) = m \times x + t$; with t = weaning age, x = decline, m = slope). The error caused by the biological variation in tooth development is determined by using dentine formation ages for both early and late tooth development (Fig. 3B).

This approach can be used to estimate individual weaning ages (as exemplarily shown in Fig. 3B) but it can also be applied to evaluate weaning age on a population level by combining data from all individuals or groups, enabling the assessment of inter- or intra-population differences (also see Figure S1.2.2).

Data on linear enamel hypoplasia (LEH) were collected on permanent maxillary and mandibular teeth. Rather than

analyzing general stress levels, we aimed to investigate a link between LEH formation and weaning. Thus, we only analyzed teeth that develop during the supposed weaning period: incisors and canines. We recorded the number and position of observable palpable LEH (a rippled pattern was detected by scratching the surface with the fingernail) on teeth from the right side of the mouth, using the corresponding teeth from the left side only if the right tooth was unavailable. Teeth that had lost over 50% of their crown height, e.g., due to wear, or were missing, were marked “not assessable.”

The SNSB, Bavarian State Collection of Anthropology, which houses the human remains, specifies in its osteological record form (Harbeck et al. 2020) that the position of LEH should be assessed by dividing the tooth into three equal parts: top, middle, and bottom. For some of the individuals in our study, this data was already available in this format, and we recorded LEH for the remaining individuals following the same manner. To determine the age span during which these crown parts are formed, we provide enamel growth curves for early, normal, and late tooth development in incisors and canines of the upper and lower jaw, based on AlQahtani et al. (2010) (see S1.3 for more details). Linear trend line equations from these curves were used to calculate the age span during which a crown part is most commonly formed (normal development) and the error resulting from the biological variation in tooth development (early and late development).

Statistical data analysis was performed using IBM SPSS 29.00 and RStudio 1.4.1717 for Windows. Statistical tests on metric data were exclusively non-parametric (independent-sample Mann–Whitney *U* and Kruskal–Wallis tests) due to small sample sizes. Nominal data was analyzed by a non-parametric chi-square test. For multiple comparisons, significance values have been adjusted by the Bonferroni correction

Fig. 4 Carbon and nitrogen isotope profiles ($n=38$). Colored frames: dating of burials (orange=second half fourth century to first half fifth century AD; blue=around 500 AD; green=seventh century). Data points: $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of the first molar dentine sections assuming normal dentine development, small dashed lines highlight changes in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ between successive sections. Error bars: variations in early or late tooth development. Horizontal red and blue dotted lines represent individual ^{13}C or $\delta^{15}\text{N}$ bone values. Shaded red and blue areas display intra-population variability ranges of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in Erding ($\delta^{13}\text{C}$: -20.7 to -18.5‰ , $\delta^{15}\text{N}$: 7.9 to 11.1‰), Straubing ($\delta^{13}\text{C}$: -20.8 to -18.9‰ , $\delta^{15}\text{N}$: 8.6 to 10.3‰), and Regensburg ($\delta^{13}\text{C}$: -21.2 to -18.7‰ , $\delta^{15}\text{N}$: 7.8 to 10.5‰) (Velte et al. 2023). Vertical green lines: big dashed line=calculated individual weaning age, smaller dashed lines=variation assuming early or late tooth development. Dental charts show the present teeth (filled teeth), sampled molars (yellow), and evaluated anterior teeth for the presence of LEH (dark grey). The formation of LEH is shown below the isotope profiles. Filled bars represent the age span in which the corresponding crown third was formed (assuming normal enamel development), error bars display variations assuming early or late tooth formation. Red signs indicate individuals with incomplete anterior dentition, so the absence of LEH must be evaluated with caution.

to counteract alpha error accumulation. Graphics were created using QGIS 3.22 and Microsoft Excel for Windows.

All statistical analysis and more detailed results are given in the Supplementary (S1.2–3).

Results

Collagen quality

Collagen quality of dentine ($n=350$) and bone specimens ($n=11$) was monitored, generally accepting C/N atomic ratios between 2.9 and 3.6 (DeNiro 1985), as well as a

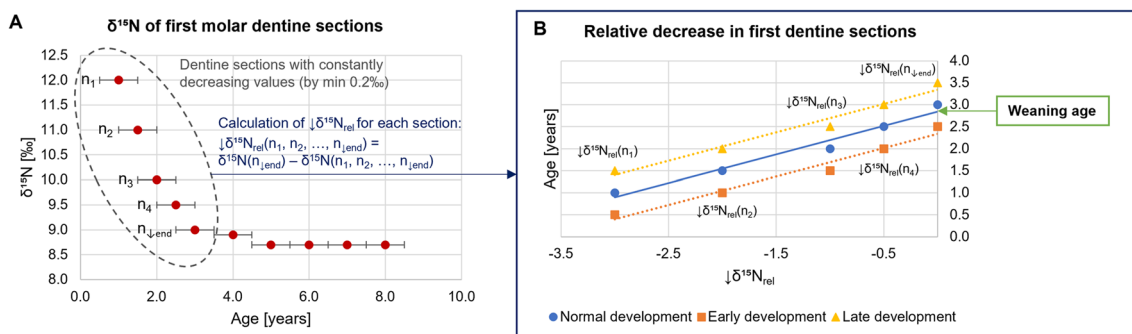
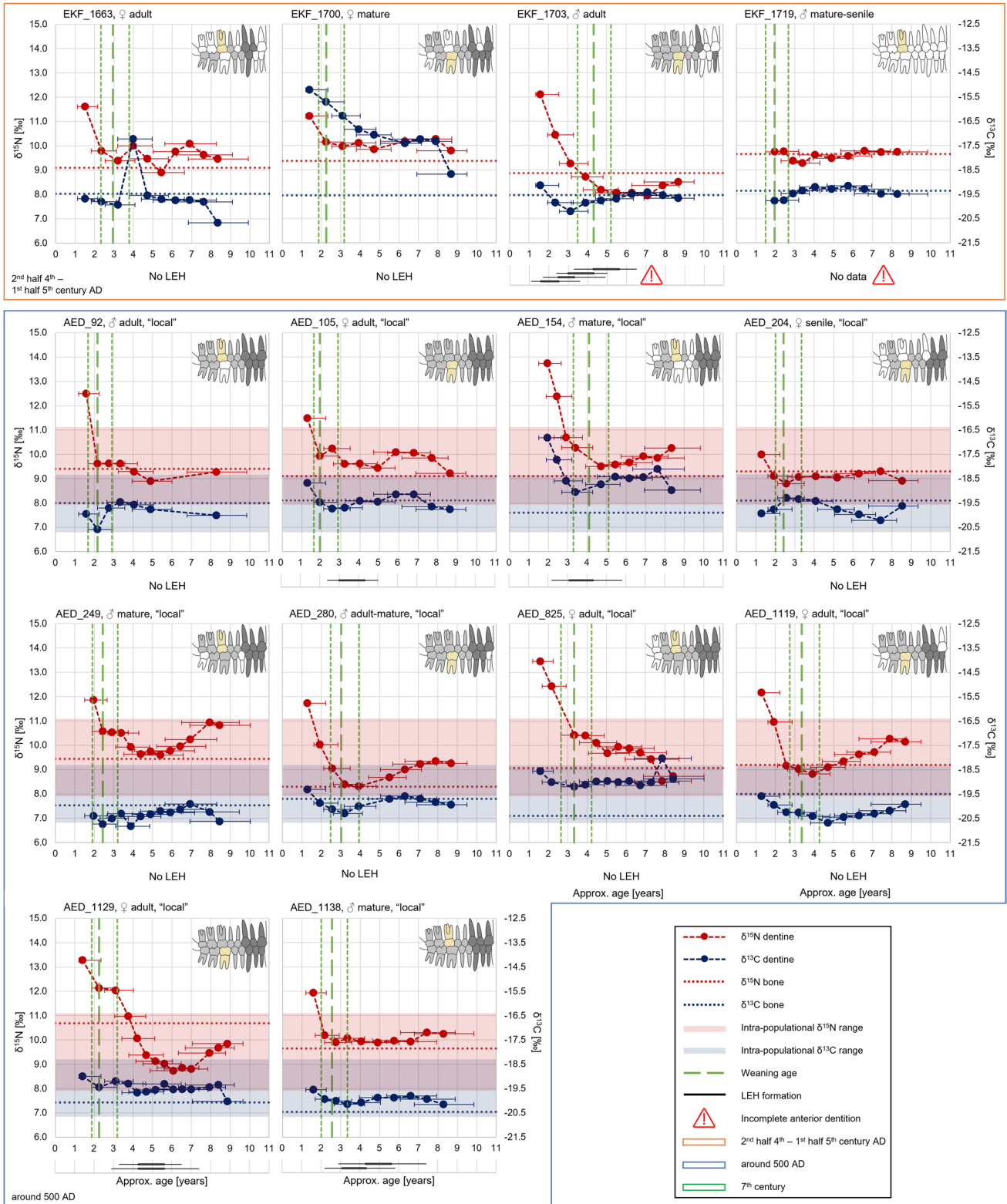


Fig. 3 Schematic illustration of the model used to estimate weaning ages based on the relative decrease in $\delta^{15}\text{N}$ ratios of successive dentine sections. **A** Example of dentine sections from a stable light isotope profile. Only dentine sections with a constant decline in $\delta^{15}\text{N}$ ratios (by min 0.2‰) at the beginning of the isotope profiles are selected. In this example down to a total drop of 3.2‰ . Samples are given a new value, representing the relative decrease in $\delta^{15}\text{N}$. This value is calculated as the difference between the ratio of the last section in the series (n_{Jend}) with continuously decreasing

values and the ratio of the respective section ($\downarrow\delta^{15}\text{N}_{\text{rel}}(n_1, n_2, \dots, n_{\text{Jend}}) = \delta^{15}\text{N}(n_{\text{Jend}}) - \delta^{15}\text{N}(n_1, n_2, \dots, n_{\text{Jend}})$). **B** Plotting of the $\downarrow\delta^{15}\text{N}_{\text{rel}}$ ratios of dentine sections against the chronological formation age of dentine samples (early, normal, and late development) including linear trend lines. Weaning age is defined as the intersection of the linear trend line for normal development and the y-axis: $f(x) = m \times x + t$, with t = weaning age, x = decline, m = slope/linear trend. The error is shown by the functions for early and late development.



