Spatially-Resolved Ca Isotopic and Trace Element Variations in Human Deciduous Teeth Record Diet and Physiological Change

Qiong Li, Alessia Nava, Linda M. Reynard, Matthew Thirlwall, Luca Bondioli & Wolfgang Müller

To cite this article: Qiong Li, Alessia Nava, Linda M. Reynard, Matthew Thirlwall, Luca Bondioli & Wolfgang Müller (2020): Spatially-Resolved Ca Isotopic and Trace Element Variations in Human Deciduous Teeth Record Diet and Physiological Change, Environmental Archaeology, DOI: 10.1080/14614103.2020.1758988

To link to this article: https://doi.org/10.1080/14614103.2020.1758988

Published online: 13 May 2020.
Spatially-Resolved Ca Isotopic and Trace Element Variations in Human Deciduous Teeth Record Diet and Physiological Change

Qiong Li a*, Alessia Nava b,c, Linda M. Reynard d, Matthew Thirlwall e, Luca Bondioli e and Wolfgang Müller b,c

aDepartment of Earth Sciences, Royal Holloway University of London, Egham, UK; bDANTE Laboratory for the Study of Diet and Ancient Technology, Sapienza University of Rome, Rome, Italy; cBioarchaeology Service, Museo delle Civiltà, Rome, Italy; dDepartment of Human Evolutionary Biology, Harvard University, Cambridge, MA, USA; eInstitute of Geosciences, Goethe University Frankfurt, Frankfurt am Main, Germany

ABSTRACT

Dental enamel represents an important mineralized archive of an individual’s early life. Previously, isotopic (Ca) or trace element ratios (Sr/Ca, Ba/Ca) have been used to reveal dietary and weaning histories, although few studies have utilized both proxies to evaluate the respective results. Here we report histologically-defined, spatially-resolved Ca-isotope (laser-cut & TIMS) and trace element ratio (Sr/Ca, Ba/Ca; LA-ICPMS) profiles along the enameldentine-junction in three deciduous dental crowns of three early twentieth century Italian infants (Modern-22, 27, 29). Modern-27 and Modern-29 display overall similar patterns of Ca-isotope variation and reflect an overall increase of >1.0‰ in δ44/40Ca across and after birth. Whilst the Sr/Ca and Ba/Ca signals of Modern-27 suggest a mixed breast-formula feeding, the two elemental proxies in Modern-29 hint at nearly exclusive breastfeeding until ~4 months, followed by introduction of formula. A ~0.5‰ δ44/40Ca decrease across and after birth together with Sr/Ca ratios of Modern-22 suggest a dominant breastfeeding history for the first ~5–8 months. Enamel Ca-isotope data alone are not sufficient to distinguish between breastfed or formula-fed infants. In addition, Ca-isotope profiles in deciduous enamel suggest a connection between prominent physiological stress like birth and negative Ca-isotope excursions, underlining the physiological overprint of Ca-isotope signatures.

Introduction

Both isotopic and trace element ratios in dental enamel have been used to reconstruct the feeding behaviour and weaning practice of past human populations in early life (Austin et al. 2013; Humphrey 2014; Humphrey et al., 2008a, 2008b; Smith et al. 2018; Tacail et al. 2017, 2019). Such studies are pivotal for our understanding of health, fertility, demography, developmental biology and the evolution of life-history strategies of human groups. Light isotopes (e.g. C, N and S) in bone collagen are routinely used to trace breastfeeding and weaning patterns in archaeological populations (Beaumont et al. 2015; Fuller et al. 2006; Jaouen and Pons 2017; Nehlich et al. 2011; Richards, Mays, and Fuller 2002; Tsutaya 2017; Tsutaya and Yoneda 2013, 2015; Wright and Schwarz 1998), based on the idea that the child is one trophic level higher than the mother. However, collagen in bones is not as durable as mineralised dental tissues, particularly dental enamel, which is highly mineralised and thus shows high resistance to post-mortem diagenetic alteration (Hinz and Kohn 2010; Hoppe, Koch, and Furutani 2003). What is more, due to the appositional growth of dental enamel, it is possible to derive precise chronologies at higher resolution compared to serial sections of dentine (Beaumont 2020; Beaumont et al. 2015; Beaumont and Montgomery 2015; Müller et al. 2019).

