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Comprendre le lien entre la mère et l'enfant dans les populations humaines anciennes : Méthodes avancées d'histologie dentaire et biogéochimie compositionnelle à haute résolution

Alessia Nava [1*]

1 Department of Odontostomatological and Maxillo Facial Sciences, Sapienza University of Rome, Rome, Italie

* alessia.nava@uniroma1.it

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Cette note fait suite à une communication invitée présentée lors des 1848es journées de la Société d'Anthropologie de Paris dans le cadre de la session "Analyses invasives, micro-invasives et non-invasives des vestiges anthropobiologiques. Comment et pourquoi?"

Abstract – The close maternal-infant relationship during pregnancy and early infancy is still an under-explored aspect of past human lives. Information about this nexus, which is driven by both biological and cultural characteristics, can be derived from the study of mineralised dental tissues, since teeth preserve a permanent record of an individual's biological life history from intra-uterine life to the first years after birth and up to early adulthood. Furthermore, a record of the mother-infant dyad's health status, diet and mobility during pregnancy is preserved in tissues that formed prenatally. State-of-the-art approaches to studying infants' growth trajectories, physiological stresses and mother-infant health status include both classic and non-destructive virtual histomorphometry. Furthermore, because human growth and development, from the earliest phases of ontogenesis, correlate strongly with dietary patterns, laser-based biogeochemical analyses of dental enamel can also be used to reconstruct the maternal diet – including during pregnancy – nursing practices and mobility patterns at very high temporal resolution. Models for the incorporation of trace elements in dental enamel and their correlation with major dietary shifts have been derived from analyses of exfoliated deciduous teeth of contemporary infants whose biological life histories are well known. These models make up an interpretative toolkit to disentangle subtle variations in individual diets at sub-weekly resolution. This contribution presents an overview of state-of-the-art high-resolution compositional biogeochemistry coupled with dental histomorphometry as a tool to deepen our knowledge of the mother-infant nexus in archaeological and palaeoanthropological contexts, and to investigate, at the highest possible temporal resolution, women's and children's survival and adaptation strategies over the course of human evolution.

Keywords – Histomorphometry, laser-based elemental biogeochemistry, mother-infant dyad, dietary patterns, weaning

Résumé – La relation intime entre la mère et l'enfant pendant la grossesse et la petite enfance est un aspect encore peu exploré chez les humains du passé. L'étude des tissus dentaires minéralisés permet d'obtenir des informations sur ce lien à la fois biologique et culturel. En effet, les dents conservent un enregistrement permanent de l'histoire biologique de l'individu, de la vie intra-utérine aux premières années après la naissance et jusqu'au début de l'âge adulte. En outre, les tissus formés pendant la période prénatale enregistrent l'état de santé, le régime alimentaire et la mobilité de la dyade mère-enfant pendant la grossesse. L'histomorphométrie classique et l'histomorphométrie virtuelle non destructive constituent les approches les plus modernes pour étudier les trajectoires de croissance des nourrissons, les stress physiologiques et l'état de santé de la mère et du nourrisson. En outre, l'alimentation maternelle, les pratiques d'allaitement, le début du sevrage et les schémas de mobilité peuvent être reconstitués avec une haute résolution temporelle grâce à des analyses biogéochimiques élémentaires et isotopiques de l'émail dentaire basées sur le laser. En effet, la croissance et le développement humains, dès les premières phases de l'ontogenèse, sont fortement corrélés aux comportements alimentaires, y compris ceux de la mère pendant la grossesse. Des modèles sur l'incorporation des oligo-éléments dans l'émail dentaire et leur corrélation avec les principaux changements alimentaires ont été dérivés de l'analyse des dents déciduales exfoliées de nourrissons contemporains dont l'histoire biologique est bien connue. Ces modèles constituent la boîte à outils interprétative permettant de démêler les variations, même subtiles, des régimes alimentaires individuels à une résolution infra-hebdomadaire. Cette contribution présente une vue d'ensemble de la biogéochimie compositionnelle à haute résolution couplée à l'histomorphométrie dentaire en tant qu'outils permettant d'approfondir notre connaissance du lien mère-enfant à partir des tissus dentaires minéralisés et d'étudier, avec la plus haute résolution temporelle possible, les stratégies de survie et d'adaptation des femmes et des enfants.

Mots clés – Histomorphométrie, biogéochimie élémentaire basée sur le laser, lien mère-enfant, comportement alimentaire, sevrage

Introduction

The way societies perceive and support pregnant women, mothers and infants has always been crucial, particularly in times of crisis and societal turmoil. The bond between a mother and her offspring starts during pregnancy and continues to shape a child's future well into adulthood. This relationship, along with the vital role of (breast)feeding and the care provided by the mother, forms a triad that profoundly influences both infant development and maternal health (Tomori et al., 2017; Bode et al., 2020). Factors such as ecological limitations, social challenges, cultural influences and adaptive responses have all played active roles in shaping the evolution of this interconnected system over time (Thorvaldsen, 2008; Sellen, 2009; Halcrow et al., 2020).

