Early life of Neanderthals

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## Early life of Neanderthals

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**Author Contributions**

S.B. initiated and led the study; A.N., F.L., M.R., C.D., L.B., M.P., W.M., S.B. designed the study; A.CP., A.H., D.E., F.L., S.S., T.G., W.M. produced chemical/isotopic data; F.BD. and R.P. produced ecological framework; A.N., C.D., L.B. produced histology data; C.T., F.BR. produced the microtomographic record; A.H., A.N., D.E., E.BR., F.L., G.O., L.B., W.M. analyzed or assisted in analysis of data; M.P., M.R., R.D., A.L., D.D. coordinated archaeological excavations; A.CI., C.F., E.BR., E.C., G.M., G.O., I.D., S.A. curated, sampled and/or described analyzed teeth; A.N., C.D., F.L., L.B., S.B., W.M. wrote the manuscript with considerable input from D.E., M.R., F.B., M.P. and with contributions from all authors; all authors contributed to final interpretation of data

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> Main Text
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1  **Abstract**

2  The early onset of weaning in modern humans has been linked to the high nutritional

3  demand of brain development that is intimately connected with infant physiology and

4  growth rate. In Neanderthals, ontogenetic patterns in early life are still debated, with

5  some studies suggesting an accelerated development and others indicating only subtle

6  differences to modern humans. Here we report the onset of weaning and rates of enamel

7  growth using an unprecedented sample set of three late (~70-50 ka) Neanderthals

8  Neanderthals and one Upper Paleolithic modern human from Northeastern-Italy via

9    spatially-resolved chemical/isotopic analyses and histomorphometry of deciduous teeth.

10   Our results reveal that the modern human nursing strategy, with onset of weaning at 5-6

11   months, was already present among these Neanderthals. This evidence, combined with

12   dental development akin to modern humans, highlights their similar metabolic constraints

13   during early life and excludes delayed weaning as a factor contributing to Neanderthals'

14   demise.

15   **Significance Statement**

16   The extent to which Neanderthals differ from us is the current focus of many studies in

17   human evolution. There is debate about their pace of growth and early life metabolic

18   constraints, both of which are still poorly understood. Here we use chemical and isotopic

19   signatures in tandem with enamel growth rates of three Neanderthal milk teeth from

20   Northeastern Italy to explore their early life. Our study shows that these late Neanderthals

21   started to wean children at 5-6 months akin to modern humans, implying similar energy

22   demands during early infancy. Dental growth rates confirm this and follow trajectories

23   comparable with modern humans. Contrary to previous evidence, we suggest that

24   differences in weaning age did not contribute to the demise of Neanderthals.

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26

27   **Main Text**

28

29   **Introduction**

30

31   Maternal physiology, breastfeeding and the first introduction of supplementary foods are

32   key determinants of human growth (1)(1). The high nutritional demands of the human

33   brain during the first years of life has been identified as the main reason for the early

34   weaning onset in modern humans (2). Indeed, supplementary food is needed when an

35   infant's nutritional requirements exceeds what the mother can provide through breastmilk

36   only (3), an event that in contemporary non-industrial human societies occurs at a modal

37   age of 6 months (4).

38   At present, our knowledge about the link between the pace of child growth, maternal

39   behavior and the onset of weaning among Neanderthals is still scarce. Previous work

40   reported that Neanderthal tooth crowns tend to develop faster than in modern humans,

3

suggesting infant growth was generally accelerated (5). Other earlier work suggested that Neanderthal brain size was comparable to modern humans at birth, but that growth rates in early infancy were higher (6). It has also been shown (7) that Neanderthals followed modes of endocranial development largely similar to modern humans. However, a permanent first molar and a second deciduous molar from La Chaise (France, 127-116 ka and <163 ka respectively) placed rates of Neanderthal tooth growth within the range of modern humans (8). Equally, the association between dental and skeletal growth in a 7-year-old Neanderthal from El Sidròn (Spain, 49 ka) indicated that Neanderthals and modern humans were similar in terms of ontogenetic development, with only small-scale dissimilarities in acceleration or deceleration of skeletal maturation (9). Ba/Ca maps of permanent tooth sections of two early Neanderthals have been interpreted (controversially, see below) as indicators of non-breastmilk food introduction for infants at ~9 (Payre 6, 250 ka) (10) and 7 (Scladina, 120 ka) (11) months of age, later than the modal age in modern humans today. Similarly, wear stage analyses of a large number of deciduous dentitions suggested that introduction of solid food in Neanderthals was delayed by one year compared to modern humans (12).

Here we investigate such key aspects of early life in Neanderthals by combining new data on chemical detection of weaning onset with deciduous enamel growth rates. We utilize dental histomorphometry (8, 13), spatially-resolved chemical (14) and isotopic profiles (15, 16) of dental enamel to reconstruct growth rates (13), nursing practices (3) and mobility (15) during the Middle and Upper Paleolithic at high (up to weekly) time resolution. We analyzed an unprecedented set of teeth (n = 4) (*SI Appendix,* Text S1) from adjacent archaeological sites in Northeastern Italy (*SI Appendix,* Text S2), dated from the Late Middle to the Early Upper Paleolithic, from Neanderthal-modern human contexts (70-40 ka). These four exfoliated deciduous fossil teeth include three Neanderthals (Fumane 1, a lower left deciduous second molar (17), ~50 ka; Nadale 1, a lower right deciduous first molar (18), ~70 ka; Riparo Broion 1, an upper left deciduous canine (19), ~50 ka) and one Early Upper Paleolithic modern human (UPMH) as comparative specimen from the Fumane site (Fumane 2, an upper right deciduous second incisor (20), Protoaurignacian, ~40 ka) (Fig. 1).

[Insert Figure 1 here]

Exfoliated deciduous teeth derive from individuals who survived permanent tooth replacement and were thus unaffected by any mortality-related bias (23). All teeth come from the same geographic area within a ~55 km radius (Fig. 1), and Fumane 1 and 2 were recovered from different archaeological layers in the same cave, thus allowing direct comparisons in a well-constrained eco-geographical setting.

We quantified enamel incremental growth parameters such as postnatal crown formation time and daily enamel secretion rates (24), and we detected the presence of the neonatal line as birth marker (25) by optical light microscopy on thin sections of the deciduous dental crowns. Chemical weaning was investigated via Element/Ca profiles on the same histological sections along the enamel-dentine junction (EDJ) by laser-ablation inductively-coupled-plasma mass spectrometry (LA-ICPMS) (14). In order to detect mobility and/or potential non-local food sources in maternal diet, $^{87}Sr/^{86}Sr$ isotope ratio profiles were measured by LA-multi-collector-ICPMS (see Materials and Methods) (15, 16).

**Results**

Weaning onset was determined using the topographical variation of the Sr/Ca ratio along the EDJ (14, 26) (*SI Appendix,* Text S3). In exclusively breastfed newborns, the enamel Sr/Ca ratio is markedly lower relative to their prenatal levels (14, 26, 27). This is because human milk is highly enriched in Ca, i.e. Ca is selectively transferred, compared to Sr, across the mammary glands and the placenta (28, 29). Such behavior is confirmed by analyses of breastmilk and infant sera (30). In comparison to human, herbivore milk (and derived formula) is characterized by higher Sr/Ca levels, due to the lower initial trophic position (31). Our dietary model for early life (*SI Appendix,* Text S3) agrees with the expected Sr behavior (14, 27, 32), showing a decrease in Sr/Ca during exclusive breastfeeding and changes in the slope of the profile across the major dietary transitions (i.e. introduction of solid food and end of weaning) (27). This model has been tested successfully in this study on a set of contemporary children's teeth with known dietary histories, including their mothers' eating habits (*SI Appendix,* Text S3 and Fig. S6-S8). Alternative literature models for Ba/Ca (10, 11) point to an increase of Ba/Ca in postnatal enamel during breastfeeding, yet due to even stronger discrimination across biological membranes, Ba/Ca behavior is expected to be similar to Sr/Ca (27), as indeed unequivocally observed here (*SI Appendix,* Text S3 and Fig. S6-S8) and elsewhere (14, 33-35).

The neonatal lines marking birth were visible in all four archaeological specimens, despite their worn crowns (*SI Appendix,* Fig. S1), allowing the precise estimation of postnatal crown formation times (Fig. 2a). The deciduous first molar Nadale 1 and the deciduous canine Riparo Broion 1 lie within the modern human variability (36-39), while the second deciduous molar Fumane 1 shows a shorter postnatal crown formation time compared with the known archaeological and modern human range (36). The UPMH Fumane 2 deciduous lateral incisor postnatal crown formation time falls instead in the lower limit of the modern human range (37, 39). Overall, the enamel growth rates and the time to form postnatal enamel compares well with modern human data, regardless of differences in their relative tissue volumes and morphologies (5, 8, 9).

117 Daily enamel secretion rates (DSRs) of all specimens, collected in the 100 µm thickness

118 along the enamel dentine junction where laser tracks were run, are reported in Figure 2b,

119 compared with range of variation (min., mean, max.) of modern humans (36-39).

120 Neanderthal DSRs in the first 100 µm of enamel thickness are slower than the

121 corresponding modern human range of variability. However, when the entire dental

122 crown is considered, the distributions of Neanderthal DSRs lie within the lower

123 variability ranges of modern humans (Fig. 2c). The UPMH Fumane 2 DSRs fit the lower

124 portion of the modern human ranges (Fig. 2b,c). The postnatal crown formation times in

125 Neanderthals couple with slower DSRs than in modern humans, as expected given the

126 thinner enamel in Neanderthals' permanent and deciduous teeth (40, 41).