Chu et al. (2006) suggested the use of Ca isotopes in teeth (and bones) as a tracer to assess dairy consumption in past human communities. This was based on the finding that dairy products, though high in Ca concentration, have the lowest Ca isotope composition among various food components. However, Reynard, Henderson, and Hedges (2010) and Reynard, Henderson, and Hedges (2011) demonstrated larger Ca-isotope variability but lower Ca isotope composition in human bones relative to fauna at some archaeological sites both pre- and post-dating animal domestication and therefore both before and after a change in animal milk availability to humans. This seemed to indicate more complicated processes (e.g. Ca metabolism) than milk consumption or trophic level effects only. Tacail et al. (2017) reported an observable difference in Ca isotope patterns in dental enamel differentiating short breastfed/formula-fed and long-term breastfed...
infants. Specifically, the long-term breastfed individuals maintained the same $\Delta^{44/42}\text{Ca}$ values pre- and post-birth, up to a few months post-birth (no further time points were available). The mostly formula-fed infants showed an increase in $\Delta^{44/42}\text{Ca}$ after birth in most cases (Tacail et al. 2017). The pattern is plausible given the relatively low $\delta^{44/42}\text{Ca}$ in breast milk and higher $\delta^{44/42}\text{Ca}$ in commercial formulas (Chu et al. 2006; Tacail et al. 2017). An increase in $\Delta^{44/42}\text{Ca}$ after weaning has also been observed in the Ca isotope compositions of modern sheep tooth enamel (Wright 2014). Sequentially microsampled enamel enabled Li, Thirlwall, and Müller (2016) to identify the effect of physiological change (e.g. menarche) on Ca isotope ratios in a modern female third molar, namely a negative excursion in the Ca isotope composition likely related to physiological change such as the start of menstruation, change in oestrogen levels and bone accretion. Therefore, the complexity of Ca isotopic data in dental enamel accumulated so far argues that multiple proxies – both isotopic and elemental – may help in differentiating metabolic from nutritional effects during biomineralization. This is necessary before any reliable inferences of dietary or environmental signals in past human populations can be deduced.

Trace element ratios (e.g. Sr/Ca, Ba/Ca, Zn/Ca, Pb/ Ca) in tooth enamel have been investigated, together with radiogenic Sr ($^{87}\text{Sr}/^{86}\text{Sr}$), to reveal information on trophic levels, dietary transitions, weaning practice, pollution exposure and mobility in modern, archaeological and paleoanthropological samples (e.g. Austin et al. 2013; Balter et al. 2012; Humphrey et al., 2008a; Joannes-Boyau et al. 2019; Müller et al. 2003, 2019, 2009; Reynard and Balter 2014; Smith et al. 2018). These elements were chosen because of their importance for biomineralization (Zn) and ability to substitute Ca (e.g. Sr, Ba, Pb), and also because of their systematic changes (in terms of El/Ca or X/Ca ratios) through food chains (Balter et al. 2002; Burton, Price, and Middleton 1999; Kohn, Morris, and Olin 2013). Austin et al. (2013), Balter et al. (2017), and Smith et al. (2018) have noted that Ba/Ca in teeth reflects dietary history patterns in early life of humans and primates. In contrast, Müller et al. (2019) found no systematic Ba/Ca patterns analogous to those observed for Sr/Ca and Zn/Ca in the teeth of modern children with different feeding histories, and furthermore demonstrate cryptic diagenesis affecting Ba in soil-buried, otherwise well-preserved tooth enamel apatite. They show that Sr/Ca in time-resolved profiles across birth systematically decreases for breast-fed infants, whereas it always increases in case of formula-fed infants. These authors also highlighted the active biological control of Zn incorporation during biological mineralisation. As a result, Zn/Ca cannot serve as a reliable trophic level indicator. Ca is highly biopurified in human milk. Relative to Sr, Ca is selectively transferred across the mammary glands and the placenta (Humphrey et al. 2008a, 2008b; Rossipal 2000). Conversely, herbivore milk and derived formulae are higher in Sr/Ca ratio, due to the lower initial trophic position (Burton, Price, and Middleton 1999).

Here we present histologically-defined Ca isotope and trace element (Sr/Ca and Ba/Ca) enamel profiles of three twentieth century Italian deciduous canines. We chose the enamel profiles along the enamel-dentine junction (EDJ) and within 100 µm to minimise any overprint from enamel maturation (Müller et al. 2019). Our primary aim is to better understand the biological/physiological control on Ca isotopic and elemental proxies in enamel, and our second aim is to retrieve reliable dietary transition and weaning information using isotopic and elemental indicators in tandem.