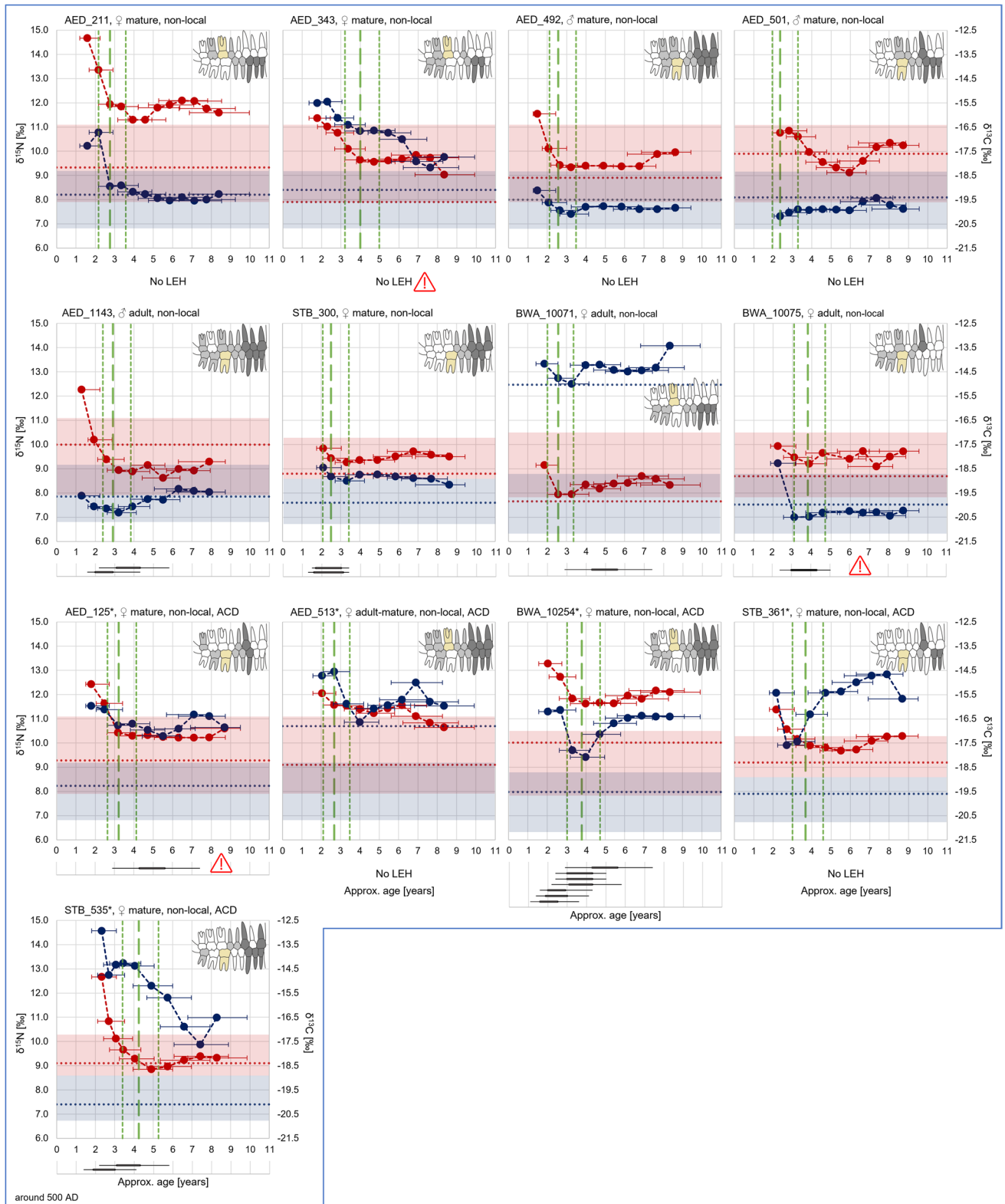


Fig. 4 (continued)

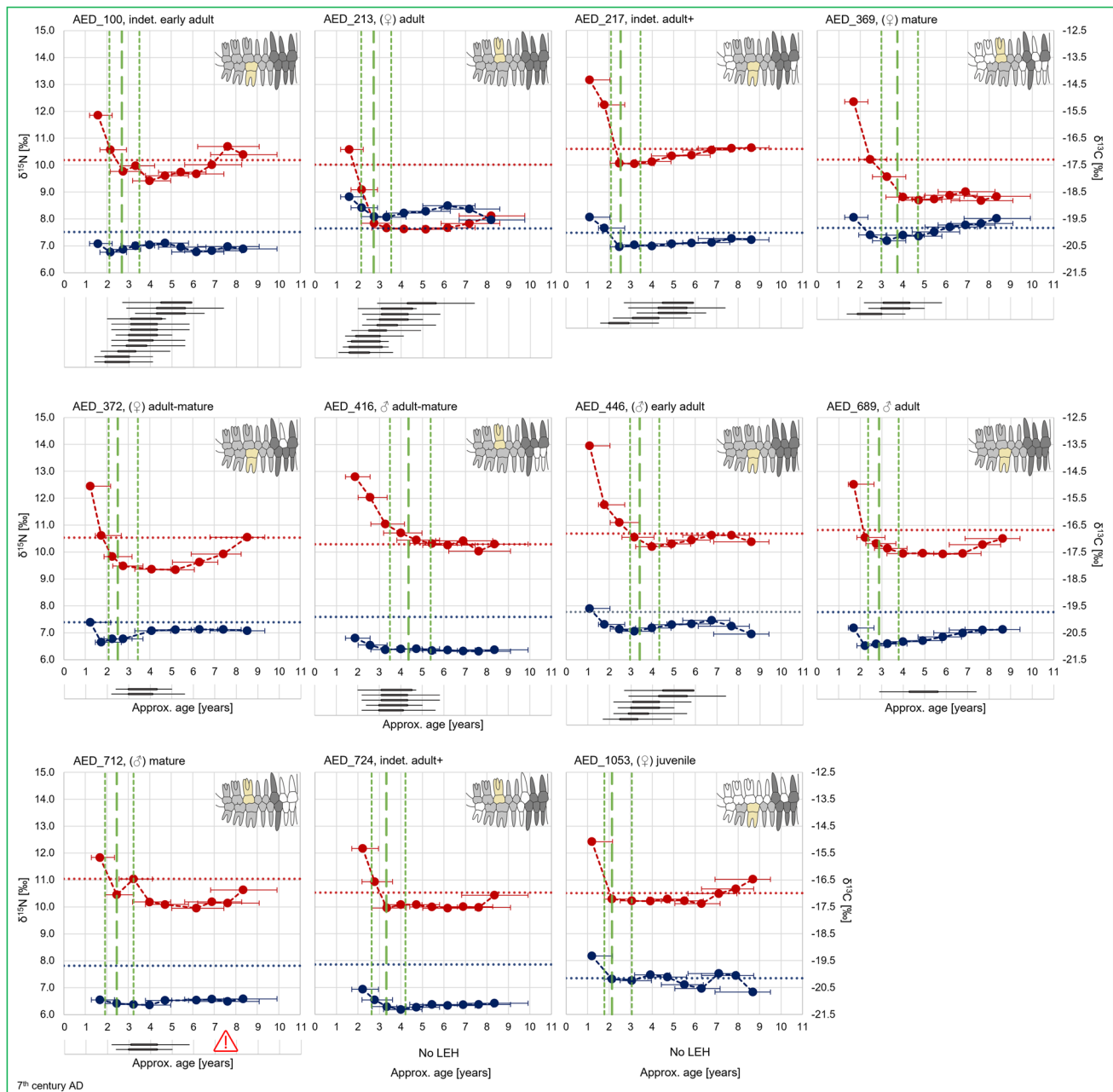


Fig. 4 (continued)

content of carbon from 13 to 50% and of nitrogen from 4.8 to 19%) (largely following DeNiro 1985, Ambrose 1990, Rand et al. 2015). Dentine samples ($n = 15$) with higher contents were excluded.

