Those who died very young—Inferences from $\delta^{15}N$ and $\delta^{13}C$ in bone collagen and the absence of a neonatal line in enamel related to the possible onset of breastfeeding

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Abstract

Objectives: Stable isotope analysis has often been used in neonatal remains from archaeological contexts to investigate the presence of a signal of breastfeeding and weaning in past populations. Tooth histology on the other hand might be used as an indicator of birth survival. This pilot study aimed to investigate the feasibility of using stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values from neonatal bone collagen to elucidate if values deviating from the adult female average could indicate breastfeeding and co-occur with the presence of a neonatal line (NNL). The combination of these independent indicators might be useful in clarifying the fate of individuals who died around birth.

Materials and Methods: Bone collagen from 21 archaeological human and animal specimens was extracted and analyzed via mass spectrometry for $\delta^{15}N$ and $\delta^{13}C$. A verification of the stable isotope results was undertaken using tooth histology on three individuals who were investigated for the presence of a NNL as an indicator of live birth and short survival.

Results: The biological age of the human samples varied between 8.5 lunar months (Lm) and 2 postnatal months (Pm) of age. All except one individual exhibited elevated $\delta^{15}N$ values compared to the female average. The histological analyses revealed no NNL for this and two further individuals (n = 3).

Discussion: The results indicate that elevated nitrogen values of very young infants relative to a female average in archaeological contexts are not necessarily associated with a breastfeeding onset signal, and therefore cannot be used exclusively as a proxy of birth survival. The elevation might be possible due to various reasons; one could be nutritional, in particular maternal stress during pregnancy or a metabolic disorder of mother and/or her child. In those cases, the evaluation of a NNL might reveal a false breastfeeding signal as seen for two individuals in our sample who have elevated nitrogen values despite the fact no NNL could be observed. Overall, our data support the growing awareness that bone collagen $\delta^{15}N$ values of neonates/infants should not be used as a proxy for breastfeeding or birth survival on its own.

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1. Introduction

Reproductive failure and neonatal or first year mortality are crucial variables for the demographic development of past and present populations (Rollet, 1997; United Nations Inter-agency Group for Child Mortality Estimation [UN IGME], 2018). From a medical or paleopathological viewpoint, stillbirth and very early life mortality are regularly regarded as the outcome of adverse influences affecting the mother and the fetus during pregnancy or at birth (Bukowski et al., 2014). These adverse influences might reflect environmental, nutritional, sanitary, and health conditions. There is also evolutionary reasoning tied to reproductive failure as fetal death might be adaptive in a sense that it forgoes investment in offspring during times of reduced chances of survival and would enable successful reproduction under improved conditions (Peacock, 1990). To untangle these different views and interpret mortality patterns, it is crucial to discern between stillborn fetuses and those who survived birth at least for a short period. Age-at-death estimation by conventional osteometric and morphognostic criteria rely on mean values obtained from reference samples of known age (Cunningham, Scheuer, & Black, 2016; Fazekas & Kósa, 1978), but the population affinity might actually be quite distant to the population under consideration (Scheuer & Black, 2004). Furthermore, relying on population means can result in the misclassification of cases in individuals that are advanced or delayed in their development compared to average percentiles (Scheuer & Black, 2004). To overcome these limitations, additional methods have to be employed.

1.1. Stable isotopes

Stable isotope analysis is regularly used in archeological contexts to identify dietary habits including breastfeeding, weaning as well as migration patterns of past populations (Ambrose, 1993; Burt, 2015; Dupras & Schwarcz, 2001; Fuller et al., 2004; Jay, 2009; Katzenberg, 2008; Katzenberg, McKenzie, Losey, Goriunova, & Weber, 2012; King et al., 2017; Lee-Thorp, 2008; Lösch, Grupe, & Peters, 2006; Lösch, Moghaddam, Grossschmidt, Risser, & Kanz, 2014; Millard, 2000; Moghaddam, Müller, Hafner, & Lösch, 2016; Schurr, 1998; Tsutaya & Yoneda, 2015; Vogel & van der Merwe, 1977). Several studies have also been conducted in order to investigate the relationship of isoto-pic composition between mother–infant pairs (De Luca et al., 2012; Dupras, Schwarcz, & Fairgrieve, 2001; Dupras & Tocheri, 2007; Fogel, Tuross, & Owsley, 1989; Fuller, Fuller, Harris, & Hedges, 2006; Herrscher, Goude, & Metz, 2017; Jay, 2009; Pearson, Hedges, Molleson, & Ozbek, 2010; Prowse et al., 2008; Reynard & Tuross, 2015; Richards, Mays, & Fuller, 2002; Romek et al., 2013; Schurr, 1998). It has been postulated that the tissue of a breastfed child expresses elevated nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) values compared to the tissue of its mother due to the trophic level effect. A breastfed individual would show this enrichment as it consumes the mothers' breastmilk, which is enriched in 15N in relation to the mother's diet and her body tissue (Fuller et al., 2006; Herrscher et al., 2017). This effect is especially seen in different body tissues for δ15N with an enrichment of about 2–3‰ between the different trophic levels and an enrichment of 1‰ of $\delta^{13}C$ (Fuller et al., 2006) while Herrscher et al. (2017) recorded a difference of less than 0.5‰ between child and mother. A problem encountered with skeletal remains is that the exact incorporation time of different elements into the collagen of developing bone is unknown and depends on the type of bone (Fahy, Deter, Pitfield, Miszkiewicz, & Mahoney, 2017) and the biological age of individuals themselves (Lehn, Rossmann, & Graw, 2015; Tsutaya & Yoneda, 2013). This complexity related to bone growth led other authors to use tooth dentin as the source for collagen since dentin growth is strictly appositional and normally lacks remodeling (Beaumont et al., 2018). The incorporation period of elements into collagen in a growing fetus is dependent on the availability of nutrients in the mother's womb and the pace of bone metabolism and turnover, and therefore reflects the mother's diet to an unknown extent. According to Lehn et al. (2015), the

measureable isotope composition around birth has been incorporated into the fetal bone between 0 and 4 weeks prior to birth. The detection of a breastfeeding signal in infants postnatally has been reported by Fogel et al. (1989) and Nitsch, Humphrey, and Hedges (2011) after 8–12 weeks in fingernails and 5–6 weeks in rib collagen, respectively. It has to be emphasized further that the trophic effect in infants always develops relative to their mothers tissues (De Luca et al., 2012; Herrscher et al., 2017; Lehn et al., 2015). However, in archaeological contexts a reliable identification of mother–infant pairs can rarely be substantiated.

1.2. Neonatal line

During birth, the level of physiological stress normally increases sub-stantially for both the mother and the fetus. Normal labor as well as birth complications leads to elevated intrauterine pressure and regu-larly to a decreasing oxygen supply resulting in acidosis that consti-tutes stress for the fetal physiology (Schneider & Kuchenbecker, 1986). The enamel forming cells, the ameloblasts, seem to be espe-cially susceptible to an elevated level of systemic stress. The impairment of cell function results in recording this experienced stress in the microstructure of the enamel, which might even be associated with hypoplasia (Hillson, 2014; Kronfeld & Schour, 1939; Stimmler, Snodgrass, & Jaffe, 1973). In the enamel of permanent teeth, different degrees of impaired enamel matrix secretion result in changes in enamel microstructure ranging from subtle alterations like accentua- tion of the incremental pattern (Witzel, Kierdorf, Schultz, & Kierdorf, 2008), the indistinctness of prims profiles (obscured prism identity, (Risnes, 1999)), the formation of aprismatic enamel, or even an abrupt cessation of enamel formation (Witzel et al., 2008). This can be related to the intensity of the stressor, modulated by ameloblast intrinsic fac- tors (e.g., age). Similar structural aberrations have also been shown for the enamel of deciduous teeth (Witzel, 2014a). Since enamel lacks remodeling, the perturbation induced by the birth process can be rec- ognized as an accentuated incremental marking referred to as the neonatal line (NNL) in teeth that start hard tissue formation in utero, which includes all deciduous teeth and first molars. This incremental marking can be analyzed retrospectively in tooth sections by light and scanning electron microscopy (SEM; Birch & Dean, 2014; Hillson, 2014; Hurnanen, Visnapuu, Sillanpaa, Loyttyniemi, & Rautava, 2017; Kurek et al., 2016; Sabel et al., 2008; Sebald, Stenzel, Gruenewald, & Grupe, 2018; Witzel, 2014b). Nondestructive techniques will poten-tially replace destructive approaches based on sectioned teeth in the future (Le Cabec, Dean, & Begun, 2017; Nava et al., 2017; Tafforeau & Smith, 2008). The NNL marks the division between the enamel secreted prenatally and postnatally. Its location is dependent on the beginning of enamel secretion in utero starting with the deciduous incisors ~15 weeks after ovulation, (Sunderland, Smith, & Sunderland, 1987) and the duration of gestation (Skinner & Dupras, 1993). The presence of a NNL can be taken as an indicator for surviving birth (Schwartz, Houghton, Macchiarelli, & Bondioli, 2010; Smith & Avishai, 2005; Witzel, 2014b), but the incomplete mineralization of recently secreted enamel matrix makes the portion along the secretory front prone to the loss of material (Antoine, Hillson, & Dean, 2009). Therefore, a period of 7–10 days of surviving birth is usually neces- sary to detect the NNL in micrographs of enamel (Whittaker & Mac- Donald, 1989; Witzel, 2014a,b). The necessity of formation of at least a small amount of postnatal enamel in order to be able to observe the NNL is the reason why its absence is not an exclusive indicator of stillbirth.

In our study, we investigate the stable isotope values ($\delta^{15}N$ and$\delta^{13}C$) of 11 fetal/neonatal remains buried intramurally in the Roman settlement of Petinesca, Switzerland (Figure 1) and compared them to the stable isotope data of the associated adult women from the nearby contemporary cemetery (Lösch et al., 2013). It is hypothesized, that lower or similar $\delta^{15}N$ values of the infants compared to the average of the adult women suggest the absence of a breastfeeding signal. Considering the lag time for the incorporation of a signal related to mother's milk into bone collagen, the age estimates of the infants would preclude the occurrence of such a signal. In cases where indi- viduals had teeth preserved, enamel was investigated microscopically for the presence of a NNL. Elevated $\delta^{15}N$ values

could only be interpreted as a breastfeeding signal when a certain period of survival after birth is documented by the presence of a NNL. This approach provides a framework to better understand and interpret elevated $\delta^{15}N$ values of bone collagen from neonates and infants.