127

128 [Insert Figure 2 here]

129

130 Nadale 1, Fumane 1 & 2 are sufficiently well-preserved from a geochemical point of

131 view, Riparo Broion 1 instead shows some diagenetic overprint (overall Ba is far more

132 affected than Sr; see *SI Appendix,* Text S4 for our diagenesis assessment strategy), but the

133 overall primary elemental signature can still be discerned. Two out of the three

134 Neanderthals, Fumane 1 and Riparo Broion 1, clearly show breastfeeding signals and a

135 decreasing trend in Sr/Ca ratio immediately post-birth, followed by slope changes with

136 the first introduction of non-breastmilk food at 115 days (3.8 months) and 160 days (5.3

137 months; Fig. 3). An even stronger signal of transitional food intake is visible in Fumane 1

138 at 200 days (6.6 months) in the form of a steep increase in Sr/Ca ratio. For the oldest

139 Neanderthal specimen Nadale 1, following a marked variability before birth, the Sr/Ca

140 profile slightly decreases until 140 days (4.7 months). We cannot determine the weaning

141 onset for this individual, who was still being exclusively breastfed by ~5 months of life.

142 The UPMH Fumane 2 has a substantial portion of the prenatal enamel preserved and only

143 a short postnatal enamel growth record (~85 days vs ~55 days respectively). This

144 precludes the chemical detection of the onset of weaning, although the Sr/Ca drop at birth

145 clearly indicates breast-feeding.

146

147    [Insert Figure 3 here]

148

149    The Sr isotope profiles of all investigated teeth show very limited intra-sample

150    variability, confirming that Sr/Ca variations likely relate to changes in dietary end-

151    members rather than diverse geographical provenance of food sources (Fig. 4). These

152    data also give insights in Neanderthal mobility and resource gathering. The $^{87}Sr/^{86}Sr$

153    ratios of all Neanderthal teeth overlap with the respective local baselines, defined through

154    archaeological micromammals (42). This suggests that the mothers mostly exploited local

155    food resources. Fumane 1 and Fumane 2, both from the same archaeological site, are

156    characterized by contrasting $^{87}Sr/^{86}Sr$ ratios (0.7094 vs 0.7087), indicative of a different

157    use of resources between Neanderthal (local resources) and early UPMH (non-local

158    resources). Such behavior might have been driven by climatic fluctuations, suggesting

159    colder conditions at ~40 ka, dominated by steppe and Alpine meadows (43).

160

161    [Insert Figure 4 here]

162

163    **Discussion**

164

165    Nursing strategies are strictly linked to fertility rates, maternal energetic investment,

166    immune development and infant mortality (44). All of these ultimately contribute to

167    demographic changes of a specific population, with key relevance to the study of human

168    evolution. Prolonged exclusive breastfeeding has a positive impact on an infant's immune

169    system; however, longer breastfeeding negatively influences women's fertility via

170    lactational amenorrhea and thus inter-birth intervals (45). It has been shown that the age

171    peak for weaning onset is reached at around 2.1 times birth weight (46), implying that

172    infants who grow more rapidly need to be weaned earlier than those with a slower pace of

173    growth. Based on modern models, a sustainable timing for infant weaning onset would

174    thus range between 3 and 5 months of age (3). However, contemporary non-industrial

8

175   societies start weaning their children at a modal age of 6 months (4). Similarly, the World

176   Health Organization recommends exclusive breastfeeding for the first six months of an

177   infant's life (47). This time frame broadly corresponds to the age at which the masticatory

178   apparatus develops, favoring the chewing of first solid foods (3). Such evidence suggests

179   that both skeletal development and infant energy demand contribute to the beginning of

180   the weaning transition. Introduction of non-breastmilk foods is also crucial in reducing

181   the energetic burden of lactation for the mother (4). Breastfeeding represents a substantial

182   investment of energy resources (total caloric content of modern human breastmilk =~60

183   kcal/100 mL) (48), entailing an optimal energy allocation between baby feeding and other

184   subsistence-related activities.

185   Our time-resolved chemical data point to an introduction of non-breastmilk foods at ~5-6

186   months in the infant diet of two Neanderthals, sooner than previously observed (10, 11)

187   and fully within the modern human pre-industrial figures (4). This evidence, combined

188   with deciduous dental growth akin to modern humans, indicates similar metabolic

189   constraints during early life for the two taxa. The differential food exploitation of Fumane

190   1 and Fumane 2 mothers - who lived in the same site and in a similar environmental

191   setting - suggests a different human-environment interaction between Neanderthals and

192   early UPMHs, as seen in Sr isotope profiles. The Fumane 2 mother spent the end of her

193   pregnancy and the first 55 days after delivery away from the site and was consuming low-

194   biopurified non-local foodstuff with elevated Sr/Ca. Conversely, all Neanderthal mothers

195   spent the last part of their pregnancies and the lactation periods locally and were

196   consuming high-biopurified local food due to the low Sr/Ca-values (see Fig. 3e).

197   The introduction of non-breastmilk food at ~5-6 months implies relatively short inter-

198   birth intervals for Neanderthals due to an earlier resumption of post-partum ovulation

199   (49). Moreover, considering the birth weight model (46), we hypothesize that

200   Neanderthal newborns were of similar weight to modern human neonates, pointing to a

201   likely similar gestational history and early-life ontogeny. In a broader context, our results

202   suggest that nursing mode and time among Late Pleistocene humans in Europe were

203   likely not influenced by taxonomic differences in physiology. Therefore, our findings do

204   not support the hypothesis that long postpartum infertility was a contributing factor to the

demise of Neanderthals (12). On the other hand, genetic evidence indicates that Neanderthal groups were limited in size (50), which is not in agreement with the shorter inter-birth interval proposed here. Thus, other factors such as e.g. cultural behavior, shorter life-span and high juvenile mortality might have played a focal role in limiting Neanderthal's group size (51, 52).

**Materials and Methods**

**Thin slices of teeth preparation**

Prior to sectioning, a photographic record of the samples was collected. Thin sections of the dental crowns were obtained using the method proposed by others (53, 54) and prepared in the Service of Bioarchaeology of the Museo delle Civiltà in Rome. The sectioning protocol consists of a detailed embedding-cutting-mounting procedure that makes use of dental adhesives, composite resins, and embedding resins. In order to be able to remove the crown from the resin block after sectioning and to restore the dental crowns, the teeth were initially embedded with a reversible resin (Crystalbond 509, SPI Supplies) that does not contaminate chemically the dental tissues and is soluble in Crystalbond cleaning agent (Aramco Products, Inc.). A second embedding in epoxy resin (EpoThin 2, Buehler Ltd) guarantees the stability of the sample during the cutting procedure. The sample was cured for 24 hours at room temperature. Teeth were sectioned using an IsoMet low speed diamond blade microtome (Buehler Ltd). After the first cut, a microscope slide previously treated with liquid silane (3 M RelyX Ceramic Primer) was attached on the exposed surface using a light curing adhesive (3M Scotchbond Multi-Purpose Adhesive) to prevent cracks and any damage during the cutting procedure. A single longitudinal bucco-lingual thin section, averaging 250 µm thick, was cut from each specimen. Each ground section was reduced to a thickness of ~150 µm using water resistant abrasive paper of different grits (Carbimet, Buehler Ltd). Finally, the sections were polished with a micro-tissue (Buehler Ltd) and diamond paste with 1 µm size (DB-Suspension, M, Struers).

Each thin section was digitally recorded through a camera (Nikon DSFI3) paired with a transmitted light microscope (Olympus BX 60) under polarized light, with different

237 magnifications (40x, 100x, 400x). Overlapping pictures of the dental crown were

238 assembled in a single micrograph using the software ICE 2.0 (Image Composite Editor,

239 Microsoft Research Computational Photography Group) (*SI Appendix,* Fig. S1).

240 After sectioning, the crowns were released from the epoxy block using the Crystalbond

241 cleaning agent and reconstructed using light curing dental restoration resin (Heraeus

242 Charisma Dental Composite Materials).

243 **Sr isotopic analysis by solution MC-ICPMS**

244 To determine local Sr isotope baselines we analyzed archaeological rodent teeth (*SI*

245 *Appendix,* Table S1). Samples were prepared at the Department of Chemical and

246 Geological Sciences of the University of Modena and Reggio Emilia, following protocols

247 described elsewhere (15, 55) and briefly summarized here.

248 From each archaeological site we selected several rodent tooth specimens, according to

249 the stratigraphic distribution of human samples. Enamel from micromammal incisors was

250 manually removed using a scalpel. Few teeth were also analyzed as whole (dentine +

251 enamel). Before the actual digestion with 3M $HNO_3$, samples (1-5 mg in mass) were

252 washed with MilliQ (ultrasonic bath) and leached with ~0.5 M $HNO_3$. Sr of the digested

253 specimens was separated from the matrix using 30 µl columns and Triskem Sr-Spec

254 resin.