Isotope profiles

Intra-tooth isotopic patterns (isotope profiles) of all 38 individuals, illustrating changes in stable light isotope ratios throughout the first 10 years of life are shown in Fig. 4. We

proceed with the assumption that tooth development was normal and identify changes of isotope only accepting a minimum difference of at least 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Weaning phase

We observe no period of exclusive breastfeeding, indicated by the first rising $\delta^{15}\text{N}$ values (Fig. 4). In most isotope profiles ($n = 36$), $\delta^{15}\text{N}$ ratios continuously decrease from the beginning of observation (Table 1, Fig. 4), marking the

Table 1. Observation start (Ob start) AQ = approximate age of the first dentine section, with the condition of normal tooth development, whereby potential wear was taken into account for each tooth. Individual $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios of the first dentine sections ($\delta^{15}\text{N}/\delta^{13}\text{C}$ first section) compared to individual bone ratios (Diff bone) and post-weaning ratios (Diff post-w). Total $\downarrow\delta^{15}\text{N}/\downarrow\delta^{13}\text{C}$ =total decrease of $\delta^{15}\text{N}/\delta^{13}\text{C}$ in first dentine sections with continuous (con) decline (by min 0.2‰ per section) and inconstant (incon) decline including second drops. W age=estimated individual weaning ages. Individual post-weaning levels (post-w $\delta^{15}\text{N}/\delta^{13}\text{C}$) are defined as the mean value

of dentine sections within three years after the approximate individual weaning age compared to individual bone values (Diff bone). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios were measured in individual bone samples, primarily ribs ($\delta^{15}\text{N}/\delta^{13}\text{C}$ bone). Values highlighted in gray correspond to minimum and maximum values within groups. Values in bold correspond to the mean value and the standard deviation (sd) within groups and total. Groups are initially defined by their chronological dating (Late Roman, 500 AD, seventh century). Within the group of burials dating around 500 AD, the division into migrants and “locals” is made according to Velte et al. (2023)

Group	Individual *ACD	Ob start [years]	$\delta^{15}\text{N}$ first section [‰]			$\delta^{13}\text{C}$ first section [‰]			Total $\downarrow\delta^{15}\text{N}$ [‰]	Total $\downarrow\delta^{13}\text{C}$ [‰]	W age [years]	post-w $\delta^{15}\text{N}$ [‰]		post-w $\delta^{13}\text{C}$ [‰]		$\delta^{15}\text{N}$ bone [‰]	$\delta^{13}\text{C}$ bone [‰]
			value	Diff bone	Diff post-w	value	Diff bone	Diff post-w				con (incon)	con (incon)	value	Diff bone		
Late Roman	EKF 1663 ♀	1.5	11.6	2.5	2.1	-19.7	-0.2	-0.9	-2.2 (-2.7)	no	3.0	9.5	0.4	-18.8	0.7	9.1	-19.5
	EKF 1700 ♀	1.4	11.2	1.8	1.2	-15.2	4.4	1.5	-1.1	-2.2	2.3	10	0.6	-16.7	2.9	9.4	-19.6
	EKF 1703 ♂	1.6	12.1	3.2	4.0	-19.1	0.4	0.4	-3.9	-1.1	4.3	8.1	-0.8	-19.5	0.0	8.9	-19.5
	EKF 1719 ♂	2.0	9.7	0.0	0.2	-19.8	-0.4	-0.4	no (-0.4)	no	<2.0	9.5	-0.2	-19.4	0.0	9.7	-19.4
	mean (N=4)	1.6	11.2	1.9	1.9	-18.5	1.1	0.2	-2.4	-1.7	2.8	9.3	0.0	-18.6	0.9	9.3	-19.5
sd	0.2	1.0	1.4	1.6	2.2	2.6	1.1	1.4	0.8	1.1	0.8	0.6	1.3	1.4	0.4	0.1	
"Locals" ~500 AD	AED 92 ♂	1.6	12.5	3.1	3.1	-20.0	-0.5	-0.3	-2.9 (-3.6)	-0.6	2.2	9.4	0.0	-19.7	-0.2	9.4	-19.5
	AED 105 ♀	1.3	11.5	2.4	1.8	-18.7	0.7	0.9	-1.6 (-1.9)	-1.0	1.9	9.7	0.6	-19.6	-0.2	9.1	-19.4
	AED 154 ♂	2.0	13.7	4.6	4.0	-16.8	3.1	1.6	-4.2	-2.3	4.1	9.7	0.6	-18.4	1.5	9.1	-19.9
	AED 204 ♀	1.3	10.0	0.7	0.9	-19.9	-0.5	-0.4	-1.2	no	2.4	9.1	-0.2	-19.5	-0.1	9.3	-19.4
	AED 249 ♀	2.0	11.9	2.5	1.9	-20.4	-0.4	0.0	-1.3 (-2.3)	-0.3	2.4	10.0	0.6	-20.4	-0.4	9.4	-20.0
	AED 280 ♂	1.3	11.7	3.4	3.2	-19.3	0.4	0.6	-3.3	-1.0	3.0	8.5	0.2	-19.9	-0.2	8.3	-19.7
	AED 825 ♀	1.6	13.5	4.4	3.5	-18.6	1.8	0.4	-3.1 (-3.8)	-0.6	3.4	10.0	0.9	-19.0	1.4	9.1	-20.4
	AED 1119 ♀	1.3	12.2	3.0	2.8	-19.6	-0.1	0.9	-3.4	-0.7	3.4	9.4	0.2	-20.5	-1.0	9.2	-19.5
	AED 1129 ♀	1.4	13.3	2.6	3.0	-19.0	1.1	0.5	-1.2 (-4.6)	-0.4	2.3	10.3	-0.4	-19.5	0.6	10.7	-20.1
	AED 1138 ♀	1.6	11.9	2.2	1.9	-19.6	0.9	0.4	-2.0	-0.3	2.6	10.0	0.3	-20.0	0.5	9.7	-20.5
	mean (N=10)	1.5	12.2	2.9	2.6	-19.2	0.7	0.5	-2.4	-0.8	2.8	9.6	0.3	-19.7	0.2	9.3	-19.8
	sd	0.3	1.1	1.1	1.0	1.0	1.2	0.6	1.1	0.6	0.7	0.5	0.4	0.6	0.8	0.6	0.4
	mean ♂ (N=5)	1.7	12.3	3.2	2.8	-19.2	0.7	0.5	-2.7	-0.9	2.9	9.5	0.3	-19.7	0.2	9.2	-19.9
	sd	0.3	0.8	0.9	0.9	1.4	1.5	0.7	1.1	0.8	0.8	0.6	0.3	0.8	0.8	0.5	0.4
	mean ♀ (N=5)	1.4	12.1	2.6	2.4	-19.2	0.6	0.5	-2.1	-0.7	2.7	9.7	0.2	-19.6	0.1	9.5	-19.8
sd	0.1	1.4	1.3	1.0	0.6	0.9	0.5	1.1	0.3	0.7	0.5	0.5	0.6	0.9	0.7	0.5	
Migrants ~500 AD	AED 125* ♀	1.8	12.4	3.1	2.1	-16.0	3.3	1.0	-2.0	no (-0.7)	3.2	10.3	1.0	-17.0	2.3	9.3	-19.3
	AED 211 ♀	1.6	14.7	5.4	3.1	-17.3	2.0	2.0	-1.8 (-3.4)	no (-2.3)	2.8	11.6	2.3	-19.3	0.0	9.3	-19.3
	AED 343 ♀	1.8	11.4	3.5	1.7	-15.5	3.6	1.6	-1.7	no (-1.2)	4.0	9.7	1.8	-17.1	2.0	7.9	-19.1
	AED 492 ♂	1.5	11.6	2.7	2.2	-19.1	0.4	0.8	-2.2	-1.0	2.6	9.4	0.5	-19.9	-0.4	8.9	-19.5
	AED 501 ♀	2.4	10.8	0.9	0.7	-20.2	-0.8	-0.3	no (-1.8)	no	<2.4	10.1	0.2	-19.9	-0.5	9.9	-19.4
	AED 513* ♀	2.0	12.1	3.0	0.7	-14.7	2.1	1.4	-0.4	no (-2.0)	2.7	11.4	2.3	-16.1	0.7	9.1	-16.8
	AED 1143 ♀	1.3	12.3	2.3	3.4	-19.6	0.1	0.3	-3.4	-0.5	2.9	8.9	-1.1	-19.9	-0.2	10.0	-19.7
	STB 300 ♀	2.1	9.8	1.0	0.4	-18.5	1.4	0.3	-0.4	-0.5	2.5	9.4	0.6	-18.8	1.1	8.8	-19.9
	STB 361* ♀	2.2	11.4	2.2	1.7	-15.4	4.2	-0.1	-1.5	-2.2	3.7	9.7	0.5	-15.3	4.3	9.2	-19.6
	STB 535* ♀	2.3	12.7	3.6	3.5	-12.9	7.2	3.8	-3.8	-1.9	4.3	9.2	0.1	-16.7	3.4	9.1	-20.1
	BWA 10071 ♀	1.9	9.2	1.5	0.9	-14.2	0.8	0.3	-1.3	-0.8	2.6	8.3	0.6	-14.5	0.5	7.7	-15.0
	BWA 10075 ♀	2.3	9.9	1.2	0.3	-18.3	1.7	2.0	-0.7	-2.2	3.8	9.6	0.9	-20.3	-0.3	8.7	-20.0
	BWA 10254* ♀	2.0	13.3	3.3	1.5	-16.2	3.3	0.5	-1.7	no (-2.0)	3.8	11.8	1.8	-16.7	2.8	10.0	-19.5
	mean (N=13)	1.9	11.7	2.6	1.7	-16.8	2.3	1.1	-1.8	-1.3	3.2	10.0	0.9	-17.8	1.2	9.1	-19.0
	sd	0.3	1.5	1.3	1.1	2.3	2.1	1.1	1.1	0.8	0.7	1.1	1.0	2.0	1.6	0.7	1.5
mean ♂ (N=3)	1.7	11.6	2.0	2.1	-19.6	-0.1	0.3	-2.8	-0.6	2.6	9.5	-0.1	-19.9	-0.4	9.6	-19.5	
sd	0.6	0.8	1.0	1.4	0.6	0.6	0.6	0.9	0.4	0.3	0.6	0.9	0.0	0.2	0.6	0.2	
mean ♀ (N=10)	2.0	11.7	2.8	1.6	-15.9	3.0	1.3	-1.6	-1.5	3.3	10.1	1.2	-17.2	1.7	8.9	-18.9	
sd	0.2	1.7	1.4	1.1	1.8	1.8	1.2	1.1	0.8	0.7	1.2	0.8	1.8	1.5	0.7	1.7	
7 th century	AED 100 ^{indef} ♀	1.6	11.9	1.7	2.2	-20.4	-0.4	0.1	-2.1 (-2.5)	-0.3	2.7	9.7	-0.5	-20.5	-0.5	10.2	-20.0
	AED 213 ♀	1.6	10.6	0.6	3.0	-18.7	1.2	0.6	-2.8	-0.7	2.7	7.6	-2.4	-19.3	0.6	10.0	-19.9
	AED 217 ^{indef} ♀	1.1	13.2	2.6	3.0	-19.4	0.6	1.1	-3.1	-1.2	2.5	10.2	-0.4	-20.5	-0.5	10.6	-20.0
	AED 369 ♀	1.7	12.4	2.2	3.6	-19.5	0.3	0.5	-3.6	-0.8	3.7	8.8	-1.4	-20.0	-0.2	10.2	-19.8
	AED 372 ♀	1.2	12.5	2.0	3.1	-20.1	0.0	0.3	-3.0	-0.8	2.5	9.4	-1.1	-20.4	-0.3	10.5	-20.1
	AED 416 ♂	1.9	12.8	2.5	2.5	-20.7	-0.8	0.5	-2.5	-0.3	4.4	10.3	0.0	-21.2	-1.3	10.3	-19.9
	AED 446 ♂	1.1	14.0	3.3	3.6	-19.6	0.1	0.6	-3.8	-0.8	3.4	10.4	-0.3	-20.2	-0.5	10.7	-19.7
	AED 689 ♀	1.7	12.5	1.7	2.5	-20.3	-0.6	0.5	-2.4	-0.7	2.9	10.0	-0.8	-20.8	-1.1	10.8	-19.7
	AED 712 ♂	1.7	11.8	0.8	1.4	-21.0	-1.3	0.1	-1.3 (-1.6)	no	2.5	10.4	-0.6	-21.1	-1.4	11.0	-19.7
	AED 724 ^{indef} ♀	2.2	12.2	1.7	2.1	-20.6	-1.0	0.6	-2.2	-0.6	3.3	10.1	-0.4	-21.2	-1.6	10.5	-19.6
	AED 1053 ♀	1.2	12.4	1.9	2.2	-19.3	0.9	0.8	-2.1	-0.9	2.1	10.2	-0.3	-20.1	0.1	10.5	-20.2
mean (N=11)	1.5	12.4	1.9	2.7	-20.0	-0.1	0.5	-2.6	-0.7	3.0	9.7	-0.8	-20.5	-0.6	10.5	-19.9	
sd	0.4	0.9	0.8	0.7	0.7	0.8	0.3	0.7	0.3	0.7	0.9	0.7	0.6	0.7	0.3	0.2	
Total	min	1.1	9.2	0.0	0.2	-21.0	-1.3	-0.9	-4.2	-2.3	1.9	7.6	-2.4	-21.2	-1.6	7.7	-20.5
	max	2.4	14.7	5.4	4.0	-12.9	7.2	3.8	-0.4	-0.3	4.4	11.8	2.3	-14.5	4.3	11.0	-15.0
	mean (N=38)	1.7	12.0	2.4	2.2	-18.5	1.0	0.7	-2.3	-1.0	3.0	9.7	0.2	-19.2	0.4	9.6	-19.5
	sd	0.4	1.2	1.2	1.1	2.1	1.8	0.8	1.0	0.6	0.7	0.9	1.0	1.7	1.4	0.8	1.0