2. Materials and Methods

Samples from 11 infants (first to third century AD) and 14 animal bones of 7 different species of the Roman settlement Petinesca (Figure 1) were used for the analysis of stable nitrogen and carbon isotopes (Table 1). The infants were found, buried in close association with the ancient roman buildings (Figure 1). That the youngest infants were not buried at the cemetery is common in Roman times and is mentioned by different authors (Kramis & Trancik, 2014; Ulrich- Bochsler & Zwahlen, 2011). Additionally, the published data from Lösch et al. (2013) of the related cemetery were taken for comparison (two female skeletons).
The ages at death of the infants were morphologically estimated to be between 8.5 lunar months (Lm) up to 2 months of postnatal age (Ulrich-Bochsler & Zwahlen, 2011). Additionally, age was estimated based on measurements of vertical tooth height of the three individ- uals with preserved teeth compared to the data published by Deutsch, Pe'er, and Gedalia (1984).

2.1. Methods

2.1.1. Collagen extraction and stable isotope measurements

The extraction of collagen from bone was performed using a modified acid-base extraction method based on Ambrose (1990, 1993), DeNiro (1985), and Longin (1971).
The selected long bones were mechanically cleaned and treated in an ultrasound bath with distilled water (ddH2O). The samples were then dried and ground to powder. For each sample, 500 ± 3 mg of bone powder was weighed. The samples were treated with 10 mL of 1 M hydrochloric acid (HCl) for 20 min at room temperature. Samples were washed with ddH2O until neutralized and 10 mL of 0.125 M sodium hydroxide (NaOH) was added to the solution, which then was left for incubation (20 hr) at room temperature.
The samples were washed with ddH2O until neutralization and 10 mL of 0.001 M HCl was added and placed in a 90o C water bath for 10–17 hr allowing solubilization of the collagen.
The solubilized collagen was filtered and freeze-dried. The solid collagen was sent for analysis via an isotope ratio mass-spectrometry by isolab GmbH, Schweitenkirchen, Germany.
The provided results are the mean of the three measurements taken for each sample. They are expressed in δ-notation per mil (‰).
The standards used are Vienna Pee Dee Belemnite (V-PDB), for carbon and Ambient Inhalable Reservoir (AIR), for nitrogen. Internal analytical errors were recorded as 0.1‰ for δ13C and 0.2‰ for δ15N.

2.1.2. Quality of collagen

The quality criteria of collagen were (a) a value of >1% collagen por- tion of dry bone, (b) a molecular C/N ratio between 2.9 and 3.6 (Ambrose, 1993; DeNiro, 1985), and (c) no strong deviation of values of %C and %N from the abundance of 43% and 15–16%, respectively, typical for fresh bone (Ambrose, 1990). Only samples that fulfilled all three criteria were considered for interpretation.

2.1.3. Histological analyses of teeth and NNL detection

Teeth for histological sectioning were available for three individuals: SPV 12, the deciduous lower left first incisor (dLLI1); SPT 1, the decid- uous upper left first incisor (dULI1) and the deciduous upper right third premolar (dURP3); SPV 10, the deciduous lower right first incisor (dLRI1) and the deciduous lower right canine (dLRC). Teeth were measured in length and photographed prior to prepa- ration for histological analysis. Following the method described by Kierdorf, Witzel, Upex, Dobney, and Kierdorf (2012) and Witzel et al. (2008), the teeth were embedded in epoxy resin (Biodur E1/E12) and cured at 30o C. Axiobuccolingual sections were cut using a water-cooled power saw with a diamond-coated cutting wheel. Silicon carbide sandpaper (grit 600–2,000) and a motorized rotor grinder, together with a diamond suspension (particle size 3 μm) were used to polish cut surfaces. Fur- ther sample preparation varied depending on which microscope imag- ing technique was used; light microscopy or SEM.

2.1.4. Light microscopy

The polished samples were fitted onto a glass slide with Biodur epoxy resin and cured at 30°C. At ~600 μm distance from section was then cut from each mounted sample. Further grinding was performed with silicon carbide sandpaper of different grit (grit 600–4,000), with a final polishing. Resulting section thickness was~40 μm for all specimens. The slides were then cover slipped and left to cure at 30°C. Examination of the slides was performed with a Zeiss Axioskop 2 Plus microscope with an attached Zeiss Axiocam 503 color digital camera.

2.1.5. Scanning electron microscopy

For inspection in the SEM, the polished cut surfaces of the blocks of SPT 1 tooth dULI1 and dURP3, SPV 10 tooth dLRI1, and SPV 12 tooth dLLI1were viewed uncoated in a Zeiss EVO MA 15 SEM operated with the backscattered secondary electron detector and photographed.

3. Results

3.1. Stable isotope analysis

Out of the 11 human samples, one sample SPV 2 did not yield enough collagen for analysis and was therefore excluded (Table 2). For the 14 samples of the animal specimens SPV B3, SPV C/O3, and SPV O/C6 did not yield collagen and were also excluded (Table 3). This resulted in a total of 21 samples for analysis. Figure 2 presents the $\delta^{13}C$ against $\delta^{15}N$ values of the human and animal samples including the averages. The neonatal $\delta^{13}C$ values (average −18.9 ± 0.9‰) scatter around the female average −19.2 ± 0.4‰ taken from Lösch et al. (2013) for $\delta^{13}C$. For the $\delta^{15}N$, the average value of the females (9.3 ± 0.2‰) is 1.9‰ lower than the average value of the neonates (11.2 ± 0.9‰). When plotting the infant samples alone, sample SPV 10 is not aligned to the other infants (−16.6‰ for $\delta^{13}C$ and 9.2‰ for $\delta^{15}N$), but falls within the values of the female $\delta^{15}N$ average (9.3‰ ± 0.2‰; Figures 3 and 4).

3.2. Tooth histology

In total five teeth from three different individuals were available for histological analyses. Two individuals exhibiting elevated $\delta^{15}N$ values (SPV 12; SPT 1) and one exhibiting no elevated $\delta^{15}N$ value (SPV 10) compared to the mean of the adult females from the site. The age estimation based

on tooth height measurements (Table 4) revealed an age in the gestational period for SPT 1 (36 weeks—ninth Lm) and around term birth (40 weeks—tenth Lm) for SPV 10 and 12.
In the enamel of all the examined deciduous teeth, no indication of a NNL could be detected (Figure 5). Depending on the tooth type, the position of the enamel forming front at birth is expected to be continuous along the external enamel surface (canines, premolars) or restricted to the cervical margin (incisors). In the dLRI1 of SPV 10 in this location an obscured prism identity is observable underneath the surface, but no corresponding expression could be found along the external enamel sur- face of the dLRC of the same individual (Figure 5a,b). Teeth from both remaining individuals exhibit prismatic enamel structure up to the enamel surface (Figure 5c,d). In the dLLI1 of individual SPV 12, an over- all accentuation of the incremental pattern was evident (Figure 5d).
None of the five teeth used for histological analysis expressed a NNL.

4. DISCUSSION

The following assumptions concerning stable isotope data of neonates and infants with regard to breastfeeding and weaning serve as basis for our discussion (Beaumont, Montgomery, Buckberry, & Jay, 2015; Tsutaya & Yoneda, 2015):

1. The effect of an individual's metabolism on the stable isotope values is minor.
2. The female average of the archeological site can be used as a proxy for the diet and the approximate isotope values of the actual mothers to evaluate the trophic level shift of the infants.
3. The biological age of the infants is reflecting the chronological age; hence, age-at-death estimations do not affect the interpretation of stable isotope values greatly.
4. The cause of death did not affect the stable isotope values of the individual.

In addition, it must be stated whether the stable isotope values from an individual are evaluated in relation to group data or individual data to verify the capabilities of the interpretation.

4.1. $\delta^{15}N$ values as proxies for birth survival?

The observed trophic level shifts of the δ15N values from animals to females and to infants demonstrated the expected distribution in general. This is further confirmed by the δ13C values of herbivores, omnivores, and carnivores in comparison to humans. In studies on recent breastfed mother–infant pairs, δ13C values show a small enrichment in body tissue, such as nails and hair, of less than 1‰ from mother to child (Fuller et al., 2006; Herrscher et al., 2017). This was also observed for our bones with a fractionation of between mothers and their infants (Fogel et al., 1989; Fuller et al., 2006). There could be several reasons for the slightly lower Δ15N value observed in this study. One explanation might be the age of the infants who died before attaining two postnatal months (Pm). There- fore, an incompletely incorporated breastfeeding signal could be the reason. Another reason is discussed by Waters-Rist and Katzenberg (2010) who observed a lower ^{15}N enrichment than is generally expected between trophic levels, due to a positive nitrogen balance in infants who quickly grow. During growth, more nitrogen is taken up $\Delta^{13}C$ females–infants= 0.1‰ when excluding SPV 10, since SPV 10 is an than is excreted and as a result the lighter isotope (^{14}N) is incorporated more rapidly leading to a decrease of the overall $\delta^{15}N$ value outlier according to its $\delta^{13}C$ and $\delta^{15}N$ values. When including SPV 10 the $\Delta^{13}C$females–infants is 0.4‰ (Figure 4). This is in concordance with the general assumption that $\delta^{13}C$ values from (archaeological) bone collagen are not suitable for assessing breastfeeding signals (Fuller et al., 2006). On the other hand, it is postulated that a breastfeeding signal is present when δ15N values of

neonate's and infant's bone collagen are enriched compared to a female average at the same site (Tsutaya & Yoneda, 2015). At Studen Petinesca, an enrichment between the female and the infant average is observable with a fractionation of Δ^{15}Nfemales–infants = 2.0‰ when excluding SPV 10 (Δ^{15}Nfemales– infants = 1.8‰ when including SPV 10). This represents the lower mar- gin of the published values of a trophic level enrichment by 2–3‰ (Katzenberg & Lovell, 1999). Hence, a trophic level shift of 3‰ from females to infants could not be expected for the very young infants of this study. Our initial hypothesis might be true for individual SPV10 as all other neonates show increased values and they might express the onset of a breastfeeding signal (Figure 4a). However, the hypothesis cannot be verified or falsified based on our collagen δ^{15}N data solely. This is also stated by Beaumont et al. (2018) who found that δ15N of bone collagen is less reliable compared to δ^{15}N of co-forming dentin and concluded that bone collagen is not an authentic material for statements about breastfeeding and weaning. The reasons discussed in the next paragraph indicate why the interpretation of the δ^{13}C and δ^{15}N values for all neonates/infants is challenging, especially for outliers such as SPV 10 and fetal remains such as SPV 1 (Figure 4).
However, some reasons for the relatively low δ^{15}N value of SPV 10, whose age-at-death was estimated to be 10 Lm, could be:

1. A period of increased growth resulting in a positive nitrogen balance.
2. The reflection of an enriched δ^{15}N value in relation to the actual mother, whose δ^{15}N value must have been even lower and is not observed from Studen Petinesca. If this were the case, the mother would have had a diet very low in or without any animal proteins. But, none of the investigated females show δ^{15}N values indicative for a low animal protein diet, in relation to the fauna data.
3. The age-at-death estimation of the neonate does not reflect its chronological age.
4. A pathological condition, for example, a metabolic disorder, of SPV 10 and/or its mother.
5. A combination of several aforementioned reasons.