Materials and Analytical Methods

Samples, Sectioning and Enamel Histology

The dental sample consists of three early twentieth century Italian deciduous canines (Modern 22, 27 and 29) belonging to a series of clinically extracted/ exfoliated teeth collected by a dental practitioner operating in Rome at that time (courtesy of A. Coppa, Department of Environmental Biology, Sapienza University of Rome, from the reference collection of his laboratory) and temporarily kept at the Museo delle Civiltà of Rome. According to the known standards of tooth development (AlQahtani, Hector, and Liversidge 2010), the deciduous canine’s crown formation time covers a period of the individual life from the 30th week in utero to the 9th–10th month of age.

Thin sections of the dental crowns were obtained using the method by Caropreso et al. (2000) with some modifications reported in Nava et al. (2017). Each tooth was embedded in bi-component epoxy (Epo Thin, Buehler) and cut by means of a diamond blade microtome (mod. 1600, Leica AG). One longitudinal section parallel to the bucco-lingual plane and crossing the tip of the dentine horn was obtained in tandem. High resolution micrographs of the dental crowns were
obtained with a digital camera (DFC 295, Leica AG) connected to the microscope, by capturing overlapping pictures and then assembling them using the software ICE 2.0 (Image Composite Editor, Microsoft Research Computational Photography Group). ImageJ software (v.1.51k, National Institutes of Health, USA) was used for digital image processing to perform histomorphometrical analysis and track length measurements.

Dental enamel is secreted in a rhythmic manner (Hillson 2014), and its growth parameters are quantifiable in mammals through biological markers which are visible in histological longitudinal thin sections. These include daily cross striations along the enamel prisms (Zheng et al. 2013) and the nearly-weekly (in humans between 6 and 12 days) Retzius lines (Dean 2006). The birth event is recorded in the forming enamel of individuals surviving the perinatal stage as an accentuated Retzius line, known as Neonatal Line (review in Sabel et al. 2008; Zanolli et al. 2011). The individual chronology of crown formation along the EDJ was derived by calculating the enamel formation time through the cross striations count across the enamel thickness and by projecting it on the EDJ by means of the isochronous Retzius lines (see Birch and Dean 2014; Guatelli-Steinberg et al. 2012; for details on the method). This results in the construction of chronologically-defined grids across the crown that allow registration of each point of the compositional profiles with the individual (secretion) chronology in days before and after birth.

**Laser Micro-Sampling of Enamel Apatite for TIMS Ca Isotope Analysis**

Laser microsampling or microcutting (i.e. not laser-ablation) of enamel apatite was detailed in Li, Thirlwall, and Müller (2016). Laser microsampling or microcutting (i.e. not laser-ablation) of enamel apatite was described in Li, Thirlwall, and Müller (2016). NIST SRM 915b is used as the primary standard for delta value calculation. The delta notation is expressed as \( \delta^{44/40} \text{Ca}_{\text{sample}} / \delta^{44/40} \text{Ca}_{\text{SRM915a}} + \delta^{44/40} \text{Ca}_{\text{SRM915a}} \). The value of \( \delta^{44/40} \text{Ca}_{\text{SRM915b}} / \delta^{44/40} \text{Ca}_{\text{SRM915a}} \) is 0.72‰ (Heuser and Eisenhauer 2008).

**Trace Element Profiles by LA-ICPMS**

Given that most labial enamel had been removed following laser microsampling, LA-ICPMS tracks were analysed on the lingual aspect of the crowns, parallel to the EDJ and less than 100 µm from the EDJ. Analytically, LA-ICPMS closely follows the procedures in Müller et al. (2019), except that an Agilent 8900 ICP-QQQ (ICP-MS/MS) was connected to the RESolution M-50 prototype LA system. Spot size, scan speed of continuous LA tracks and laser repetition rate were 20 µm, 5 µm/s and 15 Hz, respectively. \( ^{43} \text{Ca} \) was used as an internal and NIST SRM612 glass as an external...
standard, and accuracy was monitored using STDPx glasses (Klemme et al. 2008) with biases (much) lower than 5% for the elements relevant here (Sr, Ba). The time span of the Modern 22 lingual track is much smaller (138 days after birth) than the time range calculated for δ^{44/40}Ca profile on the buccal side (~7.4 months after birth). This discrepancy is due to the loss of enamel in the most cervical part of the lingual aspect of the crown.