weaning phase with complementary feeding. A concomitant initial decrease in $\delta^{13}\text{C}$ values is also observed in many profiles ($n=28$), but which often ends earlier than the drop in $\delta^{15}\text{N}$ ($n=13$). In contrast, in some individuals, $\delta^{13}\text{C}$ remains stable ($n=6$) or increases ($n=2$) in the earliest part of the isotopic profile, while $\delta^{15}\text{N}$ simultaneously decreases (Table 1).

Overall, values and intra-tooth patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are highly variable in the initial part of the profiles. The

levels of the first dentine sections, as well as the extent and rate of the continuous decrease over two or more sections, vary markedly (Table 1).

The $\delta^{15}\text{N}$ ratios of the first dentine sections are almost universally increased compared to both bone ratios ($n=37$) and post-weaning levels ($n=38$). This is in line with the expectation that the trophic level increase due to breastfeeding can still be observed at the beginning of the intra-tooth isotopic pattern. The $\delta^{13}\text{C}$ ratios, however, are similar or

lower compared to bone ($n = 15$) and/or post-weaning levels ($n = 9$) in some cases (Table 1), as anticipated, given the lesser impact of weaning on carbon.

In most isotope profiles, the extent of the observable decrease in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ($n = 25$) falls within the range of the expected trophic level effect of breastfeeding ($\sim 3.0\text{‰}$ in $\delta^{15}\text{N}$, $\sim 1.0\text{‰}$ in $\delta^{13}\text{C}$, e.g., Fuller et al. 2006). In some cases, values decrease to a greater extent in $\delta^{15}\text{N}$ ($\geq 3.2\text{‰}$, $n = 8$) and $\delta^{13}\text{C}$ ($\geq 1.2\text{‰}$, $n = 6$) (Table 1). Non or minimally decreasing $\delta^{15}\text{N}$ values ($< 1.0\text{‰}$) are only found in individuals ($n = 5$) with missing first dentine samples, consequently showing the weaning phase only towards its end or not at all.

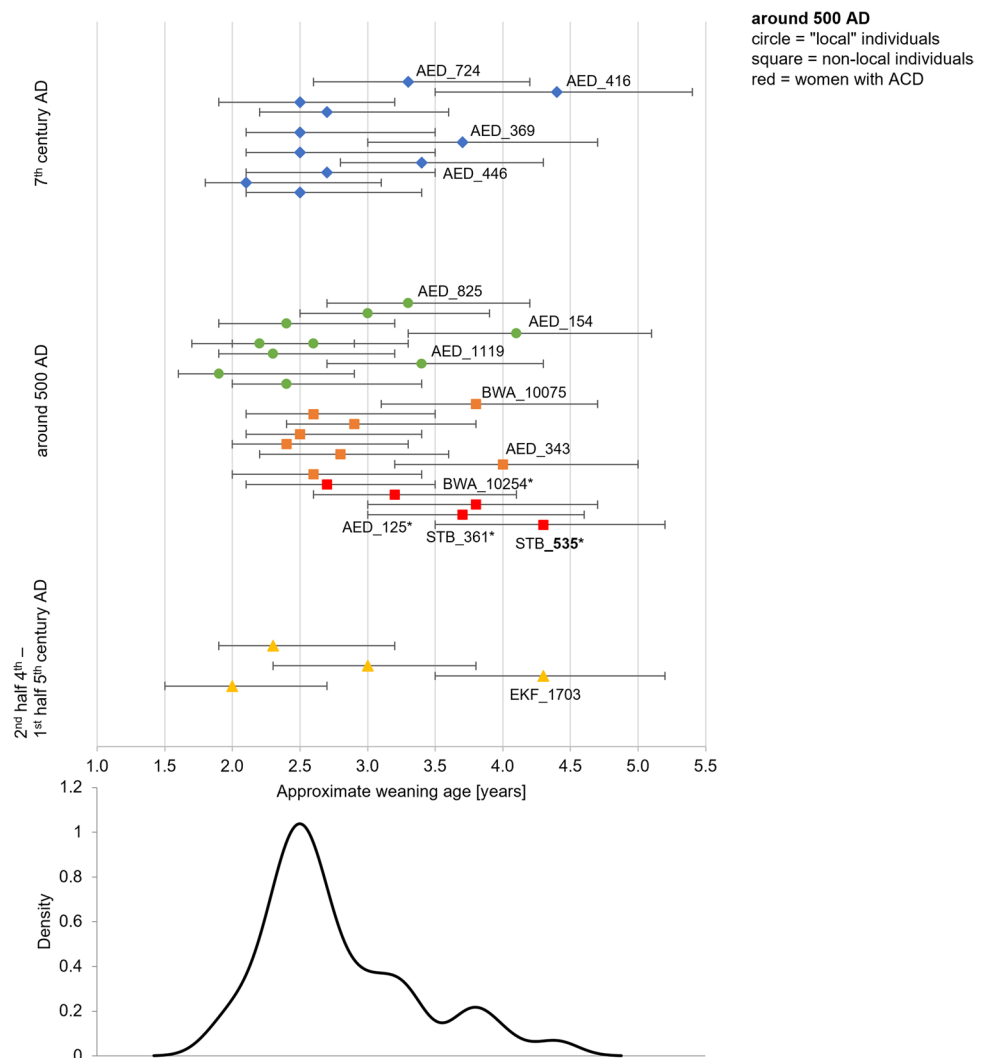
The decrease of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ is generally faster at the beginning and slows down towards the end (Table S2.2). Higher rates are predominately found in individuals whose profiles show a greater total decrease in isotope ratios, while lower rates are mostly found in profiles with a smaller total decrease (Kruskal–Wallis test $p(\delta^{15}\text{N}) < 0.001$, $p(\delta^{13}\text{C}) = 0.003$, Table S1.2.9). In some cases (AED_343,