The higher δ^{13}C value of SPV 10 compared to the other infants and the female average might be "normal" in relation to the true mother who might have had a diet rich in C_4 plants or could be indicative for a metabolic disorder of the infant. However, currently comparative data are missing to evaluate the discordant carbon value of SPV 10 in the context of birth survival and breastfeeding. In general, a death around birth is the only postulation for SPV 10, based on the stable isotope values in combination with the morphological–anthropological investigation, which can be made so far.
The enriched ^{15}N value of SPV 1 (Figure 4a) follows the trend of other reported preterm neonate (5–7 Lm) values (Kinaston et al., 2009; Richards et al., 2002; Siebke, Kanz, Witzel, & Lösch, 2016). This could be indicative for an early breastfeeding signal but is highly unlikely due to the young age and the required incorporation time of dietary elements into bone collagen. Therefore, we consider the value of SPV 1 as a "falsely interpreted breastfeeding signal" which might be due to a pregnancy-related negative nitrogen balance of the mother or a certain fluctuation of the δ^{15}N values during the fetal period as described by De Luca et al. (2012) and Fuller et al. (2006) observed for modern mother–infant pairs.

4.2. Challenges of using δ^{15}N values to identify breastfeeding signals

The interpretation of a trophic level shift between females and infants in a population as an indicator for breastfeeding is based on limited information. These limitations comprise for instance metabolic effects, the amount of food as well as the sources of food. The concept of equifinality has to be considered, as a combination of different pre-conditions can lead to the same outcomes due to genetically and environmentally determined unknown individual metabolic factors. This includes the variables affecting the incorporation and turnover rates of the lighter and heavier isotopes with

regard to stress levels and different tissue types as discussed by Beaumont et al. (2018). Namely, both, a diet rich in meat as well as periods of starvation might result in elevated nitrogen values (Mekota, Grupe, Ufer, & Cuntz, 2006).
Additionally, if the average nitrogen value of females is used as a proxy of the overall female diet, little food variability, and a negligible migration must be assumed within the population. Notwithstanding that, this is currently the most practiced method to evaluate archaeological "mother–infant" isotope relationships and has been widely accepted, as reviewed by Tsutaya and Yoneda (2015) and Reynard and Tuross (2015). The fact that the actual mothers might not be among the investigated adult females limits the interpretation of individual values. It has been discussed that pregnant and lactating females express different isotope ratios than non-pregnant and non-lactating females. Hence, using a female average whose reproductive status is unclear a sample bias might be introduced (De Luca et al., 2012; Fuller et al., 2004, 2005).
Furthermore, the morphological investigations and aspects—which are determined by genetic factors and environmental influences—such as age-at-death estimations and growth as well as the subadult mortality bias (DeWitte & Stojanowski, 2015) increase the uncertainties when studying archeological remains in three ways.

1. The age estimation represents a bias as the age at death of the individual might be over or underestimated.
2. The effect of growth or maternal nutritional stress on bone collagen, such as positive or negative nitrogen balance, requires further research to be better understood (Beaumont et al., 2018; Fuller et al., 2004; Reitsema & Muir, 2015).
3. The reasons for the infants' death (subadult mortality bias) are difficult to interpret but these may have had an effect on the collagen synthesis. Therefore, the stable isotope data may be influenced and the interpretation might be affected.

4.3. Combining stable isotopes and tooth histology—A NNL?

Tooth histology alone does not provide information about breastfeeding; however, it has been argued that an accumulation of macroscopic and microscopic hypoplastic enamel defects in certain regions of the crowns in parts of the permanent dentition can be used as a proxy for weaning (Blakey, Leslie, & Reidy, 1994; Katzenberg, Herring, & Saunders, 1996). However, tooth microstructure has been demonstrated to enable an accurate high-resolution age determination (Antoine et al., 2009; Birch & Dean, 2013; Hillson, 2014). Different regular incremental markings with a well-established periodicity in humans and their primate relatives can be counted or measured in order to provide data on the timing of tooth formation (Hupková, Dirks, Králík, & Račanská, 2015; Smith, Reid, & Sirianni, 2006; Witzel, Flohr, & Becker, 2009). If teeth of non-primate mammals mammals are to be investigated by this approach, periodicities of incremental markings have to be confirmed independently since they can deviate from the primate pattern (Kierdorf, Breuer, Witzel, & Kierdorf, 2019; Kierdorf, Kierdorf, Frölich, & Witzel, 2013). Age-at-death estimation depends on the identification of an incremental marking that is formed at the time of birth. Corresponding to the size and stage of development of the deciduous dentition and the permanent first molar at birth, a particularly prominent marking can regularly be identified in the enamel and sometimes also in the dentin of the respective teeth (Hillson, 2014; Rushton, 1933; Schour, 1936). Given the estimated ages at death of the examined individuals, only a short-term birth survival was expected. Two of the individuals (SPV 10 and SPV 12) have metric and developmental characteristics of being full-term fetuses or neonates (Tables 4 and 5). The third individual (SPT 1) was estimated even younger (c. 36 weeks of gestation) following the charts presented by Deutsch et al. (1984). Considering the $\delta^{15}N$ value of SPV 10, it was hypothesized to have died before a breastfeeding signal could have been developed, whereas SPV 12 and SPT 1 were expected to have survived birth long enough to develop an elevated $\delta^{15}N$ value due to

consumption of mother's milk (Tables 5 and 6; Figure 4a). According to this assumption, the survival period must have been within the range of about 2 months since this is the suggested turnover rate for bone collagen in infants in order to exhibit a fully established breastfeeding signal (Nitsch et al., 2011). However, histology yielded no indication for the presence of a NNL in all three individuals. Moreover, the only microstructural conspicuity was present in the dLRI1 of SPV 10 where in the cervical enamel a much less distinct prismatic enamel microstructure was located in the last pre-served enamel at the outer edge. However, no corresponding alteration could be found in the dLRC of the same individual. Low mineralization and the peripheral location of the enamel secreted last make it prone to be lost easily (Antoine et al., 2009). If the obscured prism identity in the dLRI1 of SPV 10 was caused by birth, this individual could only have survived for a few days. In the remaining two individuals, no indication of a NNL was detected although both exhibited elevated δ15N values compared to the female average. Taking into account the metrical and developmental age indicators of these individuals, they are a late fetus (SPT 1) and a full-term fetus/neonate (SPV 12). According to the lag time for the incorporation of a breastfeeding signal of about 2 months, such a period equals the growth of an enamel prism stretch of 120–150 μm (given a mean daily secretion rate of c. 4 μm, [Birch & Dean, 2009]) and an approximate crown elongation of 600–1,000 μm (given a mean daily extension rate of 20–30 μm in midcoronal or cervical crown portions,[Mahoney, 2015]). Subtracting these values from the enamel forming edge of the tooth would indicate the region where the NNL must have been located. But, these tooth regions did not exhibit indications for the presence of a NNL. Survival rates for such substantially pre-term born infants must have been much lower in history prior to intensive medical care. Instead, the elevated $\delta^{15}N$ values of SPV 12 and SPT 1 could be an indirect indication for nutritional in particular maternal stress of their actual mothers who might have had a negative nitrogen balance. Hence, not enough nitrogen is taken up than is required resulting in catabolism of the organisms own body tissue (Fuller et al., 2004; Katzenberg & Lovell, 1999). Also, the overall slight accentuation of the incremental pattern in both individuals can be interpreted as an indication for an under-supply of the organism during pregnancy. Pregnancy as a state of "accelerated starvation" can lead to an increase of nitrogen values due to nutritional stress if a pregnant woman does not meet her increased nutritional demands (Fuller et al., 2005). This could be an explanation of the increased $\delta^{15}N$ values of a fetus or neonate lacking a NNL as in the case of SPV 12 and SPT 1. Alternatively, the $\delta^{15}N$ values of SPV 12 and SPT 1 might not have been or been only slightly elevated compared to their actual mothers tissues (De Luca et al., 2012; Fuller et al., 2006). Those $\delta^{15}N$ values could have been above the female average, either due to a diet rich in animal products or due to starvation or in particular pregnancy-related negative nitrogen balance (Mekota et al., 2006). The lack of a visible NNL in both individuals with elevated $\delta^{15}N$ values compared to the female average is however contradicting the initial hypothesis that such an elevation can serve as a proxy of breastfeeding or the survival of birth for a short period.

4.4. Past and future—How to proceed?

The data in this study reveal how challenging it is to use stable isotopes from bone collagen to draw conclusions about the survival of the first postnatal weeks of neonates by means of a "breastfeeding signal." Another aspect, which should be reconsidered in the future, is the fact that for archeological remains a δ15N female average in comparison with neonatal δ15N data is not sufficient for a comprehensive interpretation of survival and breastfeeding. Our results as well as other studies (Beaumont et al., 2015, 2018; Fahy et al., 2017) show how significant the application of more than one method is in order to validate the stable isotope results (Table 6). Further studies are required to prove or falsify our hypothesis that neither nitrogen ratios solely, nor in combination with the investigation of a NNL, provide sufficient information about birth survival. High $\delta^{15}N$ values of preterm neonates require further investigation to ensure that "false breastfeeding signals" are properly interpreted in studies of past societies on birth survival, breastfeeding, and weaning.