The compositional and Ca isotopic profiles were smoothed with a locally weighted polynomial regression fit (LOWESS), with its associated standard error range (±2S.E.) for each predicted value (Cleveland, Grosse, and Shyu 1992). The statistical package R (ver. 3.6.2) was used for all statistical computations and generation of graphs (R-Core-Team 2019).

**Results**

**δ^{44/40}Ca Profiles of Modern Deciduous Teeth**

Ca isotope data of the three analysed deciduous teeth are plotted against histologically defined (secretion) time in months in Figure 2. The δ^{44/40}Ca ratio in the deciduous canine of Modern 22 is almost invariant (~−2.0‰) in the prenatal enamel, drops to ~−2.7‰ just before birth, and rises again to ~−2.5‰ after birth where it remains essentially invariant until the ~7th month after birth. The δ^{44/40}Ca data of Modern 27 are on average ~0.6‰ less negative and show a rapid in-utero decrease of 1.0‰ just before birth, followed by a gradual increase to ~−1.6‰, and a further increase to ~−1.3‰ at the ~2nd month after birth, from when the δ^{44/40}Ca decreases to ~−2‰ around 6 months of life. The Ca-isotope profile of Modern 29 reveals a broadly similar trend with the lowest δ^{44/40}Ca values just before birth, followed by a more pronounced increase to ~−1‰ around the 4th month after birth, and a final drop to ~−1.7‰ at ~9 months. Overall Modern 29 shows the highest δ^{44/40}Ca values. In all three δ^{44/40}Ca profiles, the lowest δ^{44/40}Ca value occurs just before birth. Interestingly, there is no consistent pre-birth/post-birth δ^{44/40}Ca offset among the three samples, as δ^{44/40}Ca after birth is lower in Modern 22, broadly invariant in Modern 27 and higher in Modern 29.

**Trace Element Profiles of Modern Deciduous Teeth**

Figure 3 displays the time-resolved Sr/Ca and Ba/Ca ratios of the three analysed deciduous teeth. The Sr/Ca and Ba/Ca ratios of sample Modern 22 are 50%–60% lower than those in Modern 27 & 29. The trace element profile of Modern 22 is shorter after birth (~4.6 months of life) relative to the respective Ca-isotope profile due to sampling-related loss of enamel. In Modern 22, Sr/Ca decreases by ~30% from 3
months before birth to 1 month after birth. Later, Sr/Ca increases slightly at ~2 months and then remains broadly invariant. In contrast, Ba/Ca increases between ~3 to ~2 months and then fluctuates. Overall, relative Ba/Ca variability is larger, and opposite to that of Sr/Ca across large parts of Modern 22. In Modern 27, the EDJ profiles of both Sr/Ca and Ba/Ca show increases from birth to around the 3rd month, with steeper rises between the 2nd and 3rd month. Sr/Ca then declines monotonously towards the 6th month, whilst Ba/Ca first decreases sharply between 3–5 months and then increases again towards the end of the profile. Over most of their respective profiles of Modern 29, Sr/Ca and Ba/Ca display different patterns. Whilst Sr/Ca decreases from ~1 month before to ~4 months after birth, Ba/Ca increases throughout that interval, with a further sharp rise to 7 µmol/mol until the 6th month, and a final drop to near birth values at 9–10 months. Sr/Ca increases moderately from 4 to 10 months.

Discussion

The three analysed deciduous canines represent a time interval from ~30 weeks in-utero to 9–10 months after birth (AlQahtani, Hector, and Liversidge 2010) for each of three early twentieth century central Italian infants. These infants could either have been breastfed or formula-fed, given the introduction of milk formula during the second half of the nineteenth century (Schwab 1996; Stevens, Patrick, and Pickler 2009). Pre-natal isotopic and trace elements compositions record the mother’s diet during late pregnancy, whereas the postnatal ones reflect the infant’s diet in the first few months, i.e. breast milk, animal/formula milk, solid food or the combination of all the three, depending on the individual’s feeding/weaning history.