AED_416, STB_361*, BWA_10254*), $\delta^{15}\text{N}$ decreases rather gradually at a smaller rate ($\leq 1.5\text{‰}$ per year) but still exhibits a notable extent of decline (1.5 to 2.5‰) (Table S2.2). Nevertheless, in certain cases $\delta^{15}\text{N}$ ($n = 11$) or $\delta^{13}\text{C}$ ratios ($n = 5$) decrease discontinuously, i.e., ratios decrease after a short stabilization or even increase. However, this second decrease mainly ($n = 9$) follows a prior constant decline in $\delta^{15}\text{N}$ (e.g., AED_105, AED_1129), whereas a discontinuous drop in $\delta^{13}\text{C}$ only occurs if values were either stable or increasing at the very beginning (e.g., AED_211, AED_343) (Table 1, Fig. 4).

Age of complete weaning

At the population level, weaning was completed by the age of 3.0 years (Figure S1.2.1). This corresponds to the average of individual weaning ages (3.0 ± 0.7 years (sd)). Notably, individual weaning ages vary significantly, ranging from as early as 1.9 years to as late as 4.4 years (Table 1).

Fig. 5 Estimated individual weaning ages considering normal tooth development. Error bars display deviations for early and late tooth development. The Kernel density estimation (KDE) in the lower part is based on a Gaussian kernel. The optimal bandwidth for the Gaussian kernel was calculated using the “solve the equation” method by Sheather and Jones (1991).



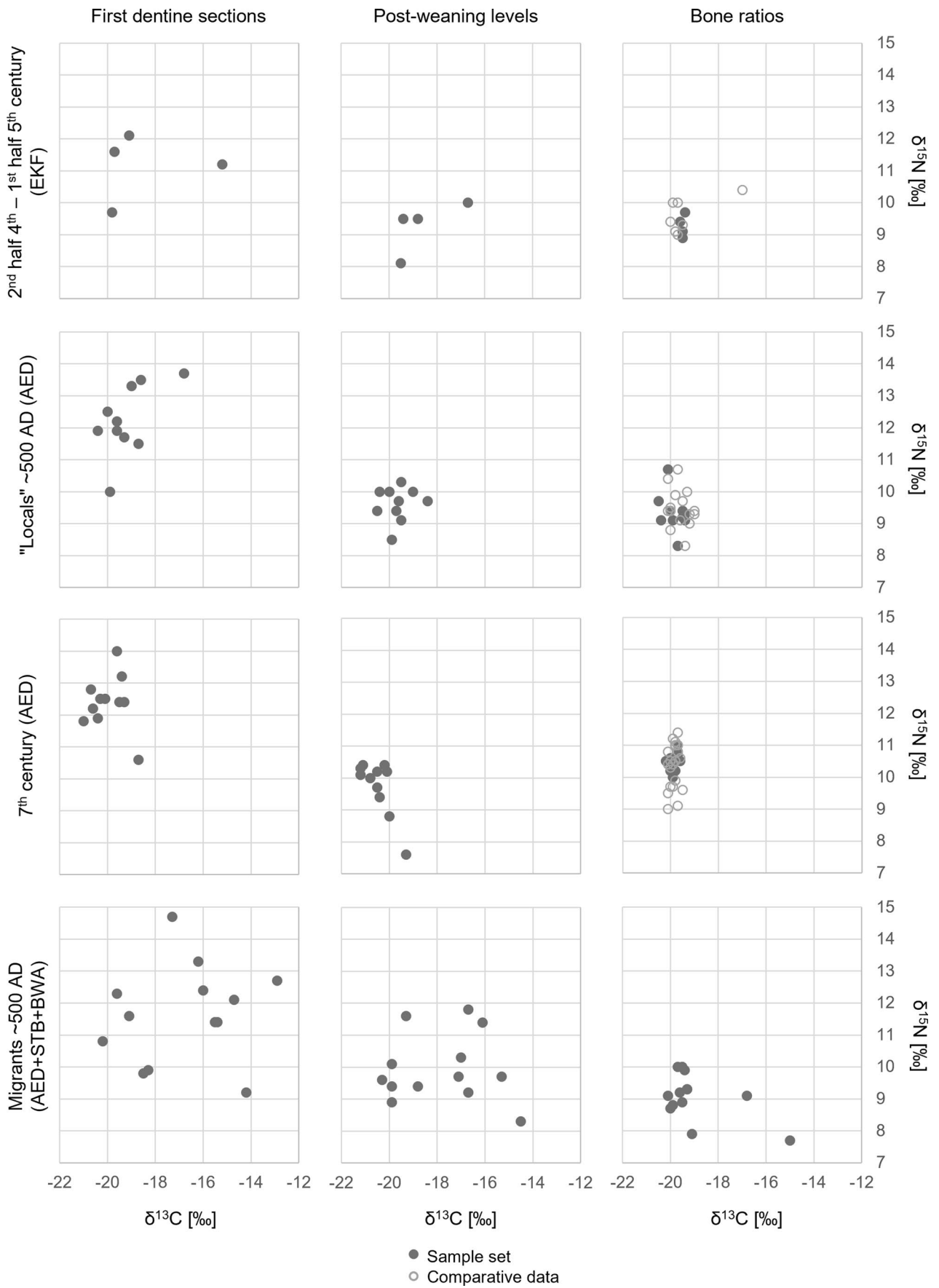


Fig. 6 Group differences of stable light isotope ratios in populations from Erding (EKF, AED). Filled data points show individual values for each stage. Additional comparative bone data are given as unfilled points. Comparative data are taken from Sofeso et al. (2012) for Late Roman individuals (EKF), Hakenbeck et al. (2010), and Velte et al. (2023) for individuals from around 500 AD, and Neidich (2023) for individuals dating from the seventh century (AED). Information about individual's provenance is taken from Velte et al. (2023).

Additionally, the Gaussian kernel density distribution of weaning ages shows two side peaks at over 3.0 years, however, a clear main peak is found around 2.5 years (Fig. 5).

Post-weaning phase

Values and intra-tooth patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are also significantly variable within the post-weaning phase. Post-weaning levels, assigned as the mean value of dentine ratios covering 3 years following individual weaning ages, show a great range and different extent and direction of fluctuations (Table 1, Fig. 4).

$\delta^{15}\text{N}$ ($n=3$) or $\delta^{13}\text{C}$ ($n=5$) post-weaning levels only rarely match individual bone ratios ($\pm 0.1\%$). Instead, they tend to be more or less equally frequent found above ($\delta^{15}\text{N}$: by 0.2 to 2.3‰, $n=20$; $\delta^{13}\text{C}$: by 0.5 to 4.3‰, $n=15$) or below bone ($\delta^{15}\text{N}$: by -0.2 to -2.4% , $n=15$; $\delta^{13}\text{C}$: by -0.2 to -1.6% , $n=18$) (Table 1). The $\delta^{15}\text{N}$ values frequently ($n=30$) exhibit an increase from the lowest point of the initial decrease, including the observed second drops, towards the end of the profiles, creating the impression of a post-weaning dip (e.g., AED_249, AED_1119) (Fig. 4). However, the visibility of this dip varies; in some cases, it is not clearly visible due to generally more fluctuating ratios (e.g., EKF_1663, BWA_10075), or because the later increase is of lower extent (e.g., EKF_1719, AED_204) or shorter time (e.g., AED_125*, AED_724) (Fig. 4). Four individuals (AED_154, AED_249, AED_501, AED_1053) show covariant decreasing $\delta^{13}\text{C}$ values during the later increase in $\delta^{15}\text{N}$.

Group differences

Intra tooth patterns are highly variable within all defined groups. However, we identified some general characteristics and group differences.