Additionally, stable isotope ratios of neonates should be discussed carefully when comparing to female data or averages of the site if a clear actual mother–neonate/infant relationship cannot be verified in archeological settings, for example, mother–infant double-burials in combination with aDNA analysis. However, further combined diagnostic methods could provide information about postnatal survival, breastfeeding, and weaning. For instance, as proposed by Beaumont et al. (2018), the use of dentin collagen as a more accurate reflection of stable isotope values of the prenatal and postnatal phases. Booth, Redfern, and Gowland (2016) investigated bone diagenesis via micro-CT to evaluate the survival of neonates. Also, trace elements, for example, Barium and Strontium extracted from teeth is an additional approach to evaluate the time of birth and dietary changes during the first months of life (Austin et al., 2013; Humphrey, Dean, Jeffries, & Penn, 2008; Tacail et al., 2017). Hence, we strongly recommended to use at least two independent methods to verify if and for how long an individual might have survived birth. Moreover, to verify if an observed fractionation could be due to breastfeeding or provides indirect information about maternal stress further baseline data is required.

5. CONCLUSION

The data presented in this study emphasize that care should be exercised in the interpretation of only stable isotope data of (very) young infants and associated females from the same archeological context, especially when discussing single individuals. With the combination of tooth histology, it is shown that elevated $\delta^{15}N$ values of fetuses and neonates are not necessarily due to the onset of a breastfeeding signal and do not have to be an indication of survival in the first postnatal weeks. They might instead be caused by other factors, such as maternal stress, metabolic disorders, or due to the absence of the actuals mother's comparable data. However, tooth histology supports the interpretation and verification of possible stillbirth or false breastfeeding signals based on stable isotope analysis of neonates/infants from archeological contexts, but it has to be applied with care due to taphonomic alteration of the material. Especially, when the $\delta^{15}N$ and $\delta^{13}C$ values of the actual mother are not available for comparison with the analyzed infant isotope ratios the values have to be interpreted carefully. This pilot study advises further investigations to establish sufficient baseline data of neonatal and infant stable isotope values from bone and dentin collagen, trace elements from teeth, micro-CT of bones as well as tooth histology. These investigations will verify the usability of combined methods as indicators of short-term postnatal survival, breastfeeding, and weaning.

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DATA ACCESSIBILITY

The raw data are provided in Tables 2 and 3 within this publication. Other data referred to in the manuscript has been cited.

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REFERENCES

Ambrose, S. H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. Journal of Archaeological Science, 17(4), 431–451.

Ambrose, S. H. (1993). Isotopic analysis of paleodiets: Methodological and interpretive considerations. In M. K. Sanford (Ed.), Investigations ofancient human tissue—Chemical analyses in anthropology (pp. 59–130). Amsterdam: Gordon and Breach Science Publishers.

Antoine, D., Hillson, S., & Dean, M. C. (2009). The developmental clock of dental enamel: A test for the periodicity of prism cross-striations in modern humans and an evaluation of the most likely sources of error in histological studies of this kind. Journal of Anatomy, 214(1), 45–55.

Austin, C., Smith, T. M., Bradman, A., Hinde, K., Joannes-Boyau, R., Bishop, D., … Arora, M. (2013). Barium distributions in teeth reveal early-life dietary transitions in primates. Nature, 498, 216–219.

Beaumont, J., Atkins, E.-C., Buckberry, J., Haydock, H., Horne, P., Howcroft, R., … Montgomery, J. (2018). Comparing apples and oranges: Why infant bone collagen may not reflect dietary intake in the same way as dentine collagen. American Journal of Physical Anthropology, 167(3), 524–540.

Beaumont, J., Montgomery, J., Buckberry, J., & Jay, M. (2015). Infant mortality and isotopic complexity: New approaches to stress, maternal health, and weaning. American Journal of Physical Anthropology, 157(3), 441–457.

Birch, W., & Dean, C. (2009). Rates of enamel formation in human deciduous teeth. Frontiers in Oral Biology, 13, 116–120.

Birch, W., & Dean, M. C. (2013). A new method of calculating human deciduous enamel formation times. Bulletin of the International Association for Paleodontology, 7(1), 47–55.

Birch, W., & Dean, M. C. (2014). A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation. Journal of Forensic and Legal Medicine, 22, 127–144.

Blakey, M. L., Leslie, T. E., & Reidy, J. P. (1994). Frequency and chronological distribution of dental enamel hypoplasia in enslaved African Americans: A test of the weaning hypothesis. American Journal of Physical Anthropology, 95(4), 371–383.

Booth, T. J., Redfern, R. C., & Gowland, R. L. (2016). Immaculate conceptions: Micro-CT analysis of diagenesis in Romano-British infant skeletons. Journal of Archaeological Science, 74, 124–134.

Bukowski, R., Hansen, N. I., Willinger, M., Reddy, U. M., Parker, C. B., Pinar, H., … for the Eunice Kennedy Shriver National Institute of Child Health and Human Development Stillbirth Collaborative Research Network. (2014). Fetal growth and risk of stillbirth: A populationbased case–control study. PLoS Medicine, 11(4), e1001633.

Burt, N. M. (2015). Individual dietary patterns during childhood: An archaeological application of a stable isotope microsampling method for tooth dentin. Journal of Archaeological Science, 53, 277–290.

Cunningham, C., Scheuer, L., & Black, S. (2016). Developmental juvenile osteology (2nd ed.). London: Academic Press.

De Luca, A., Boisseau, N., Tea, I., Louvet, I., Robins, R. J., Forhan, A., … Hankard, R. (2012). d15N and d13C in hair from newborn infants and their mothers: A cohort study. Pediatric Research, 71(5), 598–604.

DeNiro, M. J. (1985). Postmortem preservation and alteration of in vivo bone-collagen isotope ratios in relation to paleodietary reconstruction. Nature, 317, 806–809.

Deutsch, D., Pe'er, E., & Gedalia, I. (1984). Changes in size, morphology and weight of human anterior teeth during the fetal period. Growth, 48(1), 74–85.

DeWitte, S. N., & Stojanowski, C. M. (2015). The osteological paradox 20 years later: Past perspectives, future directions. Journal of Archaeological Research, 23(4), 397–450.

Dupras, T. L., & Schwarcz, H. P. (2001). Strangers in a strange land: Stable isotope evidence for human migration in the Dakhleh Oasis, Egypt. Journal of Archaeological Science, 28(11), 1199–1208.

Dupras, T. L., Schwarcz, H. P., & Fairgrieve, S. I. (2001). Infant feeding and weaning practices in Roman Egypt. American Journal of Physical Anthropology, 115(3), 204–212.

Dupras, T. L., & Tocheri, M. W. (2007). Reconstructing infant weaning histories at Roman period Kellis, Egypt using stable isotope analysis of dentition. American Journal of Physical Anthropology, 134(1), 63–74.

Fahy, G. E., Deter, C., Pitfield, R., Miszkiewicz, J. J., & Mahoney, P. (2017). Bone deep: Variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. Journal of Archaeological Science, 87(November 2017), 10–16.

Fazekas, I. G., & Kósa, F. (1978). Forensic fetal osteology. Akadémiai Kiadó: Budapest.

Fogel, M. L., Tuross, N., & Owsley, D. (1989). Nitrogen isotope tracers of human lactation in modern and archaeological populations. Annual Report of the Director, Geophysical Laboratory, Carnegie Institution of Washington 1988–1989 (pp. p111–p117). Washington, DC: Carnegie Institution of Washington.

Fuller, B. T., Fuller, J. L., Harris, D. A., & Hedges, R. E. M. (2006). Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. American Journal of Physical Anthropology, 129(2), 279–293.

Fuller, B. T., Fuller, J. L., Sage, N. E., Harris, D. A., O'Connell, T. C., & Hedges, R. E. (2004). Nitrogen balance and delta15N: Why you're not what you eat during pregnancy. Rapid Communications in Mass Spectrometry, 18(23), 2889–2896.

Fuller, B. T., Fuller, J. L., Sage, N. E., Harris, D. A., O'Connell, T. C., & Hedges, R. E. M. (2005). Nitrogen balance and δ15N: Why you're not what you eat during nutritional stress. Rapid Communications in Mass Spectrometry, 19(18), 2497–2506.

Herrscher, E., Goude, G., & Metz, L. (2017). Longitudinal study of stable isotope compositions of maternal milk and implications for the palaeodiet of infants. Bulletins et Mémoires de la Société d'Anthropologie de Paris, 29(3–4), 131–139.

Hillson, S. (2014). Tooth development in human evolution and bioarchaeology. Cambridge: Cambridge University Press.

Humphrey, L. T., Dean, M. C., Jeffries, T. E., & Penn, M. (2008). Unlocking evidence of early diet from tooth enamel. Proceedings of the National Academy of Sciences of the United States of America, 105(19), 6834–6839.

Hupková, A., Dirks, W., Králík, M., & Racanská, M. (2015). Retrieval of a developmental record from dental remains: Stress chronology and age at death assessment of a juvenile skeleton from a medieval site in Moravia, Czech Republic.

Hurnanen, J., Visnapuu, V., Sillanpaa, M., Loyttyniemi, E., & Rautava, J. (2017). Deciduous neonatal line: Width is associated with duration of delivery. Forensic Science International, 271(February 2017), 87–91.

Jay, M. (2009). Breastfeeding and weaning behaviour in archaeological populations: Evidence from the isotopic analysis of skeletal material. Childhood in the Past, 2(1), 163–178.

Katzenberg, M. A. (2008). Stable isotope analysis: A tool for studying past diet, demography, and life history. In M. A. Katzenberg & S. R. Saunders (Eds.), Biological anthropology of the human skeleton (pp. 413–441). Hoboken, NJ: Wiley-Liss.

Katzenberg, M. A., Herring, D. A., & Saunders, S. R. (1996). Weaning and infant mortality: Evaluating the skeletal evidence. American Journal of Physical Anthropology, 101(S23), 177–199.

Katzenberg, M. A., & Lovell, N. C. (1999). Stable isotope variation in pathological bone1. International Journal of Osteoarchaeology, 9(5), 316–324.

Katzenberg, M. A., McKenzie, H. G., Losey, R. J., Goriunova, O. I., & Weber, A. (2012). Prehistoric dietary adaptations among hunterfisher- gatherers from the Little Sea of Lake Baikal, Siberia, Russian Federation. Journal of Archaeological Science, 39(8), 2612–2626.

Kierdorf, H., Breuer, F., Witzel, C., & Kierdorf, U. (2019). Pig enamel revisited—Incremental markings in enamel of wild boars and domestic pigs. Journal of Structural Biology, 205(1), 48–59.