Ca Isotope Signatures in Modern Deciduous Teeth

The composition of pre-and post-natal enamel segments, clearly delineated by the neonatal line, holds vital clues on whether prominent physiological events such as birth could cause significant variations in Ca isotope ratios of tooth enamel (similar to Li, Thirlwall, and Müller 2016) in addition to nutritional signals (Tacail et al. 2017). Overall, the Ca isotope profiles of our three investigated individuals are remarkably different.

For Modern 22, the post-birth δ⁴⁴/⁴⁰Ca values are consistently ~0.5‰ lower than the pre-birth values (Figure 2). This may be expected as a trophic level effect for breastfed infants as the child is one trophic level higher than the mother. The pre-birth signal can be therefore interpreted to reflect the mother’s diet/blood in her late pregnancy and the post-birth values (0–8 months) reflect the mother’s milk, given

---

**Figure 2.** Ca isotope profiles (δ⁴⁴/⁴⁰Ca) of laser-cut microsamples from three modern Italian deciduous teeth (Modern 22, 27, and 29). The time scale refers to the chronological range of secretion of each microsample in the individual’s life, calculated using the chronological grids. Error bars are 2SD for Ca isotope values (y-axis) and the time range of the enamel portions (x-axis).
that human milk is isotopically lighter than diet/blood in Ca (Chu et al. 2006). The stability in $\delta^{44/40}$Ca during pre-birth and after birth intervals (though at different values) may also suggest the mother’s diet was constant during the final stages of pregnancy and breast-feeding period. The in-utero $\delta^{44/40}$Ca data of Modern 22 suggest a diet value of around $−0.7‰$ (or slightly higher) of the mother before giving birth, given the 1.3‰ offset between diet and skeletal tissue (Skulan and DePaolo 1999) and assuming negligible (or small) Ca isotope fractionation in the placenta or during enamel formation. This diet value would correspond to the mother’s blood $\delta^{44/40}$Ca of $∼−1.3‰$ using a blood-diet difference of $−0.6‰$ (for $\delta^{44/40}$Ca; Tacail et al. 2017). Assuming Modern 22 to have been exclusively breastfed, we then estimate the infant diet $\delta^{44/40}$Ca to be around $−1.2‰$ (and blood $\delta^{44/40}$Ca of $−1.8‰$), based on the post-birth $\delta^{44/40}$Ca data and the same assumptions above. This post-birth diet $\delta^{44/40}$Ca value seems high for a breastmilk-exclusive diet. Based on limited data, human milk values are $\sim−2.3‰$ ($\delta^{44/40}$Ca, converted from Chu et al., 2006). On the other hand, long-term breast fed infants have shown no measurable changes in $\delta^{44/40}$Ca in the first 5–10 months from Tacail et al. (2017), whereas Modern 22 reflects lower $\delta^{44/40}$Ca values after birth than the majority of the pre-birth interval. The discrepancy from enamel $\delta^{44/40}$Ca data of deciduous teeth hint at the insufficiency of using exclusively Ca isotopes to resolve the feeding patterns of infants. Further uncertainty exists in the Ca isotopic relationship between the infant’s pre-birth enamel and mother’s diet. It is possible that this relationship is variable between individuals, or is modified by physiological factors.

On average, Modern 27 and Modern 29 show lower pre-birth $\delta^{44/40}$Ca values ($−2.4$ to $−1.4‰$) than post-birth $\delta^{44/40}$Ca values ($−1.8$ to $−1.0‰$). This is especially valid for Modern 29, whose Ca-isotope profile is opposite to that of Modern 22. From the post-birth segments of Modern 27 and Modern 29 we thus calculate an infant diet $\delta^{44/40}$Ca of $−0.5$ to $+0.3‰$, given the diet-skeletal tissue offset (Skulan and DePaolo 1999) mentioned above. They appear to be incompatible with human milk but at the upper end of non-human dairy products (Chu et al. 2006). This appears also to be in line with observations by

---

**Figure 3.** Trace element profiles (Sr/Ca and Ba/Ca) along the enamel-dentine-junction (EDJ) of three modern Italian deciduous canines. The time scale refers to months after birth of each individual, calculated using chronological grids from enamel histomorphometry. The compositional profiles were smoothed with a locally weighted polynomial regression fit, shown with its associated standard error ($\pm2$ S.E.).
Tacail et al. (2017), who reported $\delta^{44/42}\text{Ca}$ increases in post-birth enamel of formula-fed or briefly breastfed infants.