255 Sr isotope ratios were measured using a Neptune (ThermoFisher) multi-collector

256 inductively-coupled-plasma mass spectrometer (MC–ICPMS) housed at the Centro

257 Interdipartimentale Grandi Strumenti (UNIMORE) during different analytical sessions.

258 Seven Faraday detectors were used to collect signals of the following masses: $^{82}Kr$, $^{83}Kr$,

259 $^{84}Sr$, $^{85}Rb$, $^{86}Sr$, $^{87}Sr$, $^{88}Sr$. Sr solutions were diluted to ~50 ppb and introduced into the

260 Neptune through an APEX desolvating system. Corrections for Kr and Rb interferences

261 follow previous works (15). Mass bias corrections used an exponential law and a $^{88}Sr/^{86}Sr$

262 ratio of 8.375209 (56). The Sr ratios of samples were reported to a SRM987 value of

263 0.710248 (57). During one session, SRM987 yielded an average $^{87}Sr/^{86}Sr$ ratio of

264 0.710243 ± 0.000018 (2 S.D., n = 8). Total laboratory Sr blanks did not exceed 100 pg.

265 **Spatially-resolved Sr isotopic analysis by laser-ablation plasma mass spectrometry**

266 **(LA-MC-ICPMS)**

LA-MC-ICPMS analyses were conducted at the Frankfurt Isotope and Element Research Center (FIERCE) at Goethe University, Frankfurt am Main (Germany) and closely follow analytical protocols described by Müller & Anczkiewicz (2016) (16); only a brief summary is provided here aiming at highlighting project-specific differences. A 193 nm ArF excimer laser (RESOlution S-155, formerly Resonetics, ASI, now Applied Spectra Inc.) equipped with a two-volume LA cell (Laurin Technic) was connected to a NeptunePlus (ThermoFisher) MC-ICPMS using nylon6-tubing and a 'squid' signal-smoothing device (58). Ablation took place in a He atmosphere (300 ml/min), with ~1000 ml/min Ar added at the funnel of the two-volume LA cell and 3.5 ml/min $N_2$ before the squid. Laser fluence on target was ~5 J/cm$^2$.

Spatially-resolved Sr isotopic analyses of dental enamel were performed on the thin sections (100-150 µm thick) used for enamel histology and trace element analysis (see below), in continuous profiling mode following the enamel-dentine-junction (EDJ) from apex to cervix (14), less than 100 µm away from the EDJ. Tuning of the LA-MC-ICPMS used NIST 616 glass for best sensitivity ($^{88}$Sr) while maintaining robust plasma conditions, i.e. $^{232}$Th$^{16}$O/$^{232}$Th <0.5% and $^{232}$Th/$^{238}$U>0.95 with RF-power of ~1360 W. In view of the low Sr concentrations in these human enamel samples (~60-100 µg/g), we utilized 130 μm spots, a scan speed of 5 μm/s and a repetition rate of 20 Hz to maintain $^{88}$Sr ion currents of ~2-3.5 x 10$^{-11}$ A. Nine Faraday detectors were used to collect the ion currents of the following masses (m/z): $^{83}$Kr, ~83.5, $^{84}$Sr, $^{85}$Rb, $^{86}$Sr, ~86.5, $^{87}$Sr, $^{88}$Sr, $^{90}$Zr. Baseline, interference and mass bias corrections follow (16). The isotopically-homogenous (Sr) enameloid of a modern shark was used to assess accuracy of the Sr-isotopic analysis and yielded $^{87}$Sr/$^{86}$Sr = 0.70916 ± 2 and $^{84}$Sr/$^{86}$Sr = 0.0565 ± 1 (2 S.D.). Raw data are reported in Dataset S1.

**Spatially-resolved elemental ratio and concentration analysis by laser-ablation plasma mass spectrometry (LA- ICPMS)**

All LA-ICPMS analyses of archaeological samples were conducted at the Frankfurt Isotope and Element Research Center (FIERCE) at Goethe University, Frankfurt am Main (Germany), using the same LA system described above, but connected via a squid smoothing-device to an Element XR ICPMS. Analytical protocols follow those by Müller

et al (2019) (14); and only a brief summary is provided here aimed at highlighting differences. LA-ICPMS trace element ratios/concentrations of the comparative contemporary teeth were obtained at Royal Holloway University of London (RHUL) using the RESOlution M-50 prototype LA system featuring a Laurin two-volume LA cell (58), coupled to an Agilent 8900 triple-quadrupole-ICPMS (ICP-QQQ or ICP-MS/MS). Compositional profiles were analyzed parallel and as close as possible to the EDJ, following the same tracks used for Sr isotope analyses. We employed 15 μm spot sizes (FIERCE) or 6 µm (MCS3, RHUL) and 34 µm (MCS1 and 2, RHUL), respectively, as well as a scan speed of 5 μm/s and a repetition rate of 15 Hz; prior to acquisition, samples were pre-cleaned using slightly larger spot sizes (22 - 57 µm), 20 Hz and faster scan speeds (25 - 50 µm/s); laser fluence was ~5 J/cm$^2$. The following isotopes (*m/z*) were analyzed: $^{25}$Mg, $^{27}$Al, $^{43}$Ca, ($^{44}$Ca), $^{55}$Mn, $^{66}$Zn, $^{85}$Rb, ($^{86}$Sr), $^{88}$Sr, $^{89}$Y, $^{138}$Ba, $^{140}$Ce, ($^{166}$Er, $^{172}$Yb), $^{208}$Pb, $^{238}$U. The total sweep times for the Element XR and the 8900 ICP-MS/MS were ~0.8 and 0.4-0.5 s, respectively; however, because of the slow scan speeds, this small difference has no effect on the compositional profiles presented here. Primary standardization was achieved using NIST SRM612. Ca was employed as internal standard ($^{43}$Ca); [Ca] at 37 %m/m was used to calculate concentrations for unknown bioapatites, although not required for X/Ca ratios. Accuracy and reproducibility were assessed using repeated analyses of the STDP-X-glasses (59) as secondary reference materials; the respective values for Sr/Ca and Ba/Ca (the element/Ca ratios of principal interest) here are $1.8 \pm 6.6\%$ and $-0.2 \pm 6.0$ % (%bias $\pm$ 2 S.D. (%)); this compares well with the long-term reproducibility for these analytes reported previously (60). Raw data are reported in Dataset S2 and S3.

The compositional/isotopic profiles were smoothed with a locally weighted polynomial regression fit (LOWESS), with its associated standard error range ($\pm$3 S.E.) for each predicted value (61). The statistical package R (ver. 3.6.2) (62) was used for all statistical computations and generation of graphs.

**Assessment of the enamel growth parameters and of the chronologies along the laser tracks**

Dental enamel is capable of recording, at microscopic level during its formation, regular physiological and rhythmic growth markers (63-65). These incremental markings are visible under transmitted light in longitudinal histological thin sections of dental crowns. Enamel forms in a rhythmic manner, reflecting the regular incremental secretion of the matrix by the ameloblasts (i.e. the enamel forming cells). The rhythmical growth of enamel is expressed in humans at two different levels: a circadian rhythm that produces the daily cross striations (66, 67) and a longer period rhythmic marking (near- weekly in humans) that give rise to the Retzius lines (68). Physiological stresses affecting the individual during tooth growth cause a disruption of the enamel matrix secretion and mark the corresponding position of the secretory ameloblast front, producing Accentuated (Retzius) Lines (ALs) (69, 70). The birth event is recorded in the forming enamel of individuals surviving the perinatal stage, and leaves - usually the first - Accentuated Line, namely the Neonatal Line (NL) (25, 71, 72).

The time taken to form the dental crown after birth was measured on each thin section adapting the methods described in literature (39, 73).

A prism segment starting from the most apical available point on the enamel dentine junction (EDJ) and extending from this point to an isochronous incremental line (i.e. the NL, an AL or a Retzius line) was measured. The incremental line was followed back to the EDJ and a second prism segment was measured in the same way. The process was repeated until the most cervical enamel was reached. The crown formation time is equal to the sum of the single prism segments. To obtain time (in days) from the prism length measurements, local daily secretion rates (24) (DSR) were calculated around the prism segments and within 100 µm from the EDJ, by counting visible consecutive cross striations and dividing it by the corresponding prism length. The chronologies of accentuated lines (ALs) in the modern sample closely match the timing of known disruptive life history events in the mother (illness, surgery) and infant, and so are well within the range or error (1.2-4.4%) observed for this histological ageing method (63).

DSRs were collected across the whole crown on spots chosen randomly in order to get the DSRs distribution. Groups of cross striations ranging from 3 to 7 were measured. For each crown the number of measured spots ranges between 49 and 233.

After LA-ICPMS analyses, a micrograph highlighting the laser tracks was acquired at 50x magnification. This was superimposed to a second micrograph of the same thin section at 100x magnification, to gain better visibility of the enamel microstructural features. The chronologies along the laser tracks were obtained matching the tracks with the isochronous lines.