Kierdorf, H., Kierdorf, U., Frölich, K., & Witzel, C. (2013). Lines of evidence–incremental markings in molar enamel of Soay sheep as revealed by a fluorochrome labeling and backscattered electron imaging study. PLoS One, 8(9), e74597.

Kierdorf, H., Witzel, C., Upex, B., Dobney, K., & Kierdorf, U. (2012). Enamel hypoplasia in molars of sheep and goats, and its relationship to the pattern of tooth crown growth. Journal of Anatomy, 220(5), 484–495.

Kinaston, R. L., Buckley, H. R., Halcrow, S. E., Spriggs, M. J. T., Bedford, S., Neal, K., & Gray, A. (2009). Investigating foetal and perinatal mortality in prehistoric skeletal samples: A case study from a 3000-year-old Pacific Island cemetery site. Journal of Archaeological Science, 36(12),

2780–2787.

King, C. L., Millard, A. R., Gröcke, D. R., Standen, V. G., Arriaza, B. T., & Halcrow, S. E. (2017). A comparison of using bulk and incremental isotopic analyses to establish weaning practices in the past. STAR: Science & Technology of Archaeological Research, 3(1), 126–134.

Kramis, S., & Trancik, V. (2014). "Extra locos sepulturae"—Literaturreview zu römerzeitlichen Perinatenfunden auf dem Gebiet der heutigen Schweiz. Bulletin der Schweizerischen Gesellschaft für Anthropologie, 20 (2), 5–26.

Kronfeld, R., & Schour, I. (1939). Neonatal dental hypoplasia. The Journal of the American Dental Association, 26(1), 18–32.

Kurek, M., Żądzinska, E., Sitek, A., Borowska-Struginska, B., Rosset, I., & Lorkiewicz, W. (2016). Neonatal line width in deciduous incisors from Neolithic, mediaeval and modern skeletal samples from north-central Poland. Annals of Anatomy, 203, 12–18.

Le Cabec, A., Dean, M. C., & Begun, D. R. (2017). Dental development and age at death of the holotype of Anapithecus hernyaki (RUD 9) using synchrotron virtual histology. Journal of Human Evolution, 108, 161–175.

Lee-Thorp, J. A. (2008). On isotopes and old bones. Archaeometry, 50, 925–950.

Lehn, C., Rossmann, A., & Graw, M. (2015). Provenancing of unidentified corpses by stable isotope techniques—Presentation of case studies. Science and Justice, 55, 72–88.

Longin, R. (1971). New method of collagen extraction for radiocarbon dating. Nature, 230(5291), 241–242.

Lösch, S., Grupe, G., & Peters, J. (2006). Stable isotopes and dietary adaptations in humans and animals at Pre-Pottery Neolithic Nevali Cori, southeast Anatolia. American Journal of Physical Anthropology, 131(2), 181–193.

Lösch, S., Gubler, R., Rüttimann, D., Moghaddam, N., Schwarz, H., & Cueni, A. (2013). Die römischen Bestattungen der Grabung Wydenpark in Studen. Eine anthropologische Untersuchung. In A. D. D. K. Bern (Ed.), Archäologie Bern/Archéologie bernoise 2013 (pp. 120–134). Bern: Erziehungsdirektion des Kantons Bern.

Lösch, S., Moghaddam, N., Grossschmidt, K., Risser, D. U., & Kanz, F. (2014). Stable isotope and trace element studies on gladiators and contemporary romans from Ephesus (Turkey, 2nd and 3rd Ct. AD)— Implications for differences in diet. PLoS One, 9(10), e110489.

Mahoney, P. (2015). Dental fast track: Prenatal enamel growth, incisor eruption, and weaning in human infants. American Journal of Physical Anthropology, 156(3), 407–421.

Mekota, A.-M., Grupe, G., Ufer, S., & Cuntz, U. (2006). Serial analysis of stable nitrogen and carbon isotopes in hair: Monitoring starvation and recovery phases of patients suffering from anorexia nervosa. Rapid Communications in Mass Spectrometry, 20(10), 1604–1610.

Millard, A. R. (2000). A model for the effect of weaning on nitrogen isotope ratios in humans. In G. A. Goodfried, M. Collins, M. L. Fogel, S. Macko, & J. Whehmiller (Eds.), Perspectives in amino acid and protein geochemistry (pp. 51–59). Oxford: Oxford University Press.

Moghaddam, N., Müller, F., Hafner, A., & Lösch, S. (2016). Social stratigraphy in late iron age Switzerland: Stable carbon, nitrogen and sulphur isotope analysis of human remains from Münsingen. Archaeological and Anthropological Sciences, 8(1), 149–160.

Nava, A., Coppa, A., Coppola, D., Mancini, L., Dreossi, D., Zanini, F., … Bondioli, L. (2017). Virtual histological assessment of the prenatal life history and age at death of the Upper Paleolithic fetus from Ostuni (Italy). Scientific Reports, 7(1), 9427.

Nitsch, E. K., Humphrey, L. T., & Hedges, R. E. (2011). Using stable isotope analysis to examine the effect of economic change on breastfeeding practices in Spitalfields, London, UK. American Journal of Physical Anthropology, 146(4), 619–628.

Peacock, N. R. (1990). Comparative and cross-cultural approaches to the study of human female reproductive failure. In C. J. De Rousseau (Ed.), Primate life history and evolution (pp. 195–220). New York: Wiley.

Pearson, J. A., Hedges, R. E. M., Molleson, T. I., & Ozbek, M. (2010). Exploring the relationship between weaning and infant mortality: An isotope case study from Asikli Hoyuk and Cayonu Tepesi. American Journal of Physical Anthropology, 143(3), 448–457.

Prowse, T. L., Saunders, S. R., Schwarcz, H. R., Garnsey, P., Macchiarelli, R., & Bondioli, L. (2008). Isotopic and dental evidence for infant and young child feeding practices in an Imperial Roman skeletal sample. American Journal of Physical Anthropology, 137(3), 294–308.

Reitsema, L. J., & Muir, A. B. (2015). Growth velocity and weaning delta (15)N "dips" during ontogeny in Macaca mulatta. American Journal of Physical Anthropology, 157(2), 347–357.

Reynard, L. M., & Tuross, N. (2015). The known, the unknown and the unknowable: Weaning times from archaeological bones using nitrogen isotope ratios. Journal of Archaeological Science, 53(January 2015), 618–625.
Richards, M. P., Mays, S., & Fuller, B. T. (2002). Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at the medieval Wharram Percy site, Yorkshire, UK. American Journal of Physical Anthropology, 119(3), 205–210.

Risnes, S. (1999). Mature enamel morphology and forming enamel dynamics: Crystal orientation, crystal continuity, prism diameter, Retziuslines, and prism cross-striations. In J. Mayhall & T. Heikkinen (Eds.), Proceedings of the 11th international symposium on dental morphology (pp. 312–322). Oulu: Oulu University Press.

Rollet, C. (1997). The fight against infant mortality in the past: An international comparison. In A. Bideau, B. Desjardins, P. Brignoli, H. P. Brignoli, & P. Brignoli (Eds.), Infant and child mortality in the past. Oxford: Oxford University Press.

Romek, K. M., Julien, M., Frasquet-Darrieux, M., Tea, I., Antheaume, I., Hankard, R., & Robins, R. J. (2013). Human baby hair amino acid natural abundance N-15-isotope values are not related to

the N-15-isotope values of amino acids in mother's breast milk protein. Amino Acids, 45(6), 1365–1372.

Rushton, M. (1933). On the fine contour lines of the enamel of milk teeth. The Dental Record, 53, 170–171.

Sabel, N., Johansson, C., Kuhnisch, J., Robertson, A., Steiniger, F., Noren, J. G., … Nietzsche, S. (2008). Neonatal lines in the enamel of primary teeth—A morphological and scanning electron microscopic investigation. Archives of Oral Biology, 53(10), 954–963.

Scheuer, L., & Black, S. (2004). The juvenile skeleton. London: Elsevier Academic Press.

Schneider, J., & Kuchenbecker, B. (1986). Normale Spontangeburt. In J. Schneider & H. Kaulhausen (Eds.), Lehrbuch der Gynaekologie und Geburtsmedizin (pp. 395–427). Stuttgart: Kohlhammer.

Schour, I. (1936). The neonatal line in the enamel and dentin of the human deciduous teeth and first permanent molar. Journal of the American Dental Association, 23, 1946–1955.

Schurr, M. R. (1998). Using stable nitrogen-isotopes to study weaning behavior in past populations. World Archaeology, 30(2), 327–342.

Schwartz, J. H., Houghton, F., Macchiarelli, R., & Bondioli, L. (2010). Skeletal remains from Punic Carthage do not support systematic sacrifice of infants. PLoS One, 5(2), e9177.

Sebald, S., Stenzel, L., Gruenewald, M., & Grupe, G. (2018). Skeletal remains of neonates from the roman cemetery of Günzburg (Bavaria, Frg)—How long did the neowborns survive? Microscopic assessment of the presence of the neonatal line in dental enamel. Anthropologie (Brno), 56(1), 1–10.

Siebke, I., Kanz, F., Witzel, C., & Lösch, S. (2016). Stable isotope investigation of mother—Infant pairs and the implication for forensic casework: Preliminary results. Las Vegas, NV: American Academy of Forensic Sciences.

Skinner, M., & Dupras, T. (1993). Variation in birth timing and location of the neonatal line in human enamel. Journal of Forensic Sciences, 38(6), 1383–1390.

Smith, P., & Avishai, G. (2005). The use of dental criteria for estimating postnatal survival in skeletal remains of infants. Journal of Archaeological Science, 32(1), 83–89.

Smith, T. M., Reid, D. J., & Sirianni, J. E. (2006). The accuracy of histological assessments of dental development and age at death. Journal of Anatomy, 208(1), 125–138.

Stimmler, L., Snodgrass, G., & Jaffe, E. (1973). Dental defects associated with neonatal symptomatic hypocalcaemia. Archives of Disease in Childhood, 48(3), 217–220.

Sunderland, E. P., Smith, C. J., & Sunderland, R. (1987). A histological study of the chronology of initial mineralization in the human deciduous dentition. Archives of Oral Biology, 32(3), 167–174.