In recent years, there has been growing recognition of the significance of the mother-infant bond from social and medical perspectives (Brüne and Schiefenhövel, 2019; Kendall et al., 2021), with increasing attention given to understanding the historical and evolutionary aspects of this bond and examining how it has evolved across different periods, regions and cultures (Gowland and Halcrow, 2019; Stefanović et al., 2019; Halcrow et al., 2020; Miller et al., 2020; Rebay-Salisbury and Pany-Kucera, 2020). Taking a broader and long-term view, changes in women's roles and status during motherhood and childcare, including alloparenting, are essential to an in-depth understanding of the evolution of our species (Halcrow et al., 2020). Maternal health and behaviour, during both pregnancy and the early years of a child's life, have evolved and adapted in response to different environments and subsistence economies, and to the resulting gender-based workloads and social limitations (Halcrow et al., 2020). In fact, the interactions between mothers and infants, encompassing nursing behaviour, maternal and infant health and caregiving strategies, have been interwoven into the most significant biocultural shifts throughout human evolution.

To date, (bio)archaeological analysis of the mother-infant nexus has been based on direct and/or indirect evidence of the individual and separate components of the dyad, which are then traced back to their indissoluble unit through inferences (e.g. Lewis, 2007; Dunne et al., 2019; Gowland and Halcrow, 2019; Stefanović et al., 2019; Halcrow et al., 2020; Penezić et al., 2020; Rebay-Salisbury and Pany-Kucera, 2020). This is because of the intrinsic limitations to analyses of infant skeletal remains, such as those arising from the Osteological Paradox (Wood et al., 1992; DeWitte and Stojanowski, 2015) which further compounds the difficulties in obtaining information about the mother-infant dyad. Individuals with the highest rates of mortality, such as infants and children in particular, are often under-represented or poorly preserved in archaeological assemblages, on the one hand because their fragile bones are more prone to taphonomic post-depositional damages, and on the other hand because of a possible differential burial treatment of the subadult individuals (Bondioli et al., 2020; Sperduti et al., 2021). Moreover, the Osteological Paradox introduces a bias in interpretations, because the skeletal remains of infants and children represent individuals who did not manage to reach adulthood. To overcome these difficulties, bioarchaeologists should employ interdisciplinary approaches, while also acknowledging the limitations imposed by the fragmentary and biased nature of the skeletal record. Nevertheless, the study of dental remains provides insights into the close-to-daily history of an individual, from the protected intrauterine period to the end of tooth (or teeth) crown formation, and can thus approximate, as well as possible in a skeletal series, a longitudinal research design that can partly overcome the limitations arising from the use of a mortality sample.

Dental enamel contains physical and chemical markers that reveal crucial information about various aspects of an individual's life, including their diet, health, mobility, biological rhythms and growth rate from birth through the first years of life (Mahoney, 2015; Mahoney et al., 2016; Mahoney et al., 2017; Nava et al., 2017a; Nava et al., 2019; McFarlane et al., 2020; Nava et al., 2020; Smith et al., 2021; Li et al., 2022). By examining specific portions of the dental enamel that form before birth (known as prenatal enamel), the mother's diet, health and mobility during pregnancy can be examined from the mineralised tissue of her offspring (Nava et al., 2017a; Nava et al., 2017b; Nava et al., 2020). Furthermore, recent methodological developments enable infant sex to be determined by analysing enamel proteins (Stewart et al., 2017; Lugli et al., 2019; Lugli et al., 2020), allowing explorations into whether the infants' sex influenced the way they were cared for and thus introducing a gender perspective into the discussion.

Reconstructing early life histories

Humans are unique among primates in that their infants are born in an immature state and have a lengthy growth and development trajectory, so that they are completely dependent on their mothers during the first years of life (Smith, 1992). Life in utero is a sheltered period when environmentally driven forces of selection are mediated and attenuated by the maternal body and immune system (Barker et al., 2002; Almond and Currie, 2011). Prenatal life and conditions during infancy – up to the first ca 1000 days after conception – deeply affect adult health and survivorship (Barker, 2001; Barker et al., 2002; Barker, 2004;

Armelagos et al., 2009; Almond and Currie, 2011). This 'Developmental Origin of Health and Disease' (DOHaD) hypothesis states that the foetus adapts to stressors by constantly changing its physiology and metabolism (Barker, 1990; 2004; Temple, 2019). Equally, key moments during infant life, such as delivery, breastfeeding, weaning and crawling, and even social and psychological pressures on the mother-infant dyad, are important determinants in shaping their life history (Barker, 2004; Temple, 2019).