**Acknowledgments**

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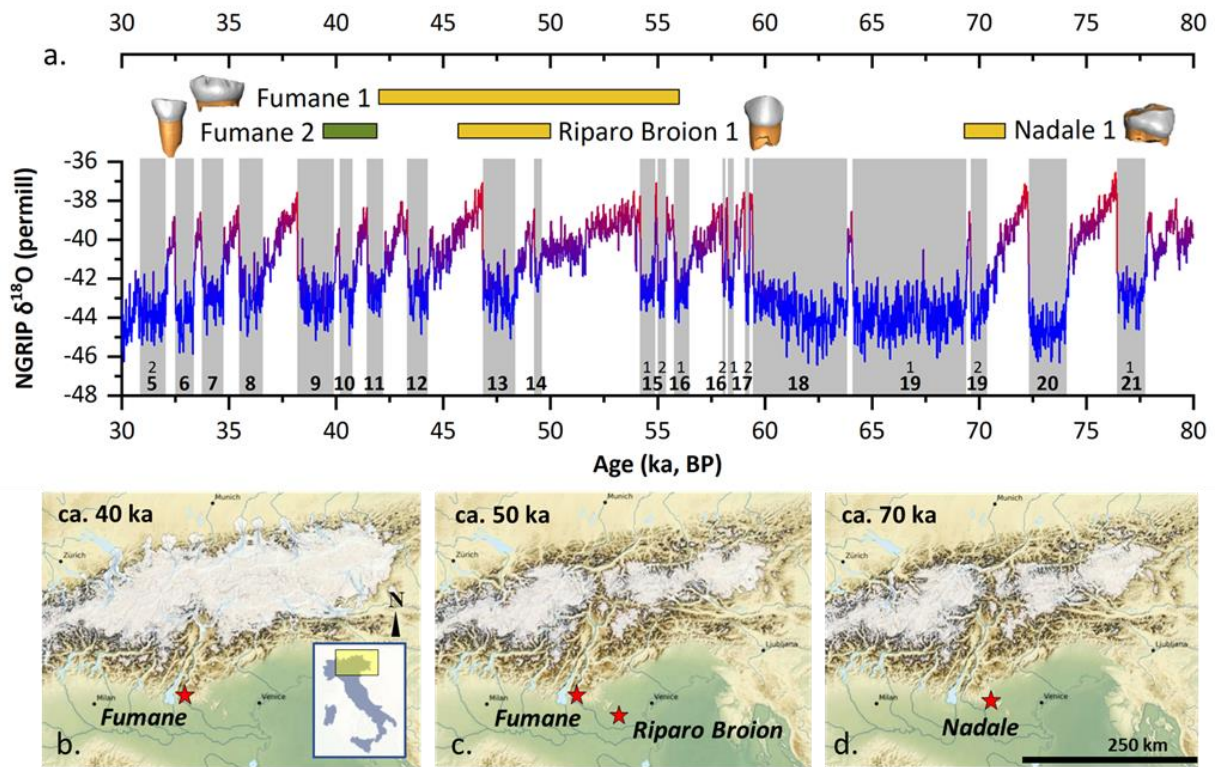593

**Figures and Tables**

**Figure 1. Geographical, paleoecological and chronological framework.** (a) Oxygen isotope curve from NGRIP (21), with Greenland Stadials 5-21 highlighted. Chronologies of the human specimens are also reported (see Supplementary Information for details); Fumane 2 is UPMH (green), while Fumane 1, Riparo Broion 1 and Nadale 1 are Neanderthals (yellow). (b,c,d) Modelled Alpine glacier extent during the time intervals of the teeth recovered at the sites of Fumane Cave (b,c), Riparo Broion (c) and Nadale (d); location within Italy is shown in the inset. Simulations show a high temporal variability in the total modelled ice volume during Marine Isotope Stages 4 (70 ka snapshot) and 3 (50, 40 ka snapshots) with glaciers flowing into the major valleys and possibly even onto the foreland (22).
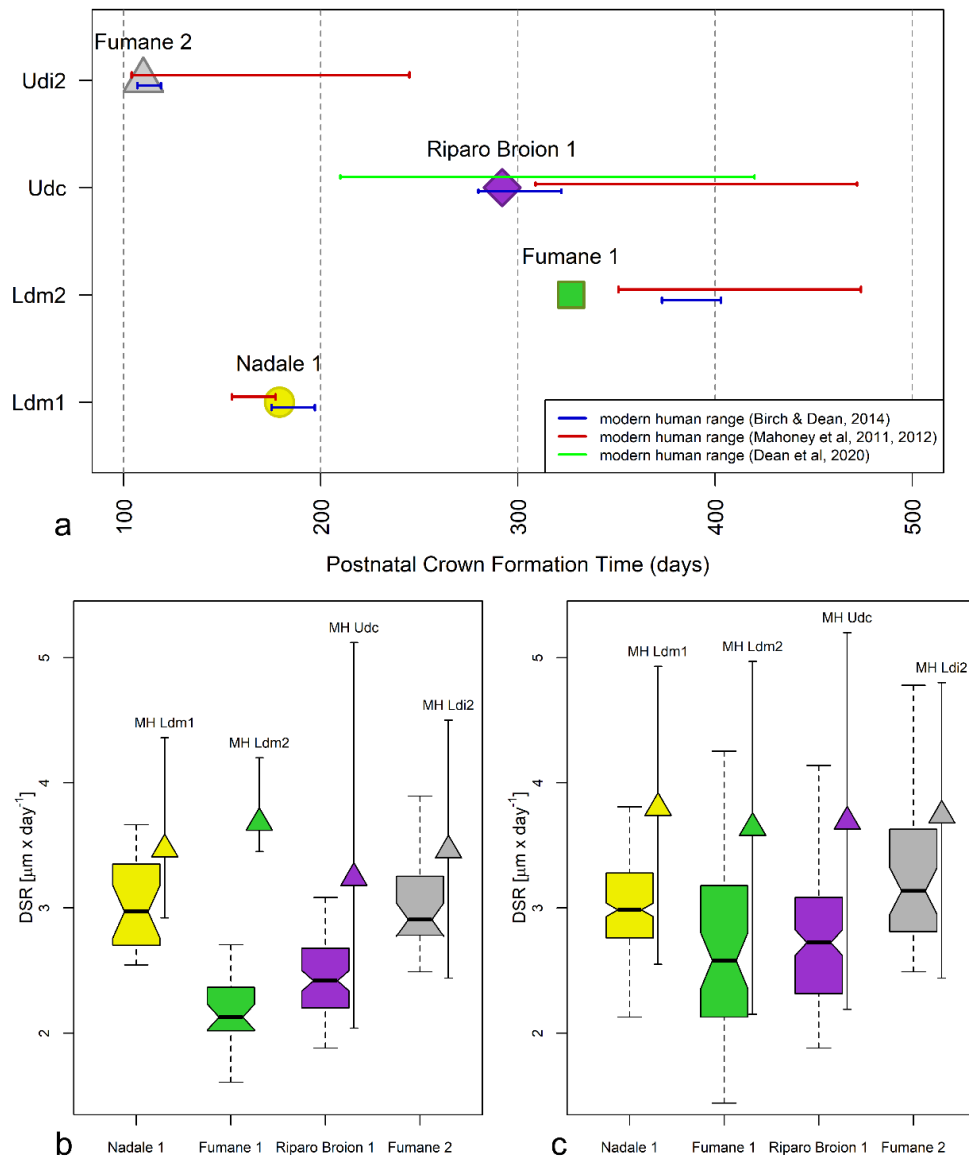
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**Fig. 2. Dental crown growth parameters.** (a) Postnatal crown formation time in days from birth for the different deciduous teeth. The range of variability reported in literature for modern and archaeological individuals is represented by red, blue, green lines. (b) Boxplot of the daily secretion rate (DSR) variation in the first 100 µm from the enamel-dentine-junction (min, second quartile, median, third quartile, max) and range of variation (min, mean, max) of modern humans (MH), re-assessed from (36-39). (c) Boxplot of the daily secretion rate variation across the whole crown (mean, second quartile, median, third quartile, max) and range of variation (min, mean, max) of modern humans (MH), re-assessed from (36-39). Ldm1 = lower deciduous first molar; Ldm2 = lower deciduous second molar; Udc = upper deciduous canine; Ldi2 = lower deciduous later incisor.
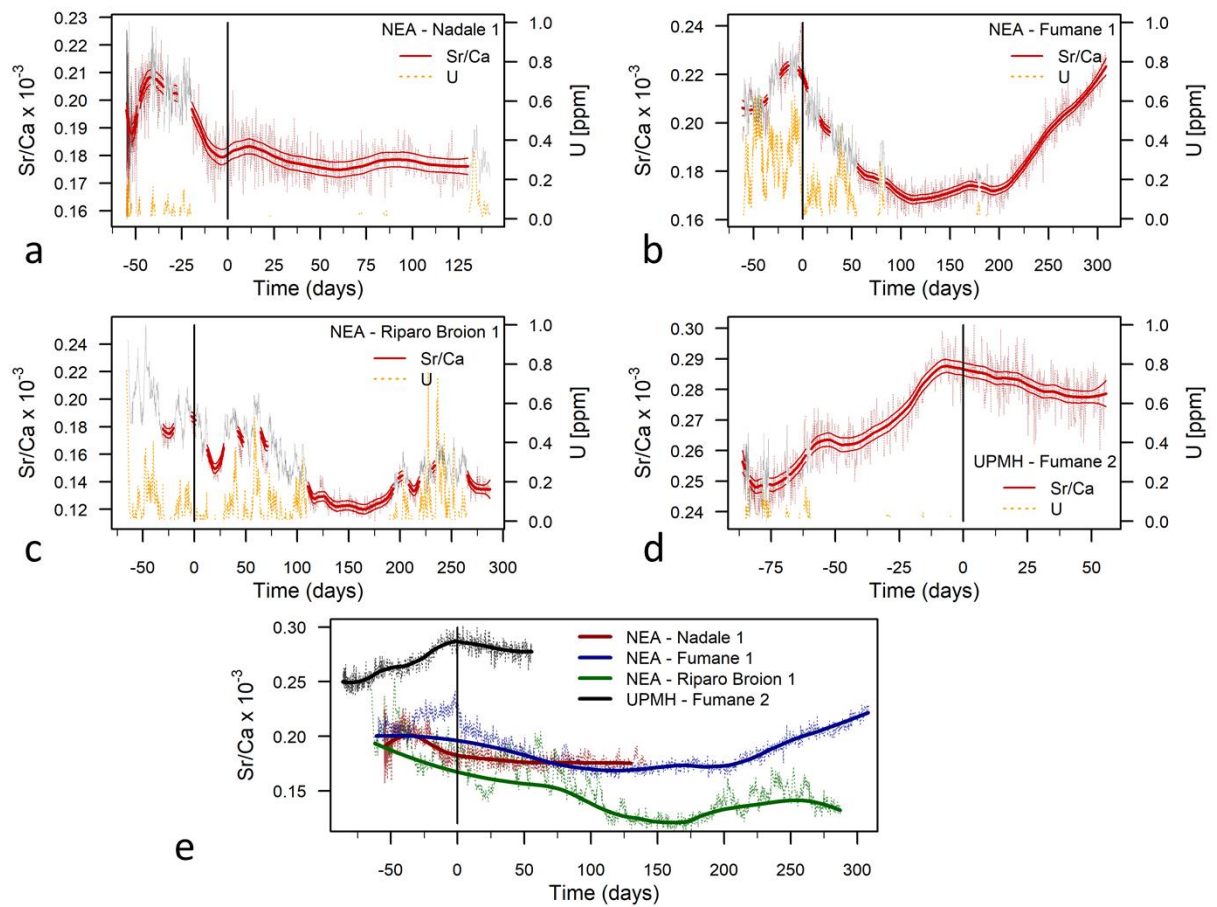
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**Fig. 3. Nursing histories from time-resolved Sr/Ca variation in Middle-Upper Paleolithic deciduous teeth.** UPMH = Upper Paleolithic modern human; NEA = Neanderthal. The elemental profiles were analyzed within enamel close to the enamel-dentine-junction (EDJ); [U] is reported as the most sensitive proxy for diagenetic alteration (14) (see *SI Appendix,* Text S4). Grey portions of the profiles represent diagenetically overprinted enamel domains, based on elevated U concentrations. The birth event is highlighted by a vertical line. (a) Nadale 1: the slight decrease of Sr/Ca indicates exclusive breastfeeding until the end of crown formation (4.7 months); (b) Fumane 1: Sr/Ca variation indicates breastfeeding until 4 months of age (fully comparable with MCS1 sample, see Supplementary Figure S6); (c) Riparo Broion 1: Sr/Ca profile indicates exclusive breastfeeding until 5 months of age; (d) Fumane 2: 55 days of available postnatal enamel shows exclusive breastfeeding. (e) Individual Sr/Ca profiles adjusted to the birth event; the interpolated modelled profiles were calculated based on those portions unaffected by diagenesis (U<limit of detection, 0.012 ppm), with strong smoothing parameters to enhance the biogenic signal. See Material and Methods section for details.