Late Roman individuals (EKF, $n=4$) and the "locals" from around 500 AD (AED, $n=10$) show similar ranges of ratios in the first dentine sections, as well as the extent or rate of decreasing values, post-weaning levels, and bone ratios (Table 1, Fig. 6). Their post-weaning levels often align or are slightly above their individual bone values (Table 1). In both of these groups, single individuals display increased $\delta^{13}\text{C}$ values in dentine, at least temporarily

(EKF_1663, EKF_1700, AED_154), corresponding with the levels observed in some migrants from around 500 AD.

In contrast, generally lower $\delta^{13}\text{C}$ dentine ratios are found in individuals dating from the seventh century (AED, $n=11$) (Table 1, Fig. 6). The $\delta^{13}\text{C}$ ratios in their first dentine sections show a trend towards lowered values, often ($n=5$) falling below their individual bone values (Table 1), which are found within a range similar to that of earlier times (Table 1, Fig. 6). Furthermore, their post-weaning $\delta^{13}\text{C}$ levels following the weaning dip are significantly decreased in comparison to the levels of the "locals" from around 500 AD (pairwise comparison $p=0.004$, Table S1.1.23). Although the mean difference is even greater when compared to Late Roman individuals (Table 1), it remains non-significant, probably due to the small sample size and increased variability within the group. Additionally, $\delta^{15}\text{N}$ ratios show similar ranges in the first dentine sections, extent of decrease, and post-weaning levels in comparison to individuals dated into Late Roman times (Table 1, Fig. 6). However, the post-weaning $\delta^{15}\text{N}$ levels are significantly lower than their individual bone values (paired samples test $p=0.004$, Table S1.2.25). Moreover, the bone ratios of individuals from the seventh century are significantly increased compared to Late Roman individuals (pairwise comparison $p=0.002$, Table S1.2.38) and the "locals" from around 500 AD (pairwise comparison $p=0.015$, Table S1.2.38).

In summary, the estimated weaning ages are remarkably variable across all periods, including prolonged weaning (weaning age > 3.0 years) (Fig. 5). Thus, no significant chronological change in weaning age is found. Instead, chronological differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios result from changes in the seventh century.

However, we found some significant differences linked to individuals' origin around 500 AD. Migrants (AED+STB+BWA, $n=13$) generally exhibit more variable results at the beginning of the observation and in post-weaning levels compared to the "locals" (AED, $n=10$). While $\delta^{15}\text{N}$ ratios of first dentine sections and post-weaning levels tend to differ only slightly from "locals," $\delta^{13}\text{C}$ ratios are, on average, significantly increased in the first dentine sections (Mann–Whitney U test $p=0.006$, Table S1.2.1) and in post-weaning levels (independent-samples Mann–Whitney U test $p=0.003$, Table S1.2.1) (Fig. 6).

Moreover, discontinuously and more strongly decreasing $\delta^{13}\text{C}$ ratios in the earliest parts of profiles are exclusively found in migrants (Table 1), but also stronger fluctuations in $\delta^{13}\text{C}$ after weaning were primarily observed in non-locals (Fig. 4). Both $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values in post-weaning dentine of migrants differ significantly from their bone ratios (paired samples test $p(\delta^{15}\text{N})=0.006$, $p(\delta^{13}\text{C})=0.019$, Table S1.2.25), which in turn do not differ from bone ratios of the "locals" (Fig. 6). Differences between post-weaning and bone ratios are more pronounced in female

migrants (AED + STB + BWA, $n = 10$) compared to male migrants (AED, $n = 3$) (independent-samples Mann–Whitney U test $p = 0.028$, Table S1.2.19). This difference can be linked to a sex-dependent difference among migrants: $\delta^{13}\text{C}$ ratios of the first dentine sections of female migrants are, on average, significantly increased compared to male migrants (Mann–Whitney U test $p = 0.007$, Table S1.2.2), as well as post-weaning $\delta^{13}\text{C}$ levels (independent-samples Mann–Whitney U test $p = 0.049$, Table S1.2.19). Additionally, more increased post-weaning $\delta^{15}\text{N}$ levels are primarily found in female migrants (Table 1). Although we did not find a significant difference between migrants and “locals” in estimated weaning ages, a clear trend towards later weaning in migrants is noticeable. Among the group of non-locals, females, particularly those with ACD, more often show prolonged weaning (weaning age > 3.0 years) (Fig. 5). Thus, the observed differences between migrants and “locals” are primarily based on non-local females, while male migrants and single female migrants do not exhibit major differences to the “local” population from around 500 AD, in which no differences between men ($n = 5$) and women ($n = 5$) were found.

Linear enamel hypoplasia (LEH)

The preservation of the anterior dentition was highly variable (Fig. 4). LEH data from individuals with an incomplete dentition (less than three teeth assessable) is labeled with an exclamation mark ($n = 6$) in Fig. 4. One individual with a completely missing dentition was excluded from the analysis (Table 2). The incidence of LEH varies, with the highest count being 12. Most individuals have fewer than four ($n = 14$), averaging to 1.9 ± 3.0 (sd) LEH. Slightly over half of the individuals display at least one LEH ($n = 21$). The development of LEH happened between 0.8–1.9 years and 4.5–5.9 years, on average at age 3.0–4.2.

The number of LEH significantly correlates with the mean formation age (midpoint of determined formation

span) of the first LEH (Pearson correlation $p = 0.014$, Table S1.3.7), thus, individuals with LEH appearing at an early age tended to develop more LEH.

The average age at the formation of the first LEH (2.7–3.8 years, Table 2) corresponds well with the mean age at the cessation of weaning. However, on an individual level, the initial formation of LEH only rarely matches the estimated weaning age ($n = 5$, Fig. 4). In most individuals, the first LEH developed with a temporal offset (≥ 0.5 years) relative to the estimated weaning ages, either before ($n = 8$) or after ($n = 8$) weaning (Fig. 4).

Regarding the total number of LEH, only a few defects appear during the weaning phase ($n = 13$, 17.6%), primarily observed in individuals ($n = 6$, 75.0%) with noticeable late weaning (≥ 3.4 years). The largest number of LEH formed around the age of complete weaning ($n = 25$, 33.8%) or in the post-weaning phase ($n = 36$, 48.7%). Apparently, they most frequently occur during the supposed post-weaning dip in $\delta^{15}\text{N}$ ratios (Fig. 4).

Group differences

We found no indications of differences in the presence, number, or timing of LEH associated with individuals’ origin or between sexes among the “locals” from around 500 AD (Table 2). Regarding the timing of defects, there is also no clear difference between the diachronic time phases (Table 2).

However, we observe a difference in individuals from the seventh century ($n = 11$, AED) compared to earlier diachronic phases (Late Roman: $n = 3$, EKF; “Locals” ~ 500 AD: $n = 10$, AED), at least in the presence and number of LEH. They display LEH more frequently (Table 2) and with significantly higher prevalence (independent samples Kruskal Wallis test $p = 0.026$, Table S1.3.10), compared to the “local” individuals from around 500 AD (pairwise comparison $p = 0.027$, Table S1.3.11), but not in comparison to Late Roman individuals, most likely due to the small sample size (Table 2).

Table 2 LEH data. Each observed LEH has a formation span not a time point. Therefore, the mean formation ages are calculated as: (mean of startpoint of formation span) – (mean of endpoint of formation span)

LEH data Group	Presence of LEH			Mean number of LEH \pm sd	Mean formation age of all LEH (years)	Mean formation age of first LEH (years)
	N evaluated	N present	% observed (95% CI)			
All individuals	37	21	56.8 (40.5–70.3)	1.9 ± 3.0	3.0–4.2	2.7–3.8
(1) Late Roman (EKF)	3	1	33.3 (0–100)	1.3 ± 2.3	3.0–4.2	1.6–2.5
(2) “Locals” ~ 500 AD	10	4	40 (10–70)	0.6 ± 0.8	3.6–4.9	3.4–4.6
Men	5	2	40 (0–80)	0.6 ± 0.9	3.7–4.9	3.1–4.3
Women	5	2	40 (0–80)	0.6 ± 0.9	3.9–5.2	3.4–4.6
(3) Migrants ~ 500 AD	13	7	53.8 (30.8–76.9)	1.2 ± 1.9	2.8–4.0	2.7–3.8
(4) Seventh century	11	9	81.8 (54.5–100)	4.3 ± 4.1	3.0–4.2	2.6–3.6

Moreover, it is noteworthy that the two individuals from the seventh century with the highest numbers of LEH (AED_213: 11, AED_100: 12) died in early adult to adult age (Fig. 4).

Discussion

General observations and limitations

Unsurprisingly, we could not detect the phase of exclusive breastfeeding or the introduction of complementary foods in any individual in our sample (see Fig. 1), as our observation begins only after the age of 6 months. From that age onward, complementary food is necessary to satisfy the growing need of the infant for energy and nutrients (e.g. Michaelsen et al. 2000, Dewey and Brown 2003). Nevertheless, the weaning process, indicated by the “weaning dip” becomes visible in almost all profiles: it is clearly noticeable in $\delta^{15}\text{N}$ but less obvious in $\delta^{13}\text{C}$. As the trophic level effect is smaller and the impact of supplementary foods is stronger on $\delta^{13}\text{C}$, the values align faster to the breastfeeder’s value (e.g., Fuller et al. 2006).