The growth and development of humans, starting from the earliest stages, are influenced by dietary patterns, including those of the mother during pregnancy, and by weaning practices. The processes of gestation and breastfeeding require significant energy investment from the mothers and are affected by access to food resources. Breastfeeding, in particular, is not a passive act but involves a mutual exchange of benefits between the mother and child (Kendall et al., 2021). Weaning, which is the introduction of solid foods into a child's diet, plays a crucial role in shaping the growth and health of infants (Humphrey, 2010): the immune coverage provided by maternal breast milk is reduced and with the gradual introduction of solid foods, infants become more exposed to pathogens (Abrams and Miller, 2011). Inadequate weaning practices can result in undernourishment, growth problems, delayed development and an increased risk of mortality (Miller, 2018), all of which can have long-term effects on adult health and ultimately lead to demographic changes (Bogin, 1999).

The timing and duration of weaning are determined by a trade-off between the energy costs to the mother during breastfeeding and the nutritional needs of the infant (Humphrey, 2010). Importantly, the age at which weaning occurs is linked to female fertility rates. For instance, early weaning shortens the intervals between childbirths in adult females, thus increasing fertility rates. However, having a greater number of slow-growing dependent children increases the mother's energy demand (Dean and Smith, 2009; Humphrey, 2010). The energetic burden on mothers, both physiological and even psychological, is partly alleviated through adaptations such as alloparenting (Hawkes et al., 1998; Kaplan et al., 2000; Tsutaya and Mizushima, 2023) and cooperative care (Foley, 1995). This includes the possibility of unburdening mothers from breastfeeding by using non- maternal milk (Tsutaya and Mizushima, 2023).

The majority of studies on weaning using the odontoskeletal remains of past human populations are based on analyses of chemical signatures of diet through nitrogen and carbon stable isotope composition ($\delta^{15}N$, $\delta^{13}C$) in bone collagen (e.g. Herring et al., 1998; Richards et al., 2002; Fuller et al., 2006; Dupras and Tocheri, 2007; Jay et al., 2008; Prowse et al., 2008; Eerkens et al., 2011; Humphrey, 2014; Beaumont et al., 2015; Britton et al., 2018; King et al., 2018). This method relies on the ability to reconstruct age at death from the skeleton, which can be imprecise (Tsutaya and Yoneda, 2015). More recent advances (Eerkens et al., 2011; Beaumont and Montgomery, 2015) use serial dentine micro-sections of the permanent dental roots to estimate weaning age through the nitrogen and carbon stable isotope ratio ($\delta^{15}N$, $\delta^{13}C$). However, this approach is still too coarse in time resolution to effectively catch subtle changes, spanning a few months, in weaning times (Tsutaya, 2020).

Recent developments in trace elements analysis of mineralised dental tissues combining dental histology and high-resolution compositional biogeochemistry (Austin et al., 2013; Humphrey, 2014; Smith et al., 2018; Joannes-Boyau et al., 2019; Müller et al., 2019; Nava et al., 2020; Li et al., 2022; Smith et al., 2022; Kubat et al., 2023), have been used to reconstruct feeding patterns and weaning practices in the early lives of past human populations.

High-resolution histology-driven compositional biogeochemistry of dental enamel

Dental histology coupled with high-resolution compositional biogeochemistry (figure 1) can be used to explore dietary shifts and nursing behaviour in early life at the highest possible temporal resolution, in order to retrieve direct evidence of one of the aspects of the mother-infant nexus in past humans (Müller et al., 2019; Nava et al., 2020; Li et al., 2022; Smith et al., 2022).

Dental enamel formation has two distinct rhythms, reflecting the regular and incremental secretion of the enamel matrix by specialised cells called ameloblasts. Firstly, a circadian rhythm results in the formation of cross-striations, indicating daily growth patterns (figure 1d) (Lacruz et al., 2012; Zheng et al., 2013). Secondly, there is a longer-term rhythmic marking, occurring approximately once a week in humans, which gives rise to what are known as Retzius lines (figure 1b) (Dean, 1987). Physiological stresses experienced by an individual during tooth development can disrupt the secretion of the enamel matrix and leave corresponding marks on the enamel surface, indicating the position of the secretory ameloblast front. These marks are referred to as Accentuated (Retzius) Lines (ALs) (Witzel et al., 2008; Nava et al., 2019). Notably, the birth event can be observed in the developing enamel of individuals who survive the perinatal stage. Typically, the first Accentuated Line that appears in the enamel is referred to as the Neonatal Line (NL) (Sabel et al., 2008; Dean et al., 2019).

The variation of trace elements in human milk and corresponding dental enamel has been well known since the late 1990s as a proxy for dietary reconstructions (e.g. Krachler et al., 1999a; Krachler et al., 1999b; Rossipal et al., 2000; Humphrey et al., 2008b; Matos et al., 2014). In the last few years, combining laser-ablation inductively-coupled-plasma mass spectrometry (LA-ICPMS) with dental histology has allowed high-resolution compositional analyses of human teeth to retrieve metabolic signals (Austin et al., 2013; Smith et al., 2018; Müller et al., 2019; Nava et al., 2020; Smith et al., 2021).

