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SHORT REPORT

Multi-isotope analysis of primary and secondary dentin as a mean to broaden intra-life dietary reconstruction. A case from Longobard Italy

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Abstract

This exploratory study proposes an original intra-life history investigation through sequential analysis of the isotopic composition of carbon, nitrogen, and sulfur (CNS) on both primary and secondary dentin of a tooth (M1). We focus on an elderly woman from Longobard Italy (6th to 8th c. CE), who showed an unprecedented case of cranial surgery, presented in a companion paper by Micarelli and colleagues. Sequential stable CNS isotope composition of first molar dentin collagen allows us to infer diet, mobility, health, and physiological stress between approximately 3 months after birth to 9.5 years old (primary dentin) and between early adulthood until death (secondary dentin). Isotopic results on primary dentin highlight the following: (i) a long weaning period (ending at approximately 4 years), followed by (ii) a specific diet, including the contribution of C_4 crops in early childhood (approximately 5.5 years), possibly concomitant with mobility. While secondary dentin shows a generally homogeneous diet during adulthood, the longitudinal analysis provided information on specific stresses that likely occurred in periods of difficult health conditions. This work emphasizes the importance of measuring complete dentin sequences (including secondary when present) for isotopic analysis to broaden intra-life histories in ancient populations.

KEYWORDS
carbon, isobiography, nitrogen, postclassical antiquity, stable isotopes analysis, sulfur

1 | INTRODUCTION

Recently, palaeodietary reconstructions by means of stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) of incremental dentin microsections proved to be relevant in exploring intra-life histories of past populations while highlighting specific cultural phenomena (e.g., weaning practices and early-life mobility) (e.g., Cocozza et al., 2021; Fernández-Crespo et al., 2020; Goude et al., 2020).

During permanent tooth development, primary dentin formation follows a relatively constant crown-to-apex deposition (4 to 6 μm per day according to tooth area) (Dean & Scandrett, 1995), providing a time interval for each microsection. Dentin isotopic data correspond to childhood dietary habits, while bone data correspond to the last decades of life, depending on the bone turnover rate. Although bone and teeth are not strictly comparable due to different physiology of tissue formation (Beaumont et al., 2018), their stable isotope composition has often been used to reconstruct intra-life dietary changes. However, when this approach is applied to mature individuals, it generates an isotopic "gap" between the end of childhood and the last years of life: decades of dietary intake that are invisible to the isotopic investigation.

This study deals with a stable isotope analysis of secondary dentin microsections, with the aim to broaden the interval of life-long dietary studies.

Secondary dentin is a dental tissue which begins to form after apex closure in a nonhomogeneous continuous deposition during life (Nanci, 2013). In permanent molars, the pulp cavity is progressively filled starting from the coronal area. Its thickness is mainly correlated to age and force distribution along the tooth and seems to act as a compensatory mechanism against mechanical loading (Nudel et al., 2021). Currently, this tissue is removed prior to incremental dentin analysis to avoid intrusive adulthood data in early-life dietary reconstruction (Scharlotta et al., 2018). Since M1 is the first permanent tooth to develop, it accounts for the longest bio-physiological information when considering both primary and secondary dentin.

A life-long dietary analysis is developed on an exceptional archeological case study from a Longobard Italian skeletal collection (Castel Trosino, 6th-8th c. CE, in Ascoli Piceno, central Italy) (Micarelli et al., 2021). The individual (CT1953), an elderly woman represented only by her cranium, was subjected to an unprecedented case of trepanation, discussed in a companion paper (Micarelli et al., submitted). Maxillary teeth show ongoing diseases: periodontal disorders, acute abscesses (RI^1, RI^2, LI^2), and severe molars wear associated with tooth loss (RM^3). The trepanation process and the oral pathologies could have somehow influenced food habits.

2 | MATERIALS AND METHODS

In this study, we analyze stable carbon, nitrogen, and sulfur isotopes on primary and secondary dentin collagen microsections of a non-pathological molar (ULM^1) belonging to the individual illustrated in the companion paper by Micarelli and colleagues (submitted).