Tacail, T., Thivichon-Prince, B., Martin, J. E., Charles, C., Viriot, L., & Balter, V. (2017). Assessing human weaning practices with calcium isotopes in tooth enamel. Proceedings of the National Academy of Sciences of the United States of America, 114(24), 6268–6273.

Tafforeau, P., & Smith, T. M. (2008). Nondestructive imaging of hominoid dental microstructure using phase contrast X-ray synchrotron microtomography. Journal of Human Evolution, 54(2), 272–278.

Tsutaya, T., & Yoneda, M. (2013). Quantitative reconstruction of weaning ages in archaeological human populations using bone collagen nitrogen isotope ratios and approximate Bayesian computation. PLoS One, 8(8), e72327.

Tsutaya, T., & Yoneda, M. (2015). Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. American Journal of Physical Anthropology, 156(S59), 2–21.

Ulrich-Bochsler, S., & Zwahlen, R. (2011). Säuglingsbestattungen im römischen Vicus Petinesca. Jahrbuch des Archäologischen Dienstes des Kantons Bern. Bern: Archäologischer Dienst (pp. 157–170).

United Nations Inter-agency Group for Child Mortality Estimation (UN IGME). (2018). Levels & Trends in child mortality: Report 2018. Estimates developed by the United Nations inter-agency Group for Child Mortality Estimation. New York: United Nations Children's Fund.

Vogel, J. C., & van der Merwe, N. J. (1977). Isotopic evidence for early maize cultivation in New York state. American Antiquity, 42(2), 238–242.

Waters-Rist, A. L., & Katzenberg, M. A. (2010). The effect of growth on stable nitrogen isotope ratios in subadult bone collagen. International Journal of Osteoarchaeology, 20(2), 172–191.

Whittaker, D. K., & MacDonald, D. G. (1989). A colour atlas of forensic dentistry. London: Wolfe.

Witzel, C. (2014a). Inkrementelle Strukturen im Schmelz der Milchzähne. Rechtsmedizin, 24(3), 165–171.

Witzel, C. (2014b). Echoes from birth—Mutual benefits for physical and forensic anthropology by applying increment counts in enamel of deciduous teeth for aging. Anthropologischer Anzeiger, 71(1–2), 87–103.

Witzel, C., Flohr, S., & Becker, J. (2009). The life in lines—Chronology of stress markers in the enamel microstructure and the possible association of pathological changes in bone in a child from the late Neolithic in Syria. American Journal of Physical Anthropology, 48(Suppl), 275.

Witzel, C., Kierdorf, U., Schultz, M., & Kierdorf, H. (2008). Insights from the inside: Histological analysis of abnormal enamel microstructure associated with hypoplastic enamel defects in human teeth. American Journal of Physical Anthropology, 136(4), 400–414.

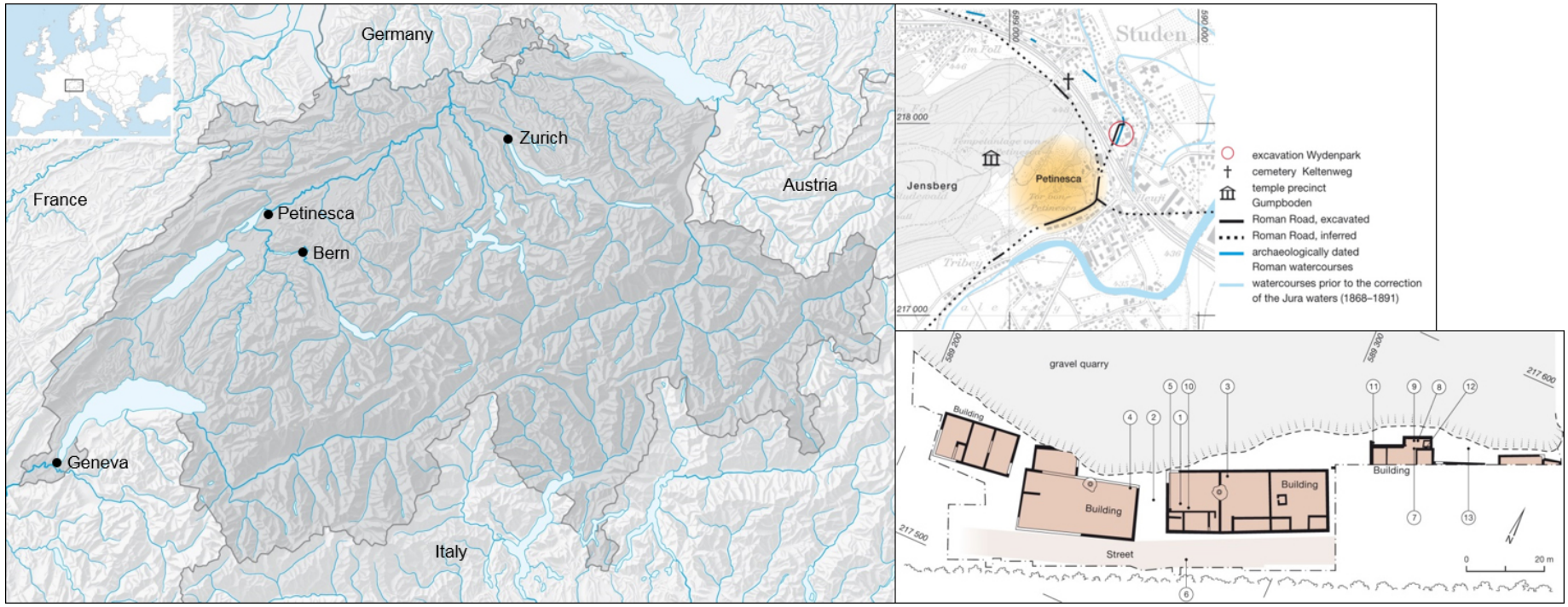

FIGURE 1: Map of Switzerland with the Roman settlement Petinesca indicated (left); general overview of the excavated area of Petinesca (upper right); finding situation of the infants in association to the ancient roman buildings (lower right). Source: Archäologischer Dienst Bern.

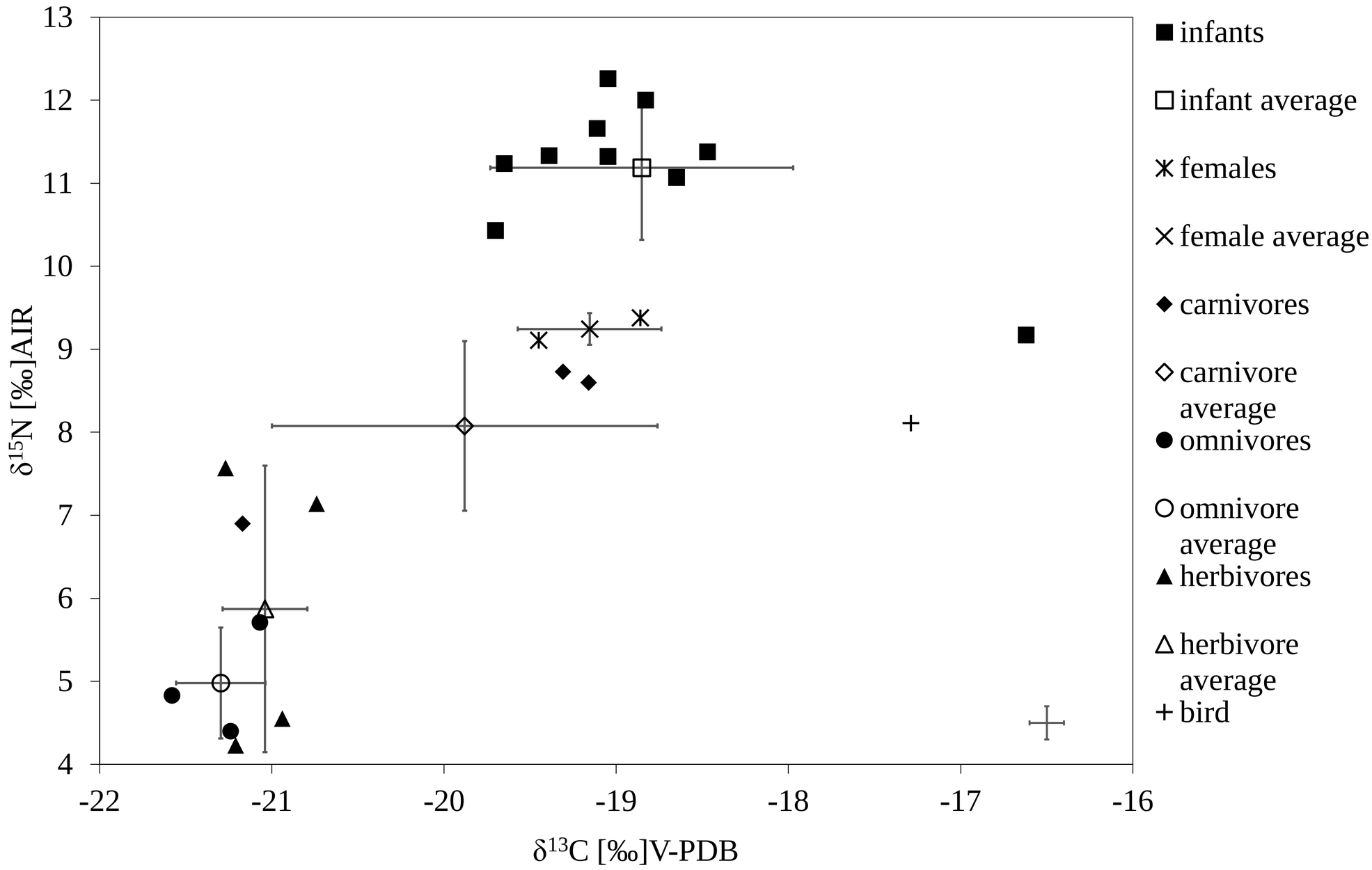

FIGURE 2: All $\delta^{13}C$ values are plotted against $\delta^{15}N$ values of both human and animal samples, n = 21 and two females (Lösch et al., 2013) for comparison (note that whiskers related to all average values represent 1σSD of the average). It is evident that the animal bones especially the omnivores and herbivores express lower $\delta^{13}C$ and $\delta^{15}N$ values than the human samples. The carnivores are close to the female average. The measurement uncertainty is indicated in the right lower corner of the graph ($\delta^{13}C$ = 0.1‰; $\delta^{15}N$ = 0.2‰).