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**Fig. 4. Mobility of the Middle-Upper Paleolithic infants via time-resolved $^{87}$Sr/$^{86}$Sr profiles of their deciduous teeth.** Grey horizontal bands represent the local Sr isotopic baselines defined via the Sr isotopic composition of archaeological rodent enamel (*SI Appendix,* Table S1). The birth event is indicated by a vertical line. (a,b) Nadale 1 / Fumane 1: exploitation of local food resources through the entire period; (c) Riparo Broion 1: possible limited seasonal mobility (non-local values between c. 25 and 75 days = 4 months); (d) Fumane 2: exploitation of non-local food resources through the entire period.

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Supplementary Information for **Early life of Neanderthals**

658

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**This PDF file includes:**

674          Supplementary text S1 to S4
675          Figures S1 to S13
676          Tables S1 to S3
677          Legends for Datasets S1 to S3
678          SI References
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680 **Other supplementary materials for this manuscript include the following:**
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682         Datasets S1 to S3
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**SUPPLEMENTARY INFORMATION TEXT S1: DENTAL MORPHOLOGY**

The deciduous dental sample here investigated consists of three Neanderthals and one Upper Paleolithic modern humans (UPMH) specimen.

Fig. S2 reports the surface rendering of the four teeth from high resolution microtomographic volumes, segmented with Avizo 9.2 (Thermo Fisher Scientific). High-resolution micro-CT images of Fumane 1 and 2 were obtained with a Skyscan 1172 microtomographic system using isometric voxels of 11.98 µm (Fumane 1 and Fumane 2) (see Benazzi et al (1) for details). High-resolution micro-CT images of Nadale 1 and Riparo Broion 1 were acquired with the Xalt micro-CT scanner using isometric voxels of 18.4 µm (see Arnaud et al (2) for details).

The Neanderthal specimen Nadale 1 is a lower right first deciduous molar (Fig. S1a), whose morphological description and morphometric analysis were provided by Arnaud et al (2). The taxonomical assessment of the Neanderthal tooth Fumane 1, a lower left second deciduous molar (Fig. S1b), was confirmed by metric data and non-metric dental traits (1), while the attribution of Fumane 2, an upper right lateral deciduous incisor (Fig. S1d), to modern human was based on mitochondrial DNA (3).

The specimen Riparo Broion 1 is unpublished, but the paper describing its morphology and morphometry is under review. Overall, Riparo Broion 1 is an exfoliated upper right deciduous canine (Fig. S1c), heavily worn, with about one-fourth of the root preserved, which suggests an age at exfoliation at about 11-12 years based on recent human standards (4). The tooth is characterized by a stocky crown, bulging buccally, and a distolingual projection of a lingual cervical eminence, ultimately producing an asymmetrical outline. Overall our data concur to align Riparo Broion 1 to Neanderthals.

Overall, considering the paucity of European human remains dating to the Middle to Upper Paleolithic transition, the dental sample here investigated represents a unique exception for 1) its provenance from a restricted region of northeast Italy, ultimately removing the geographical variable as a potential confounding factor for chemical/isotopic signatures, 2) being represented by deciduous teeth, thus allowing to evaluate diet and mobility during early infancy, 3) the presence of both late Neanderthal

715   specimens (Fumane 1 and Riparo Broion 1) and one of the earliest modern humans in

716   Europe (Fumane 2), thus providing a unique opportunity to compare subsistence

717   strategies between the two human groups around the time of Neanderthal demise.

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**SUPPLEMENTARY INFORMATION TEXT S2: ARCHAEOLOGICAL AND**

**PALEOENVIRONMENTAL CONTEXTS**

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722 <u>Nadale 1</u>

723 De Nadale Cave is a small cavity located 130m a.s.l. in the middle of the Berici Hills.

724 Research at De Nadale Cave started in 2013 when a first excavation campaign led to the

725 discovery of a cave entrance after the removal of reworked sediments. Later, six

726 campaigns were carried out between 2014 and 2017 in order to investigate the deposits

727 preserved in the cave entrance and the back (5). The excavations exposed a stratigraphic

728 sequence which includes a single anthropic layer (unit 7) embedded between two sterile

729 layers (units 6 and 8) partly disturbed by some badger's dens along the cave walls. Unit 8

730 lays on the carbonate sandstone bedrock. Besides these disturbances, unit 7 is well

731 preserved and extends into the cavity. It yielded thousands of osteological materials,

732 lithic implements, and the Neanderthal deciduous tooth (2). A molar of a large-sized

733 ungulate was U/Th dated to 70,200±1,000/900 years as a minimum age (5) placing the

734 human occupation to an initial phase of the MIS 4. The zooarchaeological assemblage is

735 largely ascribable to human activity (6). Neanderthals hunted and exploited mainly three

736 taxa: the red deer (*Cervus elaphus*), the giant deer (*Megaloceros giganteus*) and bovids

737 (*Bison priscus* and *Bos primigenius*) (6, 7), in association with other taxa consistent with

738 the paleoclimatic and paleoenvironmental reconstruction based on the small mammal

739 association, where the prominence of *Microtus arvalis* identifies a cold climatic phase

740 and correlates to a landscape dominated by open woodlands and meadows (8). A large

741 amount of anthropic traces is observed on the ungulate remains, ascribable to different

742 stages of the butchery process and to the fragmentation of the bones for marrow

743 extraction. Burnt bone fragments and charcoal accumulations have been likely related to

744 residual fire-places (6). Lithic industry from of De Nadale differentiates technologically

745 and typologically from the Mousterian elsewhere in the region, especially with regard to

746 the core reduction methods and the types of flakes and retouched tools. These are

747 represented from several scrapers with stepped-scaled invasive retouches and make the

748  De Nadale industry comparable to Quina assemblages in Italy and Western Europe (5).

749  De Nadale peculiarity is also enhanced by the high number of bone retouchers (9).

750  Research at the De Nadale Cave is coordinated by the University of Ferrara (M.P.) in the

751  framework of a project supported by the Ministry of Culture – "SABAP per le province

752  di Verona, Rovigo e Vicenza" and the Zovencedo Municipality, financed by the H.

753  Obermaier Society (2015), local private companies (R.A.A.S.M., Saf and Lattebusche),

754  and local promoters.