Following ethical principles in bioarcheology and cultural heritage conservation, a micro-CT scan of the selected tooth was performed before sampling (Figure 1a). This allowed age estimation by pulp chamber volume measurement following the method proposed by Ge et al. (2015) (Figure 1b and Method S1).

Tooth sampling followed a modified Beaumont et al. (2013) method (Figure 1c and Method S2). Dentin collagen for stable carbon, nitrogen, and sulfur isotopes analysis was extracted with a modified ABA method (Method S2).

Age assignment of primary dentin microsections ($n = 14$) was calculated by applying Czermak et al.'s (2020) method (Method S2 and

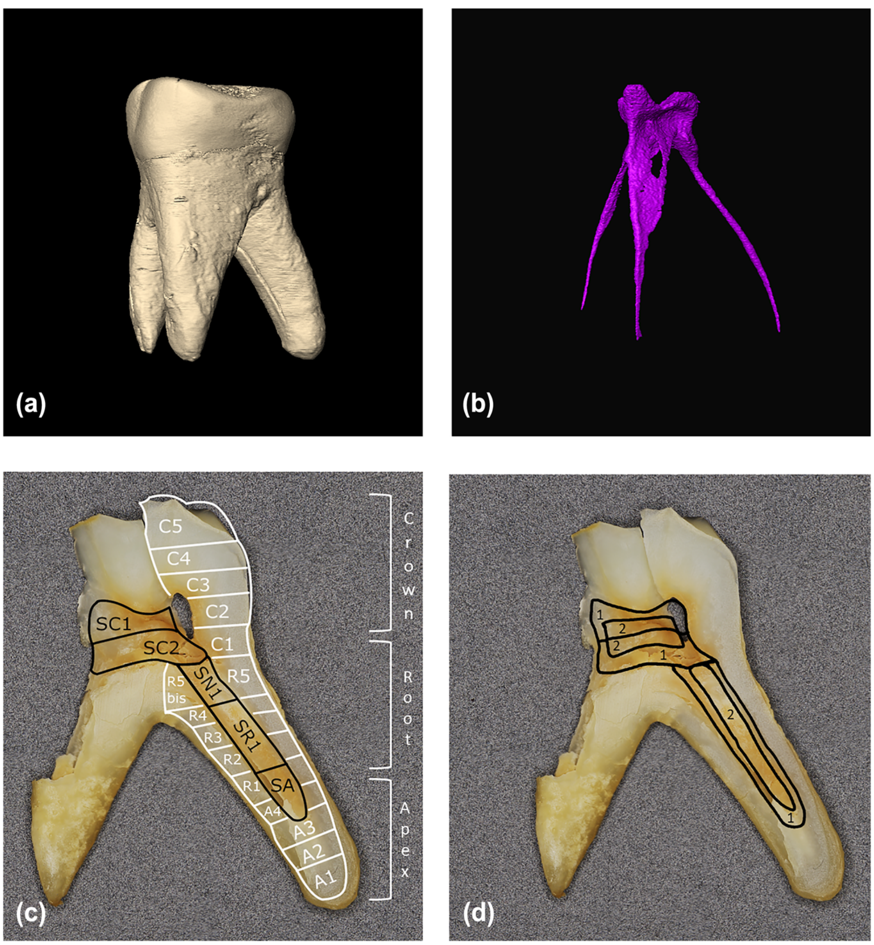

FIGURE 1 (a) Micro-CT scan of CT1953 first molar, distal view; (b) 3D image of CT1953 pulp cavity volume (distal); (c) tooth slice after chemical treatment with indication of the anatomical area and microsection sampling scheme. In white primary dentin microsections, in black secondary dentin sections; (d) tooth slice with the proposed scheme of secondary dentin microsampling. Numbers represent possible sequence considering inwards dentin deposition. [Colour figure can be viewed at wileyonlinelibrary.com]

Data S1). Accurate age correlation of secondary dentin microsections could be aleatory considering its less uniform deposition (Method S2). Therefore, the five microsections of secondary dentin (Figure 1c) represent the dietary variation from the end of tooth formation to the estimated age of death (∼10 to 50 years old). Not knowing the dietary representativity of each microsection, data from the pulp chamber samples ($n = 2$) have been merged and mean values considered (Figure 2a and Data S1), although individual measurements are reported for clarity (Figure 2b).