Deciduous exfoliated teeth from contemporary individuals with well-known and detailed feeding and anamnestic histories and who were free of problems in retrospective

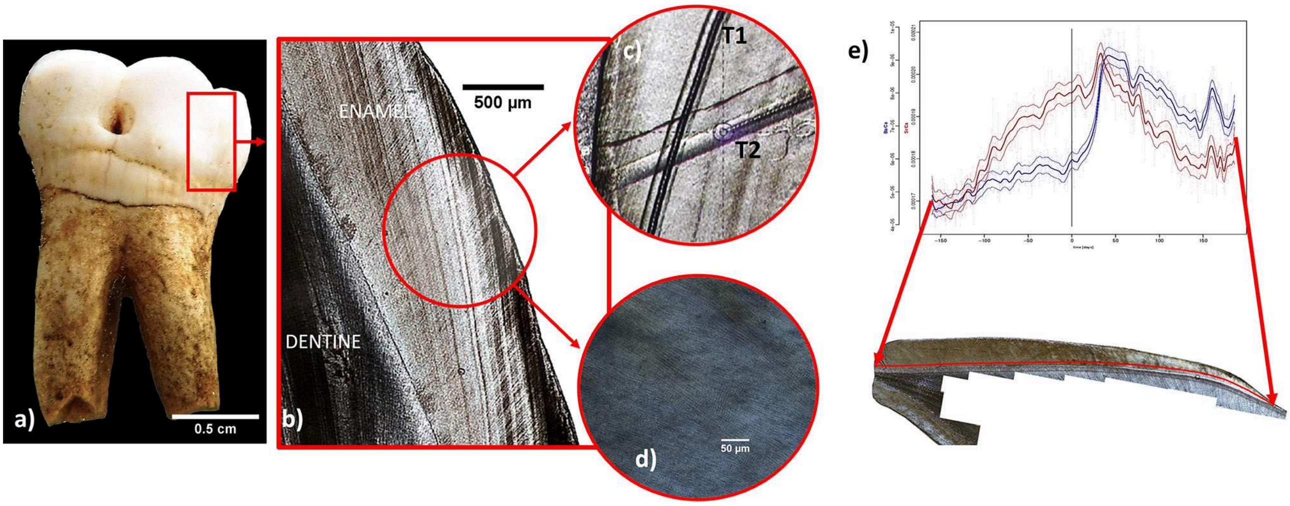

Figure 1. Schematic representation of the analytical pathway. a) Photograph of a permanent human tooth; b) Transmitted-light micrograph of a portion of a thin section of a permanent tooth showing enamel and dentine and, inside the enamel, the physiological and stress lines; c) Transmitted-light micrograph from the laser-ablation (LA) system showing the LA tracks, in this case parallel to Retzius lines (T1) and enamel prisms (T2) (from Müller et al., 2019); d) Transmitted-light micrograph of a portion of a thin section of a permanent tooth showing the daily increments; e) Transmitted-light micrograph of a deciduous tooth with highlighted LA path (bottom, red line) and the corresponding Sr/Ca and Ba/Ca profiles (top). The vertical black line marks the birth event and corresponds to the neonatal line in dental enamel. The distances along the LA path were transformed into days of life using the microstructural reading of the thin section / *Représentation schématique illustrant la méthode d'analyse. a) Photographie d'une dent humaine permanente ; b) Acquisition par microscopie électronique en transmission d'une partie d'une lame mince d'une dent permanente montrant l'émail et la dentine et, au sein de l'émail, les lignes physiologiques et les lignes de stress ; c) Acquisition par microscopie électronique en transmission après ablation laser (AL) montrant les traces de AL, dans ce cas parallèles aux lignes de Retzius (T1) et aux prismes de l'émail (T2) (d'après Müller et al., 2019) ; d) Acquisition par microscopie électronique en transmission d'une partie d'une lame mince d'une dent permanente montrant les incréments journaliers ; e) Acquisition par microscopie électronique en transmission d'une dent déciduale avec la trajectoire AL représentée (en bas, ligne rouge) et des profils Sr/Ca et Ba/Ca correspondants (en haut). La ligne noire verticale marque l'événement de naissance et correspond à la ligne néonatale dans l'émail dentaire. Les jours de vie correspondants ont été déterminés par les distances le long du profil AL sur la lame mince*

reports (Gillespie et al., 2006) of breastfeeding practices are pivotal to building interpretative dietary models from trace element profiles *vs* individual lifetimes (Müller et al., 2019; Nava et al., 2020). These models can be applied to dental remains from archaeological or palaeoanthropological horizons to disentangle dietary life histories during the early life of past individuals (Austin et al., 2013; Smith et al., 2018; Joannes-Boyau et al., 2019; Nava et al., 2020; Li et al., 2022; Kubat et al., 2023). Details on the histological approach and biogeochemical analysis can be found in (Müller et al., 2019; Nava et al., 2019; Nava et al., 2020).