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756  <u>Fumane 1 and 2</u>

757  Grotta di Fumane (Fumane Cave) is a cave positioned at the western fringe of the Lessini

758  plateau in the Venetian Pre-Alps. The site preserves a finely layered late Middle and early

759  Upper Paleolithic sequence with evidence of cultural change related to the demise of

760  Neanderthals and the arrival of the first Anatomically Modern Humans (3, 10-12). Teeth

761  Fumane 1 and Fumane 2 were found in Middle Paleolithic unit A11 and Upper

762  Paleolithic unit A2 associated to Mousterian and Aurignacian cultures respectively.

763  Of the late Mousterian layers, unit A11 is a stratigraphic complex composed of an

764  ensemble of thin levels with hearths that was surveyed in different years at the eastern

765  entrance of the cave over a total area of 10 sqm. The chronometric position of A11 is

766  provided by only one U/Th date to 49,000±7,000 years for level A11a, given unreliability

767  to the radiocarbon dataset currently available (13) but see (14). New radiocarbon

768  measurements are in progress. Paleoecological indexes calculated on the composition of

769  the micromammal assemblage point for a temperate and relatively moist period related to

770  an interstadial before HE5 (15), in a landscape dominated from open-woodland

771  formations in accordance with the previous indications based on the zooarchaeological

772  assemblage. Cervids (red deer, giant deer and roe deer) largely prevail on bovids and

773  caprids (ibex and chamois) and other mammal species (16). No taphonomic analyses

774  have still been conducted to confirm the anthropogenic nature of the accumulation of the

775  animal bone remains. Lithic artifacts belong to the Levallois Mousterian. The use of this

776  technology is recorded by high number of flakes, cores and by-products shaped into

777  retouched tools like single and double scrapers, also transverse or convergent and few

778  points and denticulates (11).

779  Aurignacian layer A2 records an abrupt change in material culture represented from lithic

780  and bone industry (10, 17, 18), beads made of marine shells and bone (10, 19), use of red

781  mineral pigment (20). Bone and cultural remains have been found scattered on a

782  paleoliving floor with fire-places, toss zones and intentionally disposed stones (21). A

783  revised chronology of the Mid-Upper Paleolithic sequence (14) has shown that the start

784  and the end of level A2 date respectively to 41,900-40,200 cal BP and 40,300-39,400 cal

785  BP at the largest confidence interval. Macro- and micro-faunal remains show an

786  association between forest fauna and cold and open habitat species typical of the alpine

787  grassland steppe above the tree line in a context of climatic cooling (15, 22, 23). Hunting

788  was mostly targeted adult individuals of ibex, chamois and bison and occurred

789  seasonally, from summer to fall (22, 24).

790  Research at Fumane is coordinated by University of Ferrara (M.P.) in the framework of a

791  project supported by the Ministry of Culture – "SABAP per le province di Verona,

792  Rovigo e Vicenza", public institutions (Lessinia Mountain Community - Regional

793  Natural Park, Fumane Municipality, BIMAdige, SERIT) and by private institutions,

794  associations and companies. Research campaigns 2017 and 2019 have received funding

795  from the European Research Council (ERC) under the European Union's Horizon 2020

796  research and innovation programme (grant agreement No 724046 – SUCCESS,

797  http://www.erc-success.eu).

798  <u>Riparo Broion 1</u>

799  The Berici Mounts are a carbonatic karst plateau at low altitude at the southern fringe of

800  the Venetian Pre-Alps in the Alpine foreland. This is a large alluvial plain that was

801  formed initially during the Middle and Late Pleistocene by a number of major rivers,

802  including the Po, the Adige and those of the Friulian-Venetian plain. The western zone of

803  the Berici is a gentle landscape which conjoins to the alluvial plain. Conversely, along its

804  eastern slope the plateau connects abruptly to the alluvial plain. Here, caves and

805  rockshelters have been archaeologically investigated since the XIX century up to present

806  days by teams from the University of Ferrara. Of these cavities, Riparo del Broion is a

flagship site for the late Middle and early Upper Paleolithic in this area. It is situated at 135m a.s.l. at the base of a steep cliff of Mount Brosimo (327 m a.s.l.) along a terraced slope for cultivation during recent historical times. The shelter is 10m long, 6m deep and 17m high and originated from rock collapse along a major ENE-WSW oriented fault that developed from thermoclastic processes and chemical dissolution comparably to other cavities in the area (25, 26). Two additional Paleolithic cavities were investigated on the western side of the same cliff, Grotta del Buso Doppio del Broion and Grotta del Broion (27, 28).

The sedimentary deposits of Riparo Broion were partially dismantled in historical times by shepherds with use to store hay and wood. Further damage occurred in 1984 when unauthorized excavators removed sediments from pits and trenches on a total area of 14sqm down to 2m at the deepest. Archaeological excavations were initially directed by Alberto Broglio (1998 -2008) and by two of us (M.P. and M.R.) in 2015 on a 20sqm area bounded to north and west from the rock walls. Faunal remains and Middle and Upper Paleolithic (Uluzzian, Gravettian and Epigravettian) cultural material was uncovered (29-31). The bedrock has not yet been reached. Sediments are mostly small stones and gravel with large prevalence on loams: 16 stratigraphic units planarly bedded have been identified. The lowermost (11, 9, 7 and 4) contain Mousterian artefacts, faunal remains and clearly differentiate in dark-brownish color from the other units.

The human canine was discovered in unit 11 top. This unit has been 14C dated to 48,100±3100 years BP with range from 50.000 to 45.700 years cal BP as the most likely age (31). Stone tools are too low in number to propose an attribution to one or another Mousterian cultural complex. Preliminary zooarchaeological data report a variety of herbivores such as elk, red deer, roe deer, megaceros, wild boar, auroch/bison, a few goats and horses, and common beaver associated sparse remains of fish and freshwater shells. This association reflects the presence of a patchy environmental context, with closed to open-spaced forests, Alpine grasslands and pioneer vegetation complemented by humid-marshy environments and low-energy water courses, wet meadows and shallow lacustrine basins.

Paleoenvironmental contexts

The paleoenvironmental contexts during the time intervals of the teeth recovered at the sites of Nadale, Fumane cave and Riparo Broion (~ 70, 50 and 40 ka) can be inferred on the basis of two high-resolution paleoecologically records from NE-Italy: Lake Fimon (Berici Hills) and Palughetto basin (Cansiglio Plateau, eastern Venetian Pre-Alps). Pie charts presented in Fig. S3 show the relative abundances of different vegetation types at 5000 years' time-slice intervals. Pollen % are calculated based on the sum of terrestrial taxa and represent mean values. Pollen taxa are grouped according to their ecology and climatic preferences. Eurythermic conifers (EC): sum of Pinus and Juniperus; Temperate forest (TF): sum of deciduous Quercus, Alnus glutinosa type, Fagus, Acer, Corylus, Carpinus, Fraxinus, Ulmus, Tilia and Salix; Xerophytic steppe (XS): sum of Artemisia and Chenopodiaceae. Other herbs: sum of terrestrial herbs, Chenopodiaceae excluded. Original pollen data used for % calculation for the Palughetto basin are from (32).

On a long-term scale, the paleoecological record from Lake Fimon points to persistent afforestation throughout the Early to Middle Würm in the Berici Hills (i.e., Nadale, Fumane and Riparo Broion sites). Moderate forest withdrawals occurred during Greenland stadials (GSs), possibly enhanced during GSs hosting Heinrich Events (HEs) (33).

Between 75 and 70 ka, at the end of the second post-Eemian interstadial, the landscape was dominated by a mosaic of boreal forests with eurytermic conifers (46%) and

865 subordinated temperate taxa (10%). Open environments are identified by pollen of

866 herbaceous taxa and steppe/desert forbes-shrubs (23%).

867 During the 50-45 ka and 45-40 ka time-slices, steppic communities further increase (7-

868 8%) as a result of enhanced dry/cold conditions during Greenland stadials (GSs). Pollen

869 of eurythermic conifers sum up to 37-38%. Temperate trees, notably Tilia, persisted in

870 very low percentages (4%) up to ~40 ka (34).

871

**SUPPLEMENTARY INFORMATION TEXT S3: TOWARDS A CONCEPTUAL MODEL FOR Sr/Ca AND Ba/Ca BEHAVIOR IN HUMAN INFANTS: THEORETICAL FRAMEWORK AND EMPIRICAL EVIDENCE FROM CONTEMPORANEOUS INFANTS WITH KNOWN FOOD INTAKE**

Strontium and barium are non-bioessential trace elements with no major metabolic functions in the human body. Strontium and Ba mimic Ca, given their coherent behavior as alkaline earth elements with respect to their divalent charge, but are characterized by larger ionic radii (Sr: 1.18, Ba: 1.35, Ca: 1.00 Å ($10^{-10}$ m); (35). Overall, they both follow the Ca metabolism but due to their larger ionic size are discriminated against in the gastrointestinal tract (GIT) (36, 37). Given the larger size, Ba is even more strongly discriminated against relative to Sr (37, 38). Similarly, kidneys tend to excrete Sr and Ba more rapidly compared to Ca (39). From plasma, Sr, Ba and Ca are mainly fixed in bones and teeth with a likely further bias in favor of Ca (39, 40). Taken together, these factors cause Ca-normalized concentrations of Sr and Ba in skeletal tissues to be lower than those of the diet, a process known as 'biopurification' (36). Burton and Wright (41) demonstrated that Sr/Ca of bones is approximately 5 times lower than the respective Sr/Ca value of the diet. Such evidence has been also demonstrated empirically by many studies (36-38, 42, 43). These pioneering studies also emphasized that Sr/Ca and Ba/Ca might be used as tools for paleodiet and trophic chain reconstruction (36).