Despite these variable pre-/post-birth Ca isotope shifts amongst the three individuals, all three $\delta^{44/40}\text{Ca}$ profiles consistently display the lowest $\delta^{44/40}\text{Ca}$ values just before birth. This hints at a relationship between physiological change or stress and negative $\delta^{44/40}\text{Ca}$ excursions, which has also been reported for a third molar in a modern female (Li, Thirlwall, and Müller 2016). Overall the Ca isotope data alone seem to record prolonged breastfeeding in Modern 22, and may indicate that Modern 27 and especially Modern 29 had a predominantly formula-based diet.

**Trace Element Signature in Modern Deciduous Teeth**

The focus in this section is on the variation of both Sr and Ba (expressed as Sr/Ca and Ba/Ca) in deciduous enamel and their relationships to feeding and weaning practice (e.g. Humphrey et al., 2008a; Müller et al. 2019). Overall, Sr/Ca shows consistent and systematic secular variation related to infant nutrition, in contrast to Ba/Ca (see below). It has been noted (e.g. Humphrey et al., 2008a; Müller et al. 2019) that enamel Sr/Ca decreases at birth in a breastfed infant but increases after birth in a formula-fed child. Accordingly, for an exclusively breastfed individual, enamel Sr/Ca ratios would be expected to decrease across birth until food other than human milk – characterised by higher Sr/Ca – is introduced. For a formula-fed infant, on the other hand, the Sr/Ca signal increases after birth owing to the higher Sr content in formula (from herbivores) and the less well developed discrimination against Sr due to the immature digestive system of the infant. During weaning, Sr/Ca ratios can either increase or decrease depending on the food components consumed in this transition period.

For enamel Ba/Ca, Austin et al. (2013) reported an increase after birth and during exclusive breastfeeding, a further rise with introduction of formula milk, and finally a decrease during weaning in a human deciduous tooth. Similar patterns, excluding formula milk introduction, have also been observed in permanent first molars (M1s) of naturally weaned macaques by the same authors.

**Combined $\delta^{44/40}\text{Ca} – X/\text{Ca}$ Profiles of Three Modern Italian Deciduous Teeth**

With the assistance of the above observations and explanations, we aim at reconstructing the individuals’ feeding pattern for the three Italian deciduous canines based on combined Ca-isotope and trace element ratio data (Figures 2 and 3). Given the more systematic Sr/Ca patterns (see above), in the following we place greater reliance on Sr/Ca relative to Ba/Ca. Considering the nature of laser microsampling combined with analytical requirements for precise Ca isotope analysis, Ca isotope data unsurprisingly have lower time-resolution than X/Ca-ratio data obtained by LA-ICPMS.

(1) Modern 22

The absolute Sr/Ca and Ba/Ca values are ~50%–60% lower than in Modern 27 & 29, which ties in with the equally lower $\delta^{44/40}\text{Ca}$ values for Modern 22. This may indicate a different food source (e.g. more meat) or different location for Modern 22 relative to Modern 27 & 29. The pattern of decreasing Sr/Ca across birth until the second month (while Ba/Ca increases) suggests (exclusive) breastfeeding for the first 1–1.5 months. In view of the Sr/Ca and Ba/Ca increase and fluctuation from the 2nd month onwards, limited introduction of formula-based food is likely. The Ca isotope profile represents a longer time period (~2–8 months) than the X/Ca-profile due to the loss of lingual enamel, but no significant Ca isotopic variations is discernible for the final seven months. Taken together, both Ca isotope and trace element signals suggest breastfeeding for this individual, with limited formula-addition from the 2nd month onwards. The decrease in $\delta^{44/40}\text{Ca}$ across birth is consistent with this presumed trophic level effect, but not with the Ca isotope variation reported for long-term breastfed infants by Tacail et al. (2017).

(2) Modern 27

The nearly invariant Sr/Ca and Ba/Ca ratios across birth and for the first two months suggest breastfeeding with limited formula intake, as exclusive breastfeeding would result in a more apparent Sr/Ca decrease over this interval. The Sr/Ca and Ba/Ca ratio increase from 2 to 3.5 months suggests predominant formula intake, followed by exclusive breastfeeding until the end of the profile at 5 months, given the sudden Sr/Ca drop at ~3.5 months. Interestingly, the post-birth $\delta^{44/40}\text{Ca}$ profile generally mirrors this X/Ca variation and also argues for more non-human milk between months 2–4. The final $\delta^{44/40}\text{Ca}$ decrease is consistent with human milk with its most negative $\delta^{44/40}\text{Ca}$ composition. The Ca isotope signals for the first four months seem to be consistent with Tacail et al. (2017) who reported a systematic increase in $\delta^{44/42}\text{Ca}$ of non-breastfed individuals during the first few months.