Identifying breastfeeding *vs* formula feeding

Strontium and barium are non-bioessential trace elements with no major metabolic functions in the human body (see discussion in Müller et al., 2019; Nava et al., 2020). Strontium and barium follow the calcium metabolism but are discriminated against in the gastrointestinal tract due to their larger ion size (Elias et al., 1982; Burton et al., 1999). Ba is discriminated against even more strongly relative to Sr (Elias et al., 1982; Balter, 2004) due to its larger size. Sr, Ba and Ca are fixed in bones and teeth from plasma, with a likely preference for Ca (Kshirsagar et al., 1966; Dahl et al., 2001). The sum of these factors causes concentrations of Sr/Ca and Ba/Ca in odontoskeletal tissues to be lower than those in the diet, due to the process known as 'biopurification' (Burton et al., 1999). Empirical evidence indicates that discrimination for Sr by the mammary gland is higher than by the placenta, which yields average breastmilk Sr/Ca values that are lower than umbilical cord (foetal) values (ICRP, 2004). This depletion in Sr/Ca is documented in the formation of dental tissues as a decrease in the Sr/Ca signal during exclusive breastfeeding, as shown by high-spatial-resolution chemical analysis of teeth (Humphrey et al., 2008b; Müller et al., 2019; Nava et al., 2020). Conversely, formula milk (which is made with derivates of herbivore milk or plant-based substitutes) has a higher Sr and Ba content than breast milk and therefore induces an increase in the Sr/Ca signal.

Figure 2 shows spatially-resolved chemical data from two contemporary individuals whose dietary patterns are known.

Two deciduous teeth, representing two different nursing histories, were analysed by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS, details of the methods reported in Nava et al., 2020). These individuals were selected because of the detailed and reliable reporting of their feeding practices during nursing. In figure 2a is represented the dietary history, derived from the deciduous second molar, of an exclusively breastfed boy who was born in Switzerland; Figure 2b shows the elemental profile across a deciduous canine of an exclusively formula-fed boy from central Italy (data from Müller et al., 2019; Nava et al., 2020). Neither of the mothers of these two infants travelled for significant periods during the time of formation of the teeth analysed.

The profile in figure 2a shows a steady decrease in the elemental ratios until ~5 months (154 days), which corresponds to the reported period of exclusive breastfeeding. As soon as supplementary food was introduced once a day into the infant's diet (i.e. onset of weaning), the slope of the profile changed and gradually became shallower. From ~6 months (182 days) some formula milk was introduced along with breastmilk and supplementary food. This event is reflected in a slight change in the Sr/Ca profile slope. At ~7 months (day 209) a further reduction in breastfeeding during the daytime is reported, and corresponds to a sharp increase in the Sr/Ca values. At 8.5 months (258 days after birth), the breastfeeding period of the individual came to an end and the diet continued with solid food and formula milk. The Sr/Ca profile at this point shows a further increase. The Sr/Ca signal observed in the last part of the profile (after day ~340) probably reflects the effect of the maturation overprint (Müller et al., 2019) due to the thin enamel closest to the crown neck rather than a biogenic dietary signal. The Ba/Ca profile broadly follows the trend observed for Sr/Ca, decreasing, though to a lesser extent, from birth until ~5 months (160 days) during the reported period of exclusive breastfeeding. Thereafter, the Ba/Ca profile increases slightly until ~8 months (day 235), then shows a steep increase up to 9.5 months (day 290). It then decreases until ~10 months (day 315), before a final steady increase until the end of crown formation. This fluctuation in the last part of the profile cannot be explained by any event in the known dietary/health history of this individual.

The profile in figure 2b belongs to an exclusively formula-fed individual. The Sr/Ca profile shows a steady increase after birth until ~4 months (130 days), which is the reported period of exclusive formula milk use. After this period, the profile starts to decrease during the reported period of the onset of weaning. In this case, the Ba/Ca profile follows the Sr/Ca profile quite closely.

Trophic level and maternal diet

Figure 3 shows the Sr/Ca concentrations in the prenatal enamel of two contemporary children with a well-known dietary and anamnestic history, whose mothers had different dietary preferences during pregnancy. One mother reported consuming large amounts of meat during this period, while the other had a predominantly vegetable-based (i.e. plant-based) diet. The reason for these choices was simply personal taste and specific cravings during pregnancy. Assuming that placental biopurification was comparable between the two mothers, there is an evident trophic level effect (Pate, 1994; Balter et al., 2012; Kubat et al., 2023): the mother with a large meat component in her diet during pregnancy shows a higher trophic level (lower Sr/Ca content) than the mother with a plant-dominated diet.