The observed variability of both isotope ratios within the weaning phase can be attributed to various factors, including the potential onset of the weaning before observation starts, the progression of the weaning process after the start of observation, the nutrition and physiology of the breastfeeding female, different breastfeeding practices, different weaning foods, as well as variations in the physiology and health of the infant (e.g., Fuller et al. 2006; Crowder et al. 2019; Henderson et al. 2022). However, this variability increased significantly with the inclusion of individuals who grew up in different geographic locations or during different historical periods.

A distinct pattern is apparent in most profiles in all groups: increasing $\delta^{15}\text{N}$ ratios after cessation of weaning creates the impression of a “post-weaning dip.” Depleted $\delta^{15}\text{N}$ values in the post-weaning phase before a subsequent rise were observed in many other studies across diverse populations from various periods and socio-economic backgrounds (Beaumont et al. 2012; Eerkens et al. 2011; Fernández-Crespo et al. 2018; Henderson et al. 2014). This pattern indicates that the proportion of animal protein only later reaches a level comparable to that of adults. However, post-weaning values below bone level must not exclusively be linked to diet but may also be influenced by a general cause, such as physiological features (e.g., Henderson et al. 2022) e.g., related to growth (e.g., Waters-Rist and Katzenberg 2010). In our study, $\delta^{15}\text{N}$ values of the “post-weaning dip” drop less frequently below individual adult bone values in contrast to previous studies, but we assume that although less clear, this is still comparable.

In our method for calculating the weaning age, we include the lowest point of the $\delta^{15}\text{N}$ curve in the calculation. Since this point often lies within the post-weaning dip, which is also influenced by factors that are independent of breastfeeding, it can be assumed that the weaning age may be overestimated. Furthermore, some patterns within profiles can be misinterpreted using our method, e.g., discontinuously decreasing $\delta^{15}\text{N}$ ratios during the weaning process. These values may result from variable breastfeeding frequencies, changing trophic levels of weaning foods, or physiological stress (Fuller et al. 2005; Mekota et al. 2006; Reitsemá 2013). Thus, subsequent decreasing $\delta^{15}\text{N}$ ratios may also belong to the actual weaning dip but are not considered in the calculations of our weaning ages.

However, these problems are not unique to our method. Especially the inclusion of the lowest $\delta^{15}\text{N}$ ratios of the “post-weaning dip” is a common practice (e.g., Scharlotta et al. 2018). While a heavily pronounced post-weaning dip could also pose an issue for the method recently published by Ganiatsou et al. (2023), it can be assumed that this method is less sensitive to discontinuously decreasing $\delta^{15}\text{N}$ ratios. The tool they developed, the WEaning Age FiNder tool (WEAN), employs fitted curves that compensate for such fluctuations. Nevertheless, compared to Ganiatsou et al. (2023), our method offers the advantage of presenting results as age ranges, allowing for a better illustration of the uncertainty of weaning ages due to the biological variability of tooth development.

Regardless of the methodological approach, the calculation of weaning ages should only be seen as an approximation for various reasons. Changes in the protein source potentially appear with a temporal offset of several weeks to month in isotope profiles (e.g., Waters-Rist et al. 2006, Chinique de Armas et al. 2022). This individually delayed onset can hardly be reconstructed, making precise weaning age calculations nearly impossible. Additionally, the temporal resolution of the isotope profiles is limited by the sampling method and by the age assignment of the samples, resulting in rather broad age ranges for certain data points or events derived (Figs. 4 and 5).

The latter also applies to the calculation of LEH formation ages. The less precise determination of the position of the LEH used here (see Methods) further increases the error beyond normal biological variability.

Weaning time and process

Most individuals exhibit a rather abrupt weaning process, with rapidly decreasing $\delta^{15}\text{N}$ ratios. A rapid decline can result from low-trophic complementary foods with limited protein supply leading to less expressed fractionation of $^{15}\text{N}/^{14}\text{N}$ and thus to a decrease in $\delta^{15}\text{N}$ of the body’s nitrogen pool (e.g., Henderson et al. 2022). Another possibility

is that higher amounts of protein in complementary foods result in a faster decline of $\delta^{15}\text{N}$ as it more efficiently dilutes the infant's nitrogen pool (e.g., Fuller et al. 2006). However, we consider the first scenario to be more likely, as many typical weaning foods (e.g., bread and cereal porridge) are high in carbohydrates and low in protein (e.g., Sellen and Smay 2001), which is also consistent with Roman and Medieval written sources (e.g., Prowse et al. 2008, Bourbou and Garvie-Lok 2009, Dasen 2015).

We observe a high variability in weaning ages within pre-defined groups from Late Roman times, around 500 AD and the seventh century. Although some of this variation may be related to the methodological limitations mentioned above, it underscores that breastfeeding and weaning practices can differ significantly within a population, e.g., through adaptation to infant's health and needs, the availability of resources probably dependent on the season, but also social ideals and status, etc. (e.g., Bourbou and Garvie-Lok 2009, Bourbou et al. 2013, Bourbou et al. 2019). Our findings indicate that most individuals are fully weaned around the middle of the third year, although we also observe more extended weaning periods of up to 4 years.

This pattern is consistent with the historical context of the complete cessation of breastfeeding at around 3 to 4 years of age in the Roman provinces, as recommended in ancient texts by the Greek medic Galen, while at the Center of the Roman Empire, breastfeeding typically ceased earlier around 2 years, following the recommendations of another Greek physician, Soranus, which is corroborated by bioarcheological data from the Classical, Hellenistic, and Roman periods (summary in Fulminante 2015). Although mainly shorter weaning ages between 1 and 2 years were observed in Continental Europe during the Early Middle Ages (Fulminante 2015), some studies suggest that breastfeeding also ended rather late around the age of three in some communities (Haydock et al. 2013, Herrscher 2003). Dittmann and Grupe (2000) calculated the weaning age based on bone collagen values from the remains of deceased children buried in a cemetery from the same time and region of our study. Interestingly, they also found a rather late weaning age of 3 years for these "non-survivors." In contrast, it is often assumed that individuals who were not breastfed or only for a short period tend to have a lower survival rate (e.g., Stuart-Macadam & Dettwyler 1995; Bourbou et al. 2019; Fernández-Crespo et al. 2022). Consequently, one might expect to observe a higher average weaning age in our study, which in turn exclusively examined survivors.

This underscores the need for additional data on subadults and adults from the same graveyards to get a more detailed picture of infant diet and associated stress in Late Roman and Early Medieval Europe.

Weaning stress

LEH observed in our sample indicates that stress, which manifests as enamel defects, only rarely occurs during the progressing replacement of breastmilk with non-breastmilk liquids and foods. This demonstrates that the weaning process does not represent an intrinsic stress factor for infants (Kendall et al. 2021), but also that continued breastfeeding may not fully protect them from all stressors. The increase in LEH formed at the end of the weaning process or in the post-weaning phase in our sample supports the assumption that the final cessation of breast milk as a source of nutrients and immune protection initiates a high-risk phase, a "weaning conundrum," as suggested by Fernández-Crespo et al. (2022).

An increase in $\delta^{15}\text{N}$ (Fuller et al. 2005; Mekota et al. 2006; Reitsema 2013), especially with a simultaneous decrease in $\delta^{13}\text{C}$ (Beaumont et al. 2018; Beaumont and Montgomery 2016, Craig-Atkins et al. 2020) is often considered a sign of physiological stress. In our study, this pattern is observed in only a few individuals and is never clearly associated with LEH formation. This correlation is perhaps not necessarily to be expected, since different types, severities, and durations of physiological stress do not necessarily have the same effect on enamel formation and isotope composition. However, LEH formation is often correlated with the observed post-weaning dip in $\delta^{15}\text{N}$ ratios, suggesting that infants may be more susceptible to stressors during this phase, possibly related to growth or a low-trophic diet (see above). Nevertheless, far more data is needed to confirm this connection.

Furthermore, we found a positive correlation between the formation age of the first LEH and the total frequency of LEH, a trend also reported previously (e.g., Berbesque and Hoover 2018). This supports the hypothesis that individuals exposed to stressors earlier in life may be less resilient to subsequent crises due to damaged immunological competence (Goodman and Armelagos 1989; Armelagos et al. 2009, Larsen 2015). Previous studies have also linked higher LEH frequencies to an earlier age-at-death (e.g., King et al. 2005; Boldsen 2007; Armelagos et al. 2009), which could explain why the two individuals with the highest numbers of LEH died at early (adult) ages (AED_100, AED_213).

Nutrition and early life histories: links to diachronic changes

We did not find significant differences in weaning patterns or weaning ages related to the considered diachronic phases.

However, we observe variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in dentine as well as bone collagen values among individuals who potentially grew up in the same region around Erding but during different chronological times. We assume that this

is probably related to diachronic changes in infant feeding practices and/or economics.

While the isotope ratios of diachronic groups generally show great overlap (Fig. 6), we only observe increased $\delta^{13}\text{C}$ ratios in the dentine of single individuals dating into Late Roman times or around 500 AD (Figs. 4 and 6). This could be related to millet consumption, either indirectly through breastmilk or directly as weaning foods. Hence, this may also be a potential hint at a previously undetected non-local origin of these individuals. However, sporadic millet cultivation cannot be ruled out, although millet was not commonly used in Bavaria during this time (e.g., Hakenbeck et al. 2010; Velte et al. 2023).