(3) Modern 29:

The decreasing Sr/Ca values across birth and the first four months suggest predominant breastfeeding during this interval, with limited formula intake hinted
at by the small Sr/Ca peak in the 2nd month. Interestingly, Ba/Ca shows antiphase behaviour during this entire period, similar to that in Modern 22, but unlike Modern 27, where both Sr/Ca and Ba/Ca essentially covary. The nearly monotonous Sr/Ca increase for the remaining six months (until month 10, when Ba/Ca varies) suggest a switch to formula milk and/or solid food from the 4th month onwards. The $\delta^{44/40}$Ca profile of this infant suggests a non-breastfed (or only very briefly breastfed) history for the first ~6 months, given the reconstructed $\delta^{44/40}$Ca diet-value (see 4.1) and when applying the Tacail et al. (2017) model. Even though the similarly decreasing $\delta^{44/40}$Ca-value and Sr/Ca ratio at 8–9 months might hint at resumed human-milk intake, this appears rather late and thus could also have other, hitherto unknown physiological reasons.

The $\delta^{44/40}$Ca profiles appear to demonstrate the same inflection points due to putative diet change suggested by Sr/Ca (and Ba/Ca) ratios at 3 months in Modern 27 and 4 months in Modern 29, but the decrease in $\delta^{44/40}$Ca values conflicts with the potentially elevated $\delta^{44/40}$Ca in solids (weaning food) other than milk.

**Conclusions**

Our application of the methodological advances regarding controlled laser-microsampling for Ca isotope analysis (Li, Thirlwall, and Müller 2016) enables the study of secular Ca isotope variations in deciduous human teeth. Ca isotope analysis together with trace element ratios from laser-ablation mass spectrometry (LA-ICPMS: Sr/Ca, Ba/Ca) represent combined independent proxies for the reconstruction of past nursing histories at high time resolution. This tandem approach helps evaluate the strengths and weaknesses of the respective geochemical dietary proxies.

For the investigated modern deciduous canines free of diagenetic overprinting, we found contrasting secular Ca isotope patterns, namely both lower and higher post-birth $\delta^{44/40}$Ca ratios relative to the respective pre-birth values. While the former represents the expected Ca isotope trophic level shift, it was not observed in the larger sample by Tacail et al. (2017). All three modern deciduous teeth revealed that enamel $\delta^{44/40}$Ca recorded its lowest value (just) before birth, implying a control by physiological stress events like birth, similar to the observation on menarche by Li, Thirlwall, and Müller (2016). This shows that physiological effects affecting the Ca isotopic compositions need to be fully evaluated before reliable nutritional information can be extracted from fossil teeth from Ca isotopes only.

Sr/Ca (and Ba/Ca) measured along the enamel-dentine junction are consistent with the known trace element behaviour. Enamel Ca isotope data only are not sufficient to distinguish between breastfed or mixed formula-breasted infants. Examples are Modern 27 and Modern 29, which have similar Ca isotope variation patterns but different nursing histories according to trace element proxies, chiefly Sr/Ca. The combination of highly time-resolved trace element and laser-cut Ca isotope profiles in human enamel are key to uncovering detailed Ca isotope behaviour in mammals.

**Acknowledgements**

We are most grateful to the Leverhulme Trust, Research Project Grant RPG-156 (to WM and MFT), for funding of this study. LA–ICPMS work at RHUL was funded by SRIF3 (HEFCE) and NERC (NERC CC073) (to WM). Two anonymous journal reviewers are thanked for their constructive assessments of our work.

**Disclosure Statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This work was supported by Leverhulme Trust: [grant number RPG-156].

**ORCID**

Qiong Li http://orcid.org/0000-0001-5002-3989
Alessia Nava http://orcid.org/0000-0002-6319-0425
Linda M. Reynard http://orcid.org/0000-0001-5732-1532
Matthew Thirlwall http://orcid.org/0000-0002-1450-5048
Luca Bondioli http://orcid.org/0000-0002-8511-7667
Wolfgang Müller http://orcid.org/0000-0001-5368-8011

**References**