Interestingly, significant GIT discrimination of Sr and Ba over Ca ions progressively increases during human growth and becomes significant at around one year of age (44, 45). This hints that both the Sr/Ca and Ba/Ca ratios of infant plasma (<1 year) should be closer to the value of their respective dietary inputs (46). Indeed, Lough et al (46) demonstrated that the relative ratio between body Sr/Ca and dietary Sr/Ca for an infant is ~0.90. Hence, for example, in breast-fed infants, the Sr/Ca of their blood plasma should reflect the Sr/Ca of the consumed breastmilk. Studies of elemental transport in humans have shown that Ca is actively transported (47), resulting in lower Sr/Ca ratios in both umbilical cord sera and breastmilk than in mother sera due to the larger size of Sr ions compared to Ca ions. Yet, empirical evidence indicates that mammary gland discrimination for Sr (2.5-fold) is higher than placenta (1.7-fold), yielding average

902  breastmilk Sr/Ca values lower than umbilical cord (fetal) values (48). Crucially, fetal

903  blood chemistry is recorded in prenatal dental enamel and breastmilk consumption in

904  postnatal enamel and can be reconstructed via high-spatial resolution chemical analysis of

905  teeth (49, 50). Thus, higher Sr/Ca signals in prenatal domains followed by lower

906  postnatal Sr/Ca indicate breastmilk consumption (see Fig. S4). This has been previously

907  shown by the Sr/Ca distribution in teeth (50, 51), but also in elemental analyses of sera

908  samples. Krachler et al. (52) showed that Sr/Ca levels are two times higher in umbilical

909  cord sera than in breast-fed infant sera. On the other hand, due to the nominal lower

910  trophic level of herbivores, their milk has higher Sr/Ca than human milk. Hence, when a

911  child is fed through formula (largely based on cow milk), a Sr/Ca increase in the

912  postnatal enamel is expected (Fig. S4).

913  Indeed, Krachler et al. (52) reported high Sr/Ca values in formula-fed infant sera.

914  Moreover, a compilation of published Sr/Ca data of geographically dispersed human and

915  bovine/caprine milks (Fig. S5 and references in caption) indicates that human breastmilk

916  has a rather homogeneous Sr/Ca ratio of $\sim 0.1 \pm 0.01 * 10^{-3}$, 4 times lower than non-human

917  milk and formula ($\sim 0.39 \pm 0.15 * 10^{-3}$).

918  From all these inferences, the Sr/Ca ratio of both breast-fed and formula-fed infants can

919  be modelled relative to an initial Sr/Ca mother diet, set equal to 1 (Tab. S2 and Fig. S4).

920  With the introduction of transitional food in the infant diet, a change in Sr/Ca values is

921  also expected. If the child was initially breast-fed, one should predict an increase of the

922  Sr/Ca ratio during transitional feeding, because both meat and especially vegetables

923  retain higher Sr/Ca than breastmilk (see e.g. 53). In general, an increased Sr/Ca signal

924  from transitional foods is also expected for formula-fed babies. However, due to the

925  compositional variability of some formulas (e.g. soy-based) and non-human milk, a

926  decrease of the Sr/Ca ratio may occur if a highly-biopurified food (e.g. close to human

927  milk) is used for initial weaning.

928  Contrary to strontium, a reliable interpretation of Ba/Ca data is difficult due to

929  contradictory literature and the lack of studies on Ba metabolism. Austin et al. (54)

930  suggested that the increased level of both Sr/Ca and Ba/Ca ratios in breast-fed infants

931  reflected improved Sr and Ba absorption during breastfeeding. Such an increase in Sr/Ca

is in stark contrast to any other study on breastfed children (49, 50). Similarly, Krachler et al. (55) highlighted increased levels of Ba/Ca in colostrum and breast-fed infant sera compared to umbilical cord sera (Tab. S3). However, colostrum is not a good proxy for breastmilk elemental content, being highly enriched in metals (56, 57). In fact, when compared with Sr/Ca and Ba/Ca ratios from literature, colostrum values reported in Krachler et al. (55) are about 2 times higher than other human milk samples (Figure S5). Moreover, other studies suggested that only a very limited portion of the absorbed Ba (~3%) is transferred to the breast-milk (48).

Studies of dental enamel indicate that Ba overall behaves akin to Sr (50, 53, 58, 59), decreasing with breastmilk consumption and increasing along with the introduction of transitional food. Still, Müller et al. (50) noted that Ba behavior in tooth enamel is less predictable than Sr. This observation may also relate to the high variability of Ba content in human milk, colostrum and formulas (see (55) and Fig. S5). Notably, Taylor et al. (60) pointed out that in controlled-fed rats, the consumption of cow milk leads to an increase of Ca absorption, without changing the Ba absorption. This, in turn, corroborates the idea that the relative Ba/Ca ratio in rats should decrease with a milk-based diet and increase with a non-milk diet. In the same publication, the authors reported that Ba absorption increased two-fold in young starved rats, whereas Ca absorption decreased in the same individuals, pointing towards an association of Ba/Ca with dietary stress rather than weaning transitions.

Around one year of age, both Ba/Ca and Sr/Ca gradually decrease due to the progressive increase in GIT discrimination in the infant due to a preferential absorption of Ca relative to Sr and Ba (44, 45). Taken together, we conclude that models for Sr/Ca with respect to dietary transitions in early life have a stronger theoretical basis compared to Ba.

The modern reference sample

In the following we present spatially-resolved chemical data from contemporaneous individuals with known dietary behavior to evaluate the theoretical framework presented above. To avoid the problem of retrospectively reporting breastfeeding and weaning practice (61), we selected offspring from parents who reliably took and preserved notes

of the feeding practice during the nursing period (explicit written consent was obtained by all relevant people with legal authority). All individual data were treated in a fully anonymous way and it is not possible from the present results to identify the involved individuals.

Three deciduous teeth, representing three different nursing histories, were analyzed by LA-ICPMS: an exclusively breastfed individual from Switzerland (deciduous second molar dm2; MCS1), an exclusively bottle-fed individual from central Italy (deciduous canine dc; MCS2 previously published as MOD2 in (50)), a mixed breast-/bottle-fed individual from central Italy (deciduous canine dc; MCS3). The mothers of the three infants did not travel for extended periods during the interval in which these deciduous teeth were forming.

MCS1 is a lower deciduous second molar from an individual exclusively breastfeed until the fifth month of life (154 days; Fig. S6). No supplementary food was given to the infant during this period. The Sr/Ca profile analyzed parallel to the enamel-dentine junction (EDJ) shows a constant decrease in the elemental ratio until ~154 days corresponding to the reported period of exclusive breastfeeding. Just after the introduction of solid food once a day (reported from day 155), the slope of the profile becomes gradually shallower, particularly, this was coincident with the introduction of some formula milk (reported from day 182). Fifteen days after cutting down breastfeeding during daytime (reported on day 209) the profile begins to show a sharp increase of the Sr/Ca values. At 8.5 months of life (reported on 258 days) the breastfeeding period of individual MCS1 stopped and the diet continued with solid food and formula milk. The rather flat Sr/Ca signal observed in the last part of the profile (after day ~340) likely reflects the effects of maturation-overprint due to the thin enamel closest to the crown neck (50). The striking correspondence of the independently recorded dietary transitions in MCS1 with the Sr/Ca trend fully supports the use of Sr/Ca as a proxy for making nursing events. In this sense, based on modelled values reported in Tab. S2, the theoretical ratio between Sr/Ca in prenatal enamel and breastfeeding signal is ~0.7. In MCS1, this ratio is ~0.8, indicating a remarkable correspondence between the theoretical model and the observed data. The MCS1 Ba/Ca profile broadly follows the trend observed for Sr/Ca, decreasing - with

992 proportionately smaller changes in Ba levels across lifetime - from birth until ~160 days.
993 Thereafter, Ba/Ca steadily increases till day 235, steeply increases until day ~290 (9.5
994 months) to then decrease again for 25 days. Finally, Ba/Ca constantly increases to the end
995 of the crown formation. This fluctuation in the last part of the profile cannot be explained
996 by any event in the known dietary/health history of MCS1.

997 MCS2 is a deciduous canine from an exclusively formula-fed individual (Fig. S7), whose
998 results have already been partially presented in the context of enamel mineralization
999 processes as MOD2 (50). The Sr/Ca profile, run parallel to the EDJ, shows a constant
1000 increase after birth until ~130 days (~4.3 months), and then it starts to decrease as a
1001 consequence of the combined effects of the onset of the reported transitional period and
1002 maturation overprint. The absolute values of Sr/Ca through all the postnatal period are
1003 higher than $5*10^{-4}$ and thus higher than those observed in the other contemporary
1004 reference individuals (Figure S4b). The model reported in Fig. S4 and Table S2 specifies
1005 a ratio between prenatal enamel and formula Sr/Ca signal equal to ~2.2. In MCS2, this
1006 ratio is ~1.8, corroborating the hypothesis that with formula introduction the postnatal
1007 Sr/Ca should double. The Ba/Ca profile follows the same trend observed in the Sr/Ca
1008 profile, increasing from birth until ~75 days (2.5 months), then remaining stable with
1009 some fluctuation until ~175 days (5.8 months).

1010 MCS3 is an upper deciduous canine from a mixed breast- formula-fed individual (Fig.
1011 S8). This infant was exclusively breastfed for the first 30 days. After that, the mother
1012 complemented the infant diet with formula milk. Mixed feeding was carried on until 4
1013 months of age, at which time the mother underwent surgery. During this period of illness,
1014 the mother used a breast pump to continue breastfeeding. After the surgery, the mother
1015 continued to breastfeed the infant with formula milk supplements, until the onset of
1016 weaning at six months.