People living in Bavaria between Roman and Medieval times primarily consumed a C3 plant-based terrestrial diet (e.g., Hakenbeck et al. 2010, Sofeso et al. 2012, Velte et al. 2023). The ratio of animal protein to plant content can only be estimated by comparing contemporaneous material, ideally from the archaeological context. However, only animal remains are available for the Late Roman site EKF, where concentration-dependent mixture models suggest an average of 25% animal vs. 75% plant content in the human diet (Harbeck and von Heyking 2014). Similar human bone isotope values of individuals from AED dating around 500 AD suggest comparable ratios if we assume the same “baseline” with unchanged $\delta^{15}\text{N}$ values of the consumed plants and thus animals.

Assuming an unchanged baseline, the significant increase in $\delta^{15}\text{N}$ bone ratios of individuals from AED dating into the seventh century indicates a higher proportion of animal protein. Increased $\delta^{15}\text{N}$ bone ratios were also found in other burial sites in Bavaria dating to the seventh century (Strott 2007, Czermak 2011) and were interpreted as an increase in protein content associated with intensification of livestock and dairy farming (e.g., Fehr 2019). However, this increase can just as well display a baseline shift related to changes in mixed farming practices through the beginning agricultural revolution (e.g., Fehr 2019), e.g., more intensive land use and increased fertilization (e.g., Bogaard et al. 2007; Fraser et al. 2011). Confirmation of a baseline shift would require sufficient data of different animal genera from the site, which is currently lacking.

The two interpretations of the bone collagen isotopes (“higher protein” vs. “different baseline hypothesis”) also affect the understanding of the observed differences from the post-weaning diet. In individuals from Late Roman times and around 500 AD, we see post-weaning $\delta^{15}\text{N}$ levels within the range of adult collagen ratios, suggesting that infants had an adult-like diet during this time (e.g., Fournier et al. 2022). However, for the seventh century, we observe post-weaning $\delta^{15}\text{N}$ levels that are lower than the comparatively increased values we observe in adults of this time but consistent with the post-weaning $\delta^{15}\text{N}$ levels in children from the earlier

times. If we follow the “higher protein hypothesis,” i.e., the increased bone levels in adults result from a higher amount of animal protein, it raises the question of why the protein intake increased in adulthood but not in childhood in the seventh century compared to earlier times. Additionally, the post-weaning diet in the seventh century appears not different from the previous century in $\delta^{15}\text{N}$ values but in $\delta^{13}\text{C}$ ratios, which are lower. If we consider the “higher protein hypothesis,” additional explanations are needed. Lower $\delta^{13}\text{C}$ values can indicate a changing plant spectrum, e.g., towards a greater diversification (e.g., Lewit 2009, Zach 2019) or smaller amounts of specific plants (e.g., millet) or plant parts with increased $\delta^{13}\text{C}$ (e.g., cereal grains see Cernusak et al. 2009), but this should then also be reflected in bone collagen ratios, at least to some extent, which is not observed.

If we follow the “different baseline hypothesis,” in which higher environmental $\delta^{15}\text{N}$ levels are assumed for the seventh century, the observed pattern becomes more straightforward to explain. In this scenario, there was no increase in the protein content of the human diet, and the $\delta^{15}\text{N}$ values of children’s post-weaning diet can be interpreted as a low protein diet because they fall below adult bone collagen ratios (e.g., Eerkens et al. 2011). This also explains the decreased post-weaning $\delta^{13}\text{C}$ levels compared to earlier times, potentially influenced by a change in plant foods but masked by the higher proportion of animal protein and thus not visible in bone isotope values. Furthermore, the observed increase in LEH in the seventh century can also be explained by a low-trophic diet, which is not supported by the “higher protein hypothesis.” A low-trophic post-weaning diet may not be sufficient to meet the needs of children, especially during growth phases (e.g., Henderson et al. 2022), and may also make them more susceptible to external stressors.

In summary, the hypothesis that an increase in environmental $\delta^{15}\text{N}$ occurred in the seventh century and thus children of this period had a lower protein diet compared to adults, which can in turn be related to the increased formation of the LEH, seems more reliable. However, further research in the region on human remains and especially on animal bones is needed to validate this conclusion.

Early life histories: links to a foreign origin

Profiles of migrants who spent their childhood in other geographical locations, with divergent ecogeographic conditions and thus distinct isotopic baselines, as well as probably different dietary traditions, show the most variable isotope profiles. While some of the increased $\delta^{15}\text{N}$ ratios are possibly related to regions with higher nitrogen baselines, e.g., marine areas (Fig. 6), the fluctuating $\delta^{13}\text{C}$ ratios at significantly higher levels suggest a more frequent consumption of C4 plants such as millet (Fig. 4). However, these unusual signals are primarily limited to the diet during childhood,

whereas most of the bone collagen ratios of migrants adapted to the common signal of a C3 plant-based terrestrial diet (Fig. 6) (for detailed discussion see Velte et al. 2023).

Despite differences in the nutritional spectrum of migrants during childhood, it is notable that only about half of the non-local individuals were completely weaned within the third year of life, all males included. For the remaining women, especially those with ACD, the cessation of weaning was later. Additionally, three female migrants, including two with ACD, show a rather gradual weaning transition curve, which was only observed in four individuals in our sample. The difference between non-local men and women, along with the remarkable variability within the group of migrants, can be attributed to the fact that those migrants originate from different geographical regions in which people likely had a different lifestyle and culture (Velte et al. 2023). Although some migrants potentially grew up in the same cultural environment as people from southern Bavaria, women with ACD likely spent their childhood in a community with different traditions.

Cases of intentional cranial modification in pre- and early history Europe are known since the fifth to fourth centuries BC but become more widespread with the arrival of nomadic populations from the Eurasian Steppe. Thus, ACD is interpreted as an indication of nomadic incomers, e.g., the historically documented Huns and other nomadic groups associated with a pastoralist way of life (for review see Hakenbeck 2009). Some studies suggest that prolonged weaning times are found in such non-sedentary populations (e.g., Clayton et al. 2006, Waters-Rist et al. 2011). Rationales are still debated, ranging from a lack of appropriate weaning foods and the distribution of subsistence activities and workload to fertility control (e.g., Sellen and Smay 2001; Fouts et al. 2005; Sellen 2007; Bocquet-Appel 2011). Studies of pastoral societies indicate that infant diets are supplemented with animal products, including milk, meat, and blood rather than cereals or nuts (Ventresca Miller et al. 2014, 2017, Sellen 2001, Sellen and Smay 2001), which can result in a more gradual weaning curve. Furthermore, a study of pastoralists from Bronze Age Eurasia found that weaning was completed by the age of four (Ventresca Miller et al. 2017). Isotope data from an Early Medieval nomadic culture of Tuva (Southern Siberia) also suggests weaning after two to three years of age (Milella et al. 2022). Consequently, growing up in a community with a pastoralist and/or nomadic lifestyle is a possible explanation for the observed more gradual weaning pattern and prolonged weaning times observed in several migrants. However, to our knowledge, no data on weaning patterns of Early Medieval nomadic-pastoralists or individuals with ACD other than those presented here are available to substantiate this assumption.

To date, no men with ACD or who have a genetic origin or dietary pattern similar to these women have been

identified in Bavarian cemeteries. This may indicate that men and women came to the region from different areas, at least partly as a result of distinct, gender-specific migration processes.

Conclusion

The analysis of weaning patterns and childhood stress through stable light isotope analysis of serial dentine samples is an intricate task, with many aspects that are still far from being sufficiently understood.

However, our study has revealed that more abrupt weaning occurred within the third year of life of most of the individuals buried between Roman and Medieval Southern Bavaria. Although we found no differences in weaning ages between males and females or diachronic time phases, we observed a trend towards prolonged weaning in some migrants.

Moreover, we found some evidence for a diachronic change in isotope baselines linked to changes in economic practices, which likely also affect infant feeding strategies, reflected in the post-weaning diet and stress level in the seventh century. If individuals experienced stress associated with the weaning process, it manifests itself primarily in the post-weaning phase, after the complete cessation of breastfeeding. This demonstrates that the choice of foods during this time is important as low-trophic foods can potentially facilitate the susceptibility of infants to stressors.

In summary, we were able to show different weaning practices in various communities, both by chronological distance and cultural differences, although weaning patterns within a community can also vary significantly. However, more data are needed including the remains of subadults and animals to obtain a more detailed and complete picture of infant feeding strategies and related stress.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12520-023-01882-6>.

Acknowledgements The authors are grateful to Silvia Codreanu-Windauer (BLfD) for information regarding the individuals from Burgweinting. We thank George McGlynn and Albert Zink for their comments on our results. The authors also thank Gabriele Arenz for their support with the collagen extraction of some specimens and Joris Peters for providing lab space and equipment.

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Funding Open Access funding enabled and organized by Projekt DEAL. We thank the Bavarian State Office for Monument Protection and the town of Erding for financial support.

Data availability All data analyzed in this study are included in the submitted supplementary information files.

Declarations

Competing Interests The authors have no competing interests to declare that are relevant to the content of this article.

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