Considering the inward formation of secondary dentin, we propose here an alternative sampling procedure, which might allow a finer longitudinal dietary reconstruction (Figure 1d).

Stable carbon and nitrogen isotope data of bone collagen samples available from the same collection ($n = 18$, including CT1953) (Bernardini et al., 2021) have been used as a reference for the incremental dentin data interpretation.

3 | RESULTS AND DISCUSSION

The measurement of pulp chamber volume from Micro-CT scan images allowed us to estimate an age at death between 41 and 50 years old (Ge et al., 2015) (Method S1), confirming the estimates based on the observation of the cranium sutures and dental wear (approximately 50 years old) (Micarelli et al., 2021, submitted).

Analytical data and calibration of stable isotope measurements are reported in Method S2. The measurements errors are 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ and 0.4‰ for $\delta^{34}S$ (Method S2).

Stable isotope incremental dentin results from approximately 1.1–1.9 years and from two secondary dentin sections were excluded from this analysis, as the data did not meet the collagen quality indicators (DeNiro, 1985; Nehlich & Richards, 2009; van Klinken, 1999) (Data S1 and Method S2).

Isotopic results from CT1953's first years of life show a moment of dietary transition, likely associated with weaning. The transition from breastfeeding to solid food is generally represented by a constant decline in both $\delta^{15}N$ and $\delta^{13}C$ values (e.g., Eerkens et al., 2011; Sandberg et al., 2014).

Here, microsections from approximately 0.7 to 4 years show a steep decline in the $\delta^{15}N$ values by ∼5‰, with $\delta^{13}C$ only showing a minimal decrease as expected (Figure 2a). The $\delta^{15}N$ profile of CT1953 shows a significant drop that could be related to different factors. These include (i) a rapid decrease in mother's milk consumption during weaning, representing the most probable scenario, (ii) the rapid introduction of solid food with low $\delta^{15}N$ (vegetal resources), and (iii) not to exclude, a change in the mother's diet and/or physiological changes during breastfeeding, as proposed elsewhere (Herrscher et al., 2017).

In these four first years of life, $\delta^{34}S$ values oscillate from 4.5‰ to 6.2‰, reflecting a possible movement between different environments before the age of 5 (Figure 2a and Data S1). This could also be associated with breastfeeding by a woman of different origins (e.g., a wet nurse) (Mummey & Reyerson, 2011) or displacement during early-life. In Late Medieval Europe, sources report that children could be sent to the countryside for nursing (Mummey & Reyerson, 2011).

Weaning time varies according to culture, mother and child health, and wealth status (e.g., Humphrey, 2010; McDade & Worthman, 1998). For the Early Middle Ages, no written sources on breastfeeding duration are available, and the few mentions of wet nurses are associated with wealthy families (Barbiera & Dalla Zuanna, 2007). The trepanation events and her tomb position (Micarelli et al., submitted) suggest a privileged condition of this woman. Studies on Italian skeletal collections from the 4th c. CE onwards, throughout the Early Middle Ages, have recorded physiological stresses caused by weaning (i.e., linear enamel hypoplasia, LEH) between the age of 3 and 4.5 (Barbiera & Dalla Zuanna, 2007). We recorded no signs of LEH on CT1953's teeth. Although it is unknown how severe stress has to be to produce enamel defects, CT1953 survived childhood and reached adult age with isotopic values consistent with those of the other adults analyzed at Castel Trosino (Bernardini et al., 2021).