1017 The X/Ca profiles were nominally analyzed close to daily-resolution (6 µm spots vs. 10.3
1018 µm/day mean enamel extension rate), well-reflecting this complex nursing history and
1019 almost perfectly matching the main dietary shifts. Ba/Ca mirrors the Sr/Ca pattern,
1020 decreasing during the period of exclusive breastfeeding, slightly increasing during the
1021 mixed breast- bottle-feeding, and increasing further at the onset of weaning. The Ba/Ca

1022 profile follows the main dietary shifts but with less precision than Sr/Ca. Moreover, as in

1023 MCS1, the period of exclusive breastfeeding is characterized by a sharp decrease in

1024 Ba/Ca, contrary to what expected by Austin et al. (54). We note here that the small laser

1025 spot (6 µm) used during analysis resulted in lower ICPMS signals and hence overall

1026 larger analytical variability than for the other two specimens.

1027

1028 <u>The fossil Late Pleistocene human dental sample</u>

1029 <u>Nadale 1 - Neanderthal</u>

1030 In Nadale1, Sr/Ca profile slightly decreases until the end of the crown, depicting a

1031 breastfeeding signal until the end of the crown formation. Unusually, Ba/Ca shows the

1032 opposite trend to Sr/Ca (Fig. S9), and appears to follow the dietary model proposed by

1033 (54). Mg/Ca is largely invariant across the whole crown, and only very minor diagenetic

1034 alteration is apparent via U peaks at the very beginning and end of the crown that have

1035 very limited correspondence in Ba/Ca and Sr/Ca.

1036

1037 <u>Fumane 1 - Neanderthal</u>

1038 In Fumane 1, the Ba/Ca profile broadly follows that of Sr/Ca (Fig. S10), yet especially

1039 for the first ~120 days displays several pronounced, narrow peaks that correlate positively

1040 with U and negatively with Mg, respectively. These reveal localized diagenetic overprint

1041 that is far less manifested for Sr/Ca. According to our model, Sr/Ca indicates an exclusive

1042 breastfeeding signal until 115 days (4 months), followed by the first introduction of non-

1043 breastmilk food and a stronger signal visible at 200 days (6.6 months), at which point

1044 there is a steep increase in Sr/Ca that likely indicates a more important and substantial

1045 introduction of supplementary food. This profile is fully comparable to the MCS1 pattern

1046 reported above. According to (54), this individual falls outside the bounds of their model,

1047 because a decrease in Ba/Ca after birth is never detected in their data.

1048

1049 <u>Riparo Broion 1 - Neanderthal</u>

1050 In Riparo Broion 1, the Ba/Ca profile overall varies in parallel (Fig. S11), but also shows

1051 some prominent peaks that correlate positively with U and negatively with Mg,

respectively, indicating, similar to Fumane 1, that U uptake and Mg loss are indicators of localized diagenetic alteration (see Figure 3 main text). Regardless of diagenesis, both elemental ratios vary in the same way. According to our contemporary reference sample, a decrease in the Sr/Ca ratio is a consequence of exclusive breastfeeding until 160 days (5 months), after which an increase in Sr/Ca points to the first introduction of non-breastmilk food.

Fumane 2 - Aurignacian

The Ba/Ca profile of Fumane 2 follows that of Sr/Ca (Fig. S12), slightly decreasing in the first month of postnatal life and then increasing in the most cervical enamel. The short postnatal portion of available enamel (~55 days) precludes the chemical detection of the onset of weaning but a clear breast-feeding signal is detectable after birth since Sr/Ca decreases. Ba/Ca also decreases accordingly, and all is independent of diagenesis that is very low.

**SUPPLEMENTARY INFORMATION TEXT S4: ASSESSMENT OF POST-**

1068 **MORTEM DIAGENETIC ALTERATION OF BIOAPATITE**

1069

1070 In order to retrieve primary in-vivo elemental and isotopic signals from fossil teeth,

1071 preferably no alteration by post-mortem diagenetic processes should have taken place.

1072 During the post-depositional history, however, bioapatite may react with soils and

1073 underground waters, which can modify the initial biogenic chemical composition.

1074 Depending on apatite crystal-size, organic content and porosity, the distinct dental tissues

1075 behave differently in a soil environment. Bone and dentine are most susceptible to

1076 diagenetic chemical overprint, in contrast to highly-mineralized enamel (62-65). Equally,

1077 the extent of chemical overprint depends on the concentration gradient between burial

1078 environment and bioapatite tissue as well as the partition coefficient for the element(s)

1079 concerned.

1080 While alkali-earth elements (e.g. Ba, Mg and Sr) and biologically-important divalent

1081 metals (e.g. Cu, Fe and Zn) are present at mid-high concentrations (i.e. $>1 - 10^3$ µg/g) in

1082 modern bioapatite, Rare Earth Elements (REE), actinides and high-field strength

1083 elements (e.g. Hf, Th and U) have very low concentrations (lowest ng/g) in modern

1084 teeth/bones, yet are usually strongly incorporated into apatite during fossilization

1085 processes (66).

1086 In particular, uranium as water soluble (as uranyl $(UO_2)^{2+}$) and highly mobile element is

1087 readily incorporated into bioapatite (67, 68), such that uranium in fossil bioapatite,

1088 especially in bone and dentine, often shows high concentrations ($>10s - 100s$ µg/g),

1089 whereas enamel frequently displays much lower U concentrations (e.g. (69)). Given these

1090 variations at the microscale, uranium can reveal diagenetic overprint in tandem with Mn

1091 or Al. Conversely, some bio-essential trace elements in bioapatite such as Mg may

1092 decrease post-mortem due to precipitation of diagenetic phases with lower trace metal

1093 concentrations, incipient recrystallization or leaching from the dental/bone tissue (70, 71).

1094 To monitor diagenetic alterations of our fossil dental specimens, we monitored $^{25}$Mg,

1095 $^{27}$Al, $^{55}$Mn, $^{89}$Y, $^{140}$Ce, $^{166}$Er, $^{172}$Yb and $^{238}$U signals during the LA-ICPMS analyses and

1096 found that U (and Al) were the most sensitive indicators of diagenetic alteration, while

commonly utilized REEs plus Y were rather insensitive in all cases as they remained at detection limit even in domains with clearly elevated U and Al. As a result, REE + Y are not shown here and we focus on U as main proxy for post-mortem diagenesis.

Scatter plots between U and the residuals of Sr, Ba or Mg variation for the diagenetically most affected segments (Fig. S13) illustrate well the nature of element-specific diagenetic overprint of the four teeth. In samples with overall low [U] (<0.2 µg/g), i.e. Nadale 1 and Fumane 2, there are no significant positive or negative correlations discernible. In case of Riparo Broion 1 and Fumane 1, [U] rises up to 0.6 µg/g and positively correlates with Ba and negatively with Mg, while Sr only shows significant co-variation in Riparo Broion 1. It should be noted that spatially-resolved analysis by LA-ICPMS not only allows the retrieval of time-resolved chemical signals, but is equally ideally-suited for the delineation of well-preserved segments in partially diagenetically-overprinted samples. We employ the following strategy to delineate well-preserved from diagenetically overprinted segments in our enamel profiles:

1) The visible co-variation between U and Sr/Ca (Fig. 3) as well as above mentioned correlations between Sr, Ba, Mg residuals with U (Fig. S13) show that especially Ba and less so Sr (only Riparo Broion 1) were added during diagenesis, while Mg was lost. Consequently, only data segments with lowest U ([U] <~0.05 µg/g) were used for further considerations.

2) The shape and nature of the discernible peaks/troughs provide an additional constraint. Very sharp variations, over less than 5 days, in U, Ba, Mg in Fumane 1 (Fig. S10) and U, Ba, Sr, Mg in Riparo Broion 1 (Fig. S11) characterize diagenetic signals, while variations in low-U domains are far more gradual and occur over tens of days. The latter is more in line with biologically-mediated variations that are additionally modulated by the protracted nature of enamel mineralization (50), which precludes, for example, the up to fourfold variability in Ba/Ca occurring at the profile start of Fumane 1 to be of in-vivo origin (Fig. S10).

3) Diagenesis is highly sample-specific even at the same site, illustrated here for Fumane cave, which makes a 'one size fits all' approach difficult to apply. While Fumane 2 is almost not affected by diagenesis that does also not affect Ba or Mg, the only slightly

43

older Fumane 1 sample is more strongly overprinted, which manifests itself especially in Ba addition (>twofold increase) and Mg loss, while Sr is little affected.

Overall, we note that diagenesis appears to affect the early formed enamel segments more than later mineralized areas. As the former are characterized by higher enamel extension rates, one conjecture is that this may have caused slightly greater amount of porosity that in turn makes such domains more susceptible for post-mortem chemical overprint. Thus, the initial portions of Nadale 1, Fumane 1 and Riparo Broion 1 crowns show enrichments in U, Al and Mn, with a concurrent decrease of Mg (Figure 3 and S9-S12). While Sr seems only partly affected by this overprint, Ba tends to precisely resemble the small-scale chemical fluctuations of the diagenetic proxies (clearly visible in Riparo Broion 1 and Fumane 1), suggesting a lack of post-burial stability for the latter element.

Taken together, we observe that the areas of interest (i.e. weaning onset) of our specimens are sufficiently free from diagenetic alterations to reliably deduce time-resolved dietary and mobility signals based on Sr/Ca and Sr isotopic ratios, respectively.