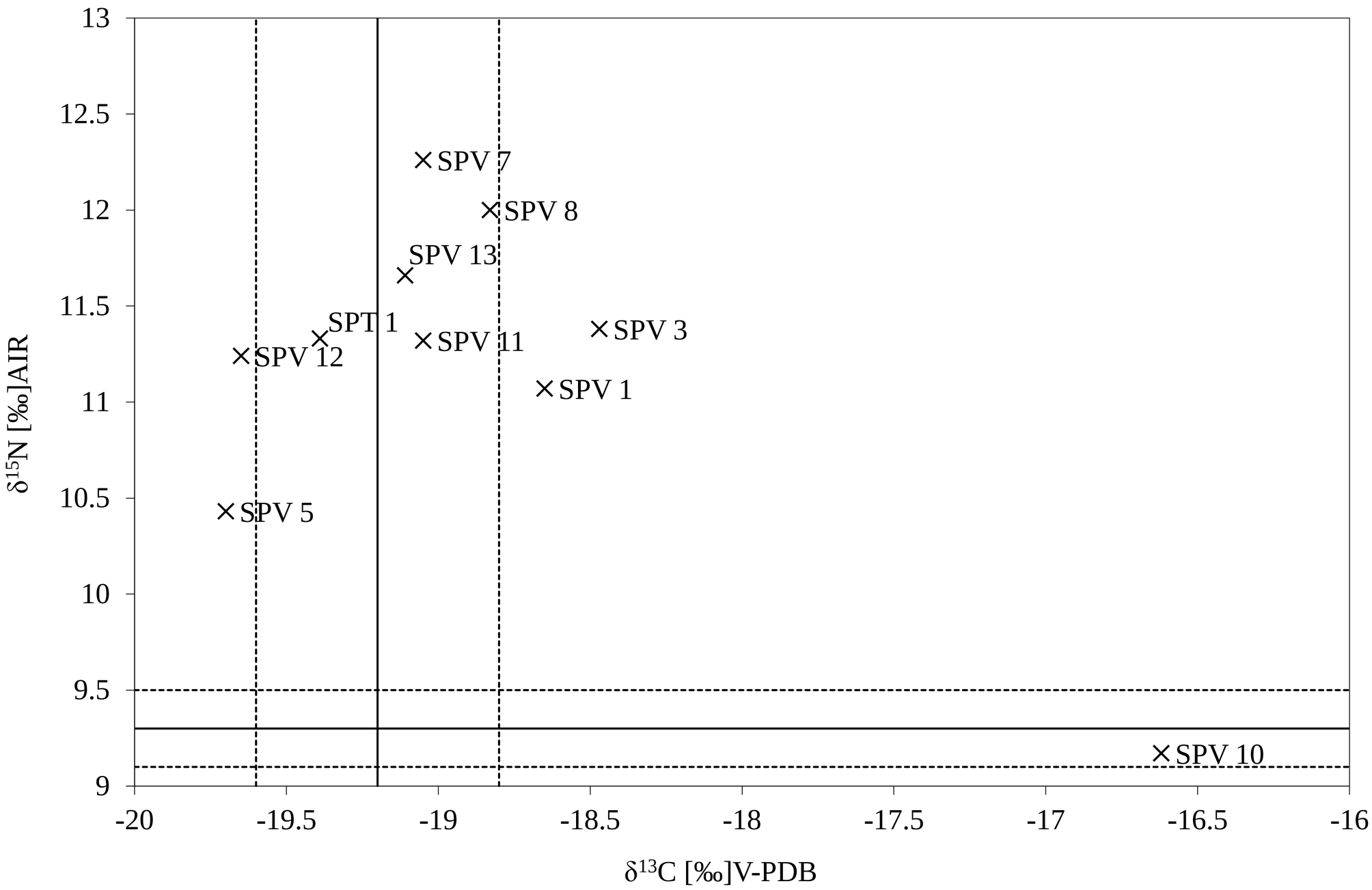

FIGURE 3: The $\delta^{13}C$ values of the infants are plotted against the $\delta^{15}N$ values. The female average is included: solid horizontal line = $\delta^{15}N$ average with associated 1σSD as dotted lines; solid vertical line = $\delta^{13}C$ average with associated 1σSD as dotted lines. It is evident that individual SPV 10 does not follow the overall trend of the infant values.

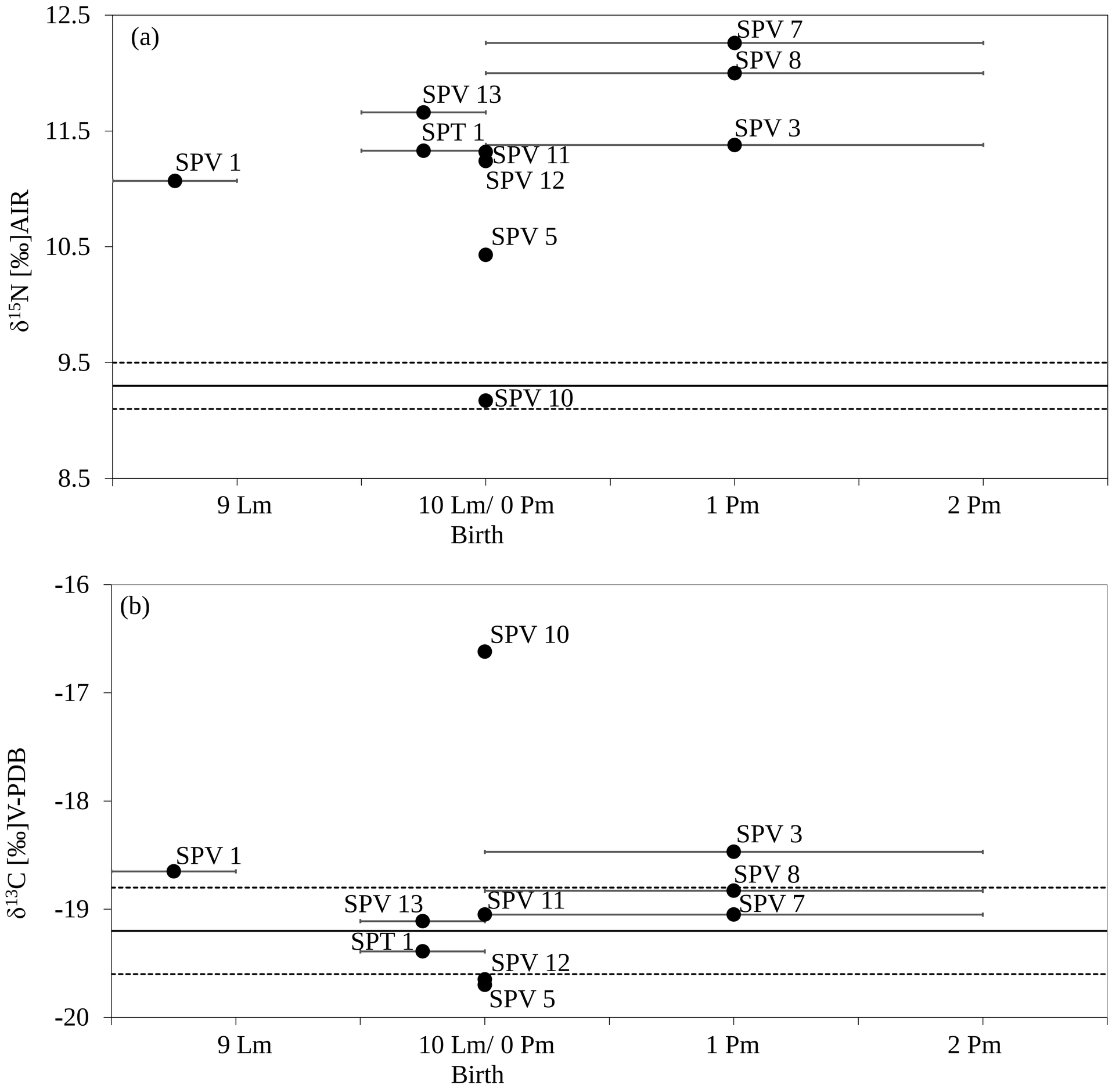

FIGURE 4: Infant $\delta^{15}N$ values (a) and $\delta^{13}C$ values (b) plotted in relation to their age estimations. Indicted are the complete estimated age ranges. The female average (solid line) with associated 1σSD (dotted lines) is indicated. Lm, lunar months; Pm, postnatal months.