After weaning, isotopic carbon and sulfur values suggest a dietary shift at approximately 5 years, parallel to childhood mobility (Figure 2a). The highest $\delta^{13}C$ value (−16.3‰) (Data S1) recorded at approximately 5.5 years might be indicative of C_4 plants consumption, possibly millet and/or sorghum, which are crops known for their tolerance to drought and poor soils. The spread of these plants has been associated with the Longobard arrival in northern Italy (Iacumin et al., 2014). This low-quality crop is not suitable for breadmaking, but in the Early Middle Ages, it was ideal for soup and porridge-like meals (Guglielmetti, 2014; Iacumin et al., 2014). The consumption of C_4-based gruels for CT1953 may be linked to her early-life displacement in an area where the reliance on C_4 crops had already taken place. It is noteworthy that $\delta^{15}N$ values do not vary in this age-range (Figure 2a); therefore, the peak in $\delta^{13}C$ should be related to dietary change rather than nutritional stress, which would have caused a parallel increase in $\delta^{15}N$ values (e.g., Reitsema, 2013). The intake of marine resources is also excluded. A consumption of seafood would have resulted in ^{13}C, ^{15}N, and ^{34}S enrichment due to the longer trophic chain and the higher carbon and sulfur δ-values of the sources in this ecosystem (e.g., Nehlich, 2015; Schoeninger & DeNiro, 1984).

A progressive decrease in carbon values is observed in later childhood, reaching the lowest $\delta^{13}C$ (−19.1‰) at approximately 8.7 years, with the subsequent profile reflecting that of the very first years of life (Figure 2a and Data S1).

Isotopic values from 7 years onwards show a homogenous trend, with no further changes until the last moment of life ($\delta^{15}N$ mean: 9.6 ± 0.2‰; $\delta^{13}C$ mean: −18.4 ± 0.5‰; $\delta^{34}S$ mean: 6.6 ± 0.2‰, $n = 6$).

When looking at secondary dentin, there is a gradual decrease in $\delta^{13}C$ and $\delta^{15}N$ values from the end of tooth formation (approximately 9.5 years) to death (Figure 2a). Mean values are consistent with data registered in later childhood for primary dentin (Figure 2a). Values measured in the apex section (Figure 1c) show a drop in both carbon and nitrogen, which are consistent with the bone data (Figure 2a and Data S1).