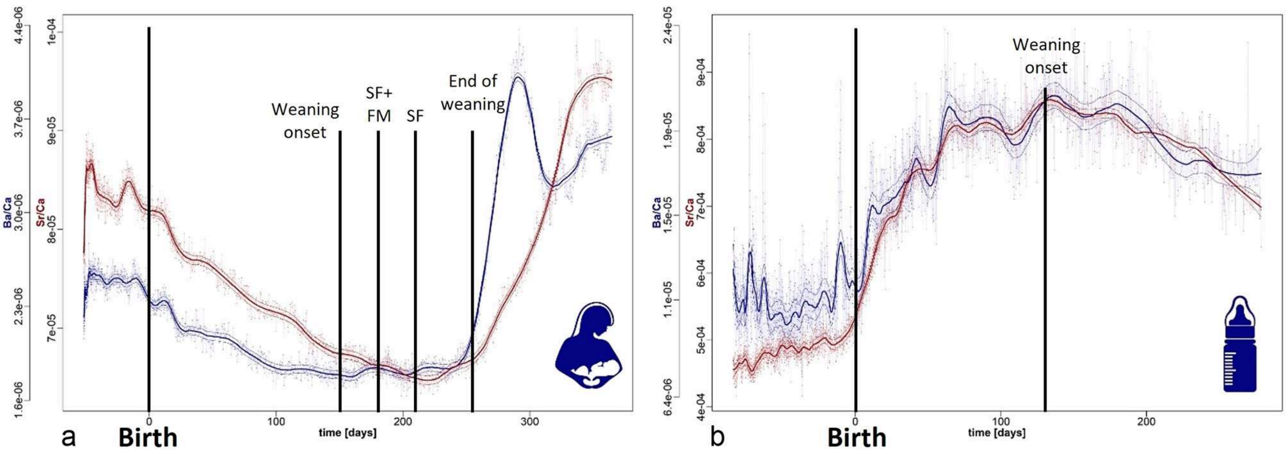

Figure 2. Elemental profiles of contemporary individuals (modified from Nava et al., 2020): a) an exclusively breastfed individual and b) an exclusively formula-fed individual. In exclusively breastfed individuals, Sr/Ca and Ba/Ca decrease until formula and/or solid foods are introduced into the infants' diet. In formula-fed individuals, conversely, Sr/Ca and Ba/Ca increase until the introduction of solid food into the infants' diet. SF=solid food introduction; FM=formula milk introduction / *Profils de compositions élémentaires d'individus actuels modifié à partir de (Nava et al., 2020) ; a) un individu exclusivement nourri au sein et b) un individu exclusivement nourri au lait maternisé. Chez les individus nourris exclusivement au sein, les ratios Sr/Ca et Ba/Ca diminuent jusqu'à l'introduction du lait maternisé et/ou d'aliments solides dans le régime alimentaire des nourrissons. Chez les individus nourris au lait maternisé, à l'inverse, les ratios Sr/Ca et Ba/Ca augmentent jusqu'à l'introduction d'aliments solides dans le régime alimentaire des nourrissons. SF=introduction d'aliments solides ; FM=introduction de lait maternisé*

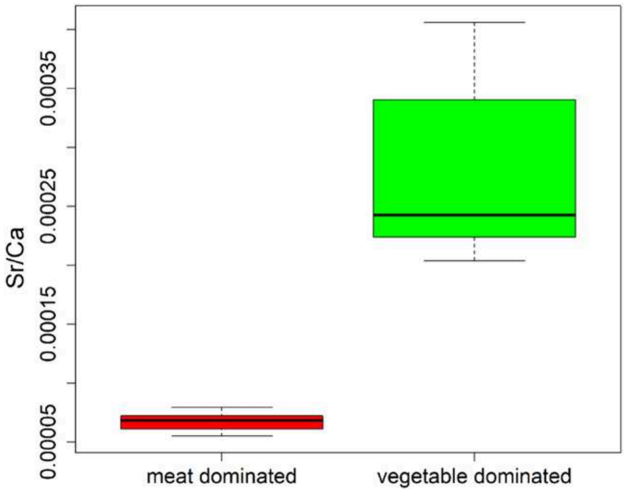

Figure 3. Sr/Ca boxplots of the prenatal enamel of two contemporary individuals whose mothers had different diets during pregnancy / *Boîte à moustaches des ratios Sr/Ca de l'émail prénatal de deux individus actuels dont les mères ont suivi des régimes alimentaires différents pendant la grossesse*

Destructive and micro-destructive analyses of human dental remains

The major issue with histological and biogeochemical approaches is the need for destructive sampling. Today, almost all advanced analyses of human bioarchaeological or palaeoanthropological specimens, such as ancient DNA, stable or radiogenic isotope analyses, serial sampling of dentine, trace element analysis of dental enamel or histological analysis of teeth and bones, rely on destructive sampling of tissue portions from archaeological or fossil specimens. Moreover, particularly when these analyses involve very old and valuable fossil remains, concerns about their integrity and the desirability of preserving them for possible future analysis using future techniques may complicate or hinder the process of obtaining permission for destructive sampling. However, without the destructive analyses that have been developed and applied in recent decades, our knowledge of the past would be much more incomplete. From this point of view, bioarchaeologists should carefully consider the balance between the damage caused and the expected scientific output before undertaking destructive/microdestructive sampling. The matter is further complicated by the different regulations in different countries or institutions, and changing policies over time.