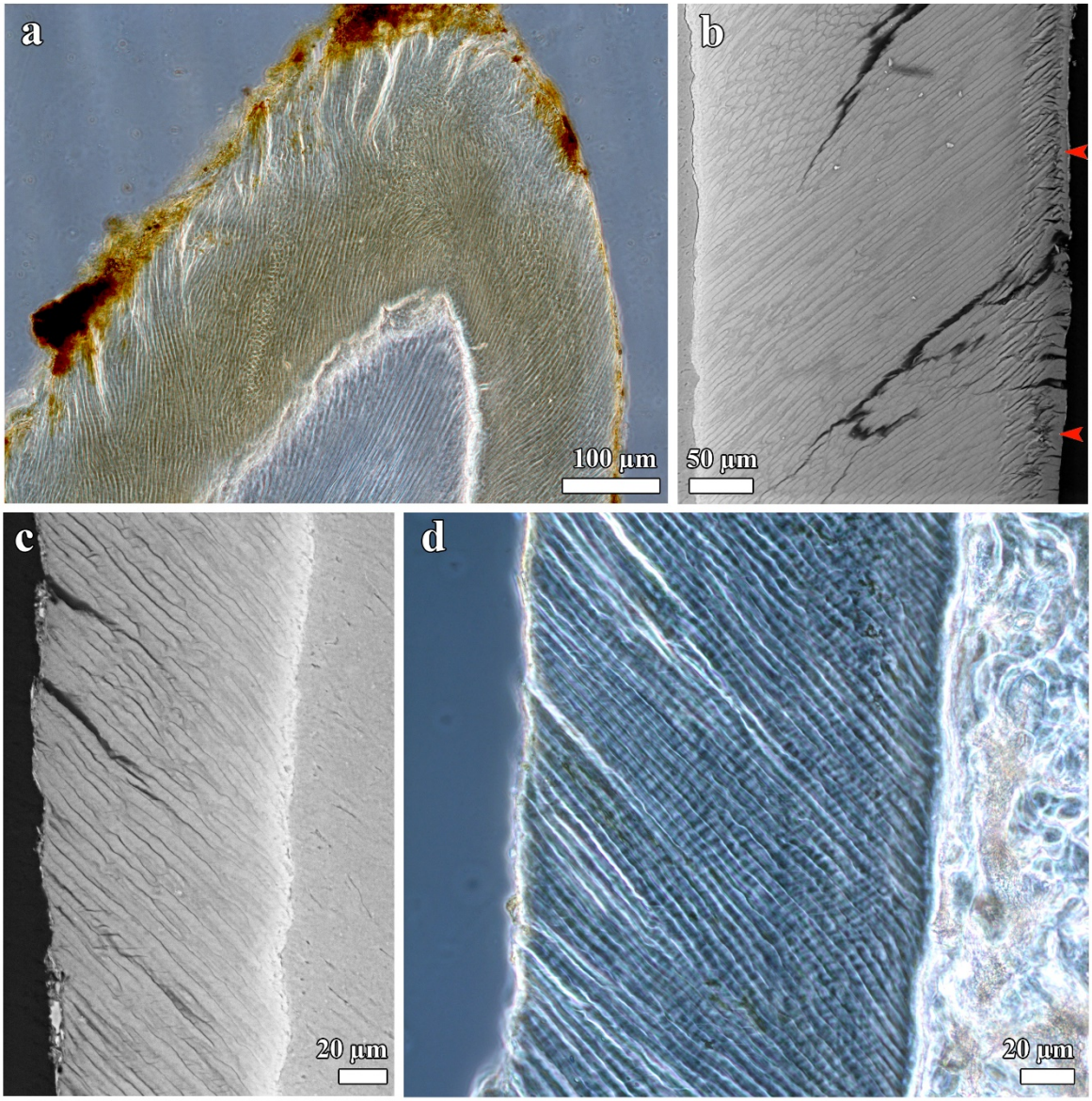

FIGURE 5: Phase contrast (a, d) and scanning electron (b, c) micrographs of Axiobuccolingual ground sections of the deciduous lower right canine (a) and deciduous lower right first incisor (b) of individual SPV 10, the deciduous upper left first incisor of individual SPT 1 (c), and the deciduous lower left first incisor of individual SPV 12 (d). Note presence of prismatic enamel up to the preserved surface in a, c, and d and obscured prism identity in the surface enamel in b (red arrowheads). Enamel prisms in d exhibit a marked prism crossstriation pattern.

Table 1: Human and animal samples from the Roman site Petinesca (1st to 3rd century AD) used for stable isotope analysis. Lm = lunar months, na = not applicable, Pm = postnatal months (10 Lm = 0 Pm), ? = uncertain age estimation due to bad bone preservation. In those cases, age estimation was based on comparison of bone fragments to a known age estimated skeleton (Ulrich-Bochsler and Zwahlen 2011).

Sample ID	**Species**	**Bone sample**	**Age**
SPV-1	*Homo sapiens*	femur	8.5 to 9 Lm
SPV-2	*Homo sapiens*	femur	0 to 2 Pm
SPV-3	*Homo sapiens*	femur	0 to 2 Pm
SPV-5	*Homo sapiens*	femur	0 Pm
SPV-7	*Homo sapiens*	ilium, ribs	0 to 2 Pm?
SPV-8	*Homo sapiens*	femur, tibia & fibula	0 to 2 Pm?
SPV-10	*Homo sapiens*	femur	0 Pm
SPV-11	*Homo sapiens*	femur	0 Pm
SPV-12	*Homo sapiens*	femur	0 Pm
SPV-13	*Homo sapiens*	femur	9.5 to 10 Lm
SPT-1	*Homo sapiens*	femur	9.5 to 10 Lm
SPV-C1	*Canis familiaries*	mandible	na
SPV-C2	*Canis familiaries*	long bone	na
SPV-V1	*Vulpus vulpus*	long bone	na
SPV-G1	*Gallus domesticus*	long bone	na
SPV-S1	*Sus domesticus*	long bone	na
SPV-S2	*Sus domesticus*	cranium	na
SPV-S7	*Sus domesticus*	cranium	na
SPV-E3	*Equus caballus*	long bone	na
SPV-B3	*Bos taurus*	cranium	na
SPV-B6	*Bos taurus*	mandible	na
SPV-B7	*Bos taurus*	mandible	na
SPV-C/O2	*Capra/ Ovis*	cranium	na
SPV-C/O3	*Capra/ Ovis*	mandible	na
SPV-C/O6	*Capra/ Ovis*	long bone	na

Table 2: Stable isotope data of infant and female samples from the Roman site Petinesca (1st to 3rd century AD)**;** Lm = lunar months, Pm = postnatal months, ? = uncertain age estimation due to bad preservation (Ulrich-Bochsler and Zwahlen 2011).

Sample ID	**Bone sample**	**Age (Lm/ Pm/years)**	**$\delta^{13}C$ [‰]$_{V\text{-}PDB}$**	**$\delta^{15}N$ [‰]$_{AIR}$**	**collagen portion %**	**%C**	**%N**	**C/N mol**
SPV 1	femur	8.5 to 9 Lm	-18.65	11.07	5.8	41.8	15.3	3.2
SPV 2	femur	0 to 2 Pm	not enough collagen					
SPV 3	femur	0 to 2 Pm	-18.47	11.38	4.5	40.2	14.6	3.2
SPV 5	femur	10 Lm	-19.70	10.43	1.5	40.9	14.0	3.4
SPV 7	ilium, ribs	0 to 2? Pm	-19.05	12.26	2.1	43.8	15.8	3.2
SPV 8	femur, tibia & fibula	0 to 2? Pm	-18.83	12.00	2.1	40.3	14.8	3.2
SPV 10	femur	10 Lm	-16.62	9.17	7.2	35.0	12.9	3.2
SPV 11	femur	10 Lm	-19.05	11.32	1.8	31.4	10.9	3.4
SPV 12	femur	10 Lm	-19.65	11.24	1.5	43.0	14.5	3.5
SPV 13	femur	9.5 to 10 Lm	-19.11	11.66	2.3	43.7	15.1	3.4
SPT 1	femur	9.5 to 10 Lm	-19.39	11.33	9.3	44.8	16.6	3.2
V 52	femur	30 to 50	-19.45	9.11	1.7	38.5	13.6	3.3
V 224	femur	30 to 50	-18.86	9.38	2.6	38.0	13.4	3.3

Table 3: Stable isotope data of animal samples from the Roman site Petinesca (1st to 3rd century AD).

Sample ID	**Species**	**Nutrition**	**$\delta^{13}C$ [‰]$_{V\text{-}PDB}$**	**$\delta^{15}N$ [‰]$_{AIR}$**	**Collagen portion %**	**%C**	**%N**	**C/N mol**
SPV C1	*Canis familiaries*	carnivore	-19.31	8.73	5.3	42.4	15.9	3.1
SPV C2	*Canis familiaries*	carnivore	-21.17	6.90	7.9	39.3	16.6	2.8
SPV V1	*Vulpus vulpus*	carnivore	-19.16	8.60	2.5	41.2	16.0	3.0
SPV G1	*Gallus domesticus*	omnivore (bird)	-17.29	8.11	4.5	43.3	16.4	3.1
SPV S1	*Sus domesticus*	omnivore	-21.58	4.83	8.6	41.8	15.5	3.2
SPV S2	*Sus domesticus*	omnivore	-21.24	4.40	4.1	40.8	14.6	3.3
SPV S7	*Sus domesticus*	omnivore	-21.07	5.71	4.7	43.1	16.4	3.1
SPV E3	*Equus caballus*	herbivore	-21.27	7.57	5.5	43.3	16.6	3.0
SPV B3	*Bos taurus*	herbivore	not enough collagen					
SPV B6	*Bos taurus*	herbivore	-20.74	7.14	8.4	43.5	16.3	3.1
SPV B7	*Bos taurus*	herbivore	-21.21	4.23	3.7	41.2	16.3	3.0
SPV C/O2	*Capra/ Ovis*	herbivore	-20.94	4.55	1.7	40.1	15.7	3.0
SPV C/O3	*Capra/ Ovis*	herbivore	not enough collagen					
SPV C/O6	*Capra/ Ovis*	herbivore	not enough collagen					

Table 4: Vertical tooth height measurements of deciduous tooth crowns with corresponding ages read from the charts of Deutsch et al. (1984) and provided by Liversidge (personal communication) based on unpublished data from the collection of Maurice Stack; Lm = lunar months.

Sample ID	tooth	height (mm)	age (Lm)
SPV 10	Decisuous lower right first incisor (dLRI1)	4.9	~ 10
	Deciduous lower right canine (dLRC)	3.0	~ 10
SPV 12	Deciduous lower left first incisor (dLLI1)	4.7	~ 10
SPT 1	Deciduous upper left first incisor (dULI1)61	4.9	~ 9
	Deciduous upper right third premolar (dURP3)	3.3	~ 9

Table 5: Individuals screened for elevated $\delta^{15}N$ values, resp. a breastfeeding signal and a NNL*;* Lm = lunar months, based on morphological analysis of bones and measurement of tooth height (table 4), NNL = neonatal line.

Sample ID	**Age (Lm)**		**Breastfeeding signal**	**NNL**
	bone	**teeth**		
SPV 10	10	10	no	no
SPV 12	10	10	yes	no
SPT 1	9.5-10	9	yes	no

Table 6: Summarized result combinations of $\delta^{15}N$ values of the infants compared to a female average in correlation with the presences or absence of a NNL and corresponding interpretations; NNL = Neonatal line.

	$\delta^{15}N$ values compared to female average	**NNL**	**Interpretation**
Possible result combinations	^{15}N not enriched	no	Infant younger than 7-10 days of age, possible stillbirth, perinatal death, an early neonatal death or the practice of infanticide
	^{15}N not enriched	yes	Infant older than 7-10 days of age, but in the range of the lag time of bone collagen turnover: Alternatively, the $\delta^{15}N$ of the mother could have been markedly below the female average
	^{15}N enriched	no	Infant younger than 7-10 days of age or the NNL is obliterated due to taphonomic reasons, $\delta^{15}N$ values possible due to metabolic reasons, true $\delta^{15}N$ value in relation to actual mother not known which could also have been markedly above the female average, possibly due to nutritional, resp. maternal stress
	^{15}N enriched	yes	Infant older than 7-10 days of age, possibly a beginning breastfeeding signal