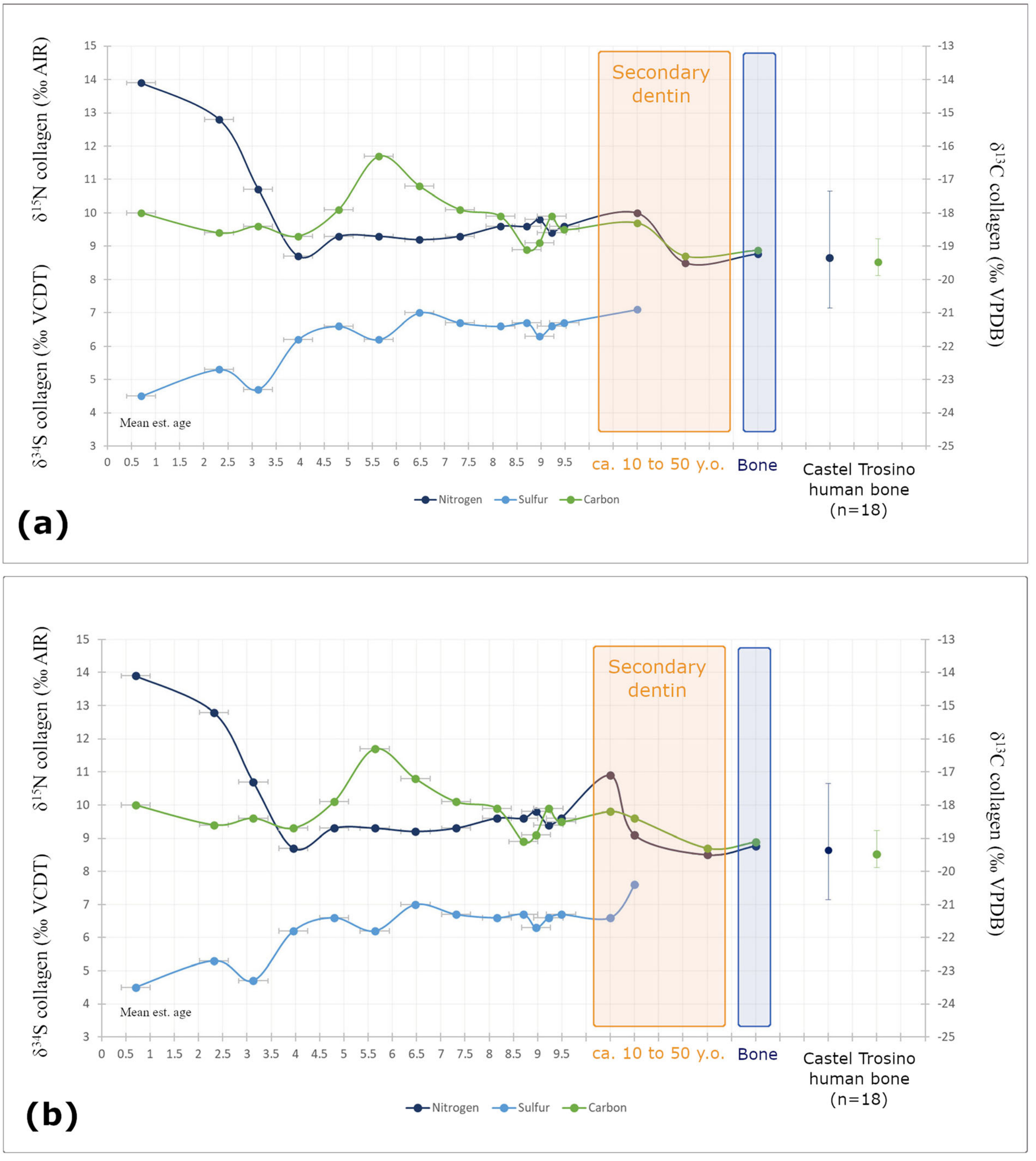

FIGURE 2 Isotopic profiles of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of CT1953 dentin collagen microsections according to sampling sequence and reported on the x-axis as estimated mean age intervals (Data S1). Error bars represent the mean error (0.3) on the estimated age intervals for each primary dentin microsection (ranging from 0.1 to 0.4). The orange area corresponds to secondary dentin values; blue area corresponds to bone values. Isotopic nitrogen (blue) and carbon (green) values from human bone available from Castel Trosino cemetery (Bernardini et al., 2021) are reported as median; bars indicate values dispersion (min. and max. values). For secondary dentin, we report mean values (a) and individual values (b). [Colour figure can be viewed at wileyonlinelibrary.com]

Considering secondary dentin sections singularly, pulp chamber data show an increase in nitrogen (+1‰) compared with primary dentin apex values, while carbon and sulfur data are consistent with the latest primary dentin data (Figure 2b and Data S1). $\delta^{34}S$ values increase (+1‰) in the section showing a parallel nitrogen decrease (Figure 2b and Data S1). From pulp to apex values, nitrogen drops by approximately 2‰, with a trend previously observed in case of physiological or nutritional stress (Beaumont & Montgomery, 2016). The severe ongoing dental disease may have limited CT1953 ability to eat a varied diet, possibly reflected in the gradual descending $\delta^{13}C$ and

$\delta^{15}N$ values during adulthood. Yet, her later protein intake (bone values) is similar to that of the other adults (Bernardini et al., 2021) (Data S1). It is worth noting that the trepanation, or the situation which led to it, could have strongly affected the woman's metabolism/physiology (e.g., Giuffra & Fornaciari, 2017; Goude et al., 2020).

Whether dietary or physiological stress is responsible for the isotopic values recorded for secondary dentin, these were able to highlight short-term variation, a change otherwise invisible in bone collagen. Indeed, we suggest considering secondary dentin analysis in long-life dietary studies.

4 | LIMITATIONS OF THE STUDY

The limit of this study relies on the difficulty of an accurate age assignment for secondary dentin sections due to its nonhomogeneous deposition driven by mechanical loading (Nanci, 2013; Nudel et al., 2021). An alternative sampling scheme is proposed in this paper, which can be applied when enough material is available. The lack of similar multi-isotopes analyses at the intra-population level (i.e., on the other crania from Castel Trosino) and inter-population level (i.e., Longobard contexts in Italy) limited the data interpretation as well. Complementary investigations of both bone and dental tissues from Italian Early Middle Age contexts are needed to evaluate whether the practices documented in CT1953 are related to a particular social status or reflect specific habits of the Italian Longobard community.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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