The classic histological approach, which is in essence a partially destructive analysis (see below on restoration), is the basic tool in the analytical approach described above. In contrast, biogeochemical analysis of mineralised dental tissues is microdestructive, but cannot be carried out without classic histology because of the limitations imposed by the need to collect data in the innermost part of the enamel, close to the EDJ (for details see Müller et al., 2019). Despite the destructive nature of the histological approach, the unique set of information about the biological life history of the mother-infant dyad that can be obtained *from a single dental element* may be worth the partially destructive sampling. Following a modified version of the ALARA principle (As Low As Reasonably Achievable [damage]), which is commonly adopted in human radiological and dental medical practice (Yeung, 2019), destructive analyses should follow a few simple but strict rules: a) No analyses should be undertaken unless they are expected to provide a net benefit; b) All destructive sampling must be kept as low as reasonably achievable, taking into account economic and social factors; c) Destructive sampling must not exceed the limits recommended in the literature and by curators for appropriate circumstances. Following this principle, after the histological thin sectioning, a possible good practice is to perform a reconstruction of the sampled dental elements to recover their original morphology. This can be done through reconstruction of the sampled portions using, for example, light-curing dental restoration resin (Nava et al., 2020) or, as proposed more recently, through reverse engineering and rapid prototyping techniques (Vazzana et al., 2022).

A possible attenuation of destructive and microdestructive sampling is the ability to perform virtual non-destructive histological analysis using synchrotron-based X-ray microtomography. The now well-established use of this technique (Tafforeau and Smith, 2008; Tafforeau et al., 2012; Le Cabec et al., 2015; Le Cabec et al., 2017; Nava et al., 2017b; Mahoney et al., 2021; Cerrito et al., 2022; Nava et al., 2022) allows analyses of incremental dental growth microstructures to be extended to ancient specimens, albeit without the biogeochemical data. It is worth noting that synchrotron-based X-ray fluorescence imaging, which is applied to analyse elemental distributions in mineralised dental tissues (Dean et al., 2019; Dean et al., 2020), requires dental thin sections.

Discussion and conclusions

The role and contribution of women and children to the formative moments of human history have only recently begun to be explored systematically (Gowland and Halcrow, 2019; Halcrow et al., 2020; Nava et al., 2020; Rebay-Salisbury and Pany-Kucera, 2020). Great effort has been invested in finding ways to give a voice to these members of past human societies who usually remain hidden in archaeological and historical records, because social and historical constraints have masked and diminished the presence of mothers and infants as key determinants of past social dynamics and relationships. A deeper understanding of mothers' and infants' roles in the past is essential for all studies concerning the evolution of social and gender inequalities, social stratification and the evolution of hierarchies. Therefore, any attempt to reconstruct prenatal and early postnatal growth trajectories, dietary habits and health status by analysing the skeletal and dental remains of infants from archaeological contexts is of fundamental importance to understand the general pattern of growth and development of past humans and their social interactions from a biocultural perspective (Nava et al., 2017a; Jovanović et al., 2019; Nava et al., 2019; Nava et al., 2020; Tsutaya and Mizushima, 2023).

Recent advances in elemental and isotopic analyses of mineralised dental tissues coupled with dental enamel histomorphometry – being based on in-depth knowledge of elemental metabolism in humans (Smith et al., 2018; Müller et al., 2019; Tacail et al., 2019; Jaouen et al., 2020; Smith et al., 2020; Smith et al., 2021; Li et al., 2022) – are producing effective tools to perform longitudinal analyses of the dietary variations, mobility, health status and growth rates of past human children and, at the same time, of their mothers. In particular, elemental analysis can identify the switch from exclusive breast-feeding to a diet that includes non-milk foods, and assess the mother's diet during pregnancy (Humphrey et al., 2008a; Humphrey, 2010; Austin et al., 2013; Humphrey, 2014; Tacail et al., 2017; Jaouen et al., 2020; Nava et al., 2020; Bourgon et al., 2021; Li et al., 2022). Alignment of these time-resolved chemical signals of the diet with high-resolution histological evidence is capable of providing the precise age when the process of weaning began (Humphrey et al., 2008b; Humphrey, 2010; Austin et al., 2013; Nava et al., 2020).

The striking correspondence between the independently recorded dietary transitions in contemporary individuals and the Sr/Ca trends (also the Ba/Ca trends to a lesser extent) fully supports the use of Sr/Ca as a proxy for nursing events and particularly for the onset of weaning. The application of these models to archaeological and palaeoanthropological specimens has shown the effectiveness of Sr/Ca variation profiles to identify dietary variations in early life, as well as in dental enamel potentially affected by a diagenetic imprint (Nava et al., 2020; Kubat et al., 2023). It is worth noting that Ba tends to be more affected by diagenesis than Sr: This, coupled with the more erratic behaviour of Ba in tissue incorporation (Müller et al., 2019), suggests the Sr signal as the preferred option to retrieve the dietary biogenic signal (figure 4). However, the use of Sr *vs.* Ba is still controversial among authors, as other researchers prefer to rely on Ba for palaeonutritional reconstructions (Austin et al., 2013; Smith et al., 2018; Smith et al., 2022).

Furthermore, Sr/Ca ratios can be used as relative trophic level proxies (Peek and Clementz, 2012; Nava et al., 2020; Kubat et al., 2023). The higher the trophic level, the smaller the quantity of Sr relative to Ca incorporated into the enamel, resulting in higher values for trace element ratios in the enamel of herbivores than in omnivores or carnivores (Kubat et al., 2023). Sr/Ca ratios from fossil teeth of carnivores, herbivores and omnivores from the Sangiran Dome (Indonesia, late Early Pleistocene, see Kubat et al., 2023) show that their content in dental enamel reflects differences in trophic level according to their expected dietary habits, and therefore suggests that trophic level determination

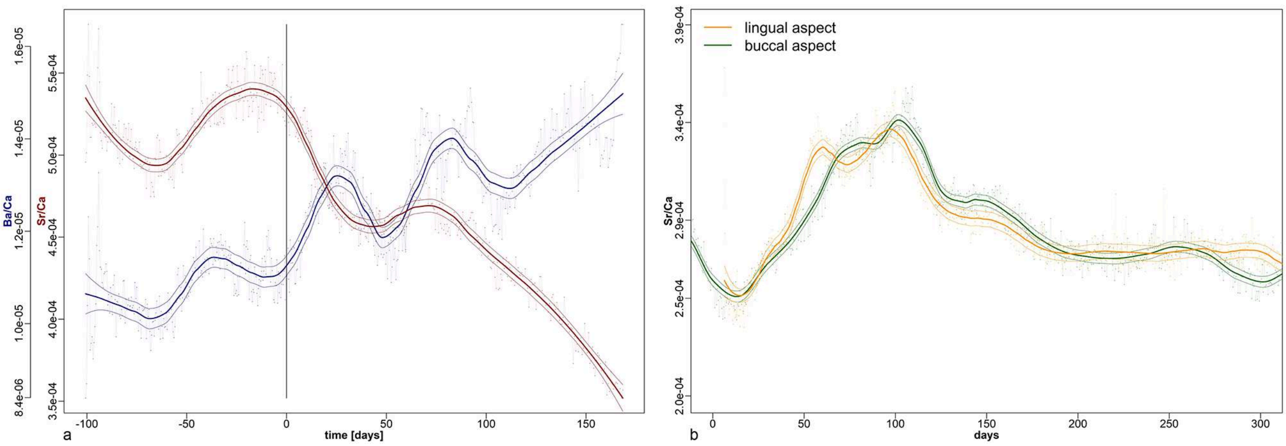

Figure 4. Ba/Ca vs Sr/Ca profiles. a) Elemental profiles of a contemporary exclusively breastfed individual, showing that Ba does not constantly co-vary with Sr, contrary to what is observable in figure 2a,b; b) Buccal *vs.* lingual profiles of the same tooth of another contemporary individual, showing the consistency between crown aspects of the retrieved Sr/Ca signal / *Profils des ratios Ba/Ca* vs. *Sr/Ca. a) Profils des compositions élémentaires d'un individu actuel nourri exclusivement au sein montrant que Ba ne covarie pas constamment avec Sr, contrairement à ce qui est observé dans la figure 2a,b ; b) Profils des compositions élémentaires au niveau vestibulaire et lingual de la même dent d'un autre individu actuel, montrant la cohérence entre les aspects de la couronne du signal des ratios Sr/Ca récupéré*

based on enamel Sr/Ca is reliable. In another study, Nava et al. (2020) suggested that Sr/Ca in prenatal enamel can point to maternal dietary differences during pregnancy. This study, performed on three Neanderthals and one Upper Palaeolithic *Homo sapiens*, showed a difference in food exploitation based on the comparative Sr/Ca profiles between Neanderthals and early *H. sapiens*, with mothers in the latter category consuming low-biopurified (e.g. vegetables) non-local foodstuffs with a high Sr/Ca content, while the Neanderthal mothers consumed highly biopurified (e.g. meat) local food (Nava et al., 2020).

The usefulness of this type of approach has already been demonstrated in a number of papers that have brought an understanding of hitherto unknown aspects of the life history of hominins (Austin et al., 2013; Smith et al., 2018; Joannes-Boyau et al., 2019; Nava et al., 2020; Kubat et al., 2023). Over and above this approach to fossil remains, it is clear that the possibility of applying this methodology to more recent contexts can be of great use in identifying cultural and/or stress-related differences in archaeological populations (e.g. Kamenov et al., 2018; Simonetti et al., 2021; Simpson et al., 2021; Oldershaw et al., 2023). The information that can be obtained is of the highest quality once the diagenesis that can affect such remains is precisely controlled (Nava et al., 2020; Rey et al., 2022). In addition, the ability to distinguish infant feeding with maternal milk from feeding with animal milk allows important conclusions to be drawn about the domestication of herbivores, alloparenting and the role of women in society. To conclude, recent advances in both high-resolution biogeochemistry and dental histology are opening up new cognitive windows on aspects of the early life of our ancestors that were previously impossible to retrieve even with powerful methods such as aDNA, and thus offer a new tool to investigate the so far scantily researched and historically neglected life of the mother-infant dyad in the past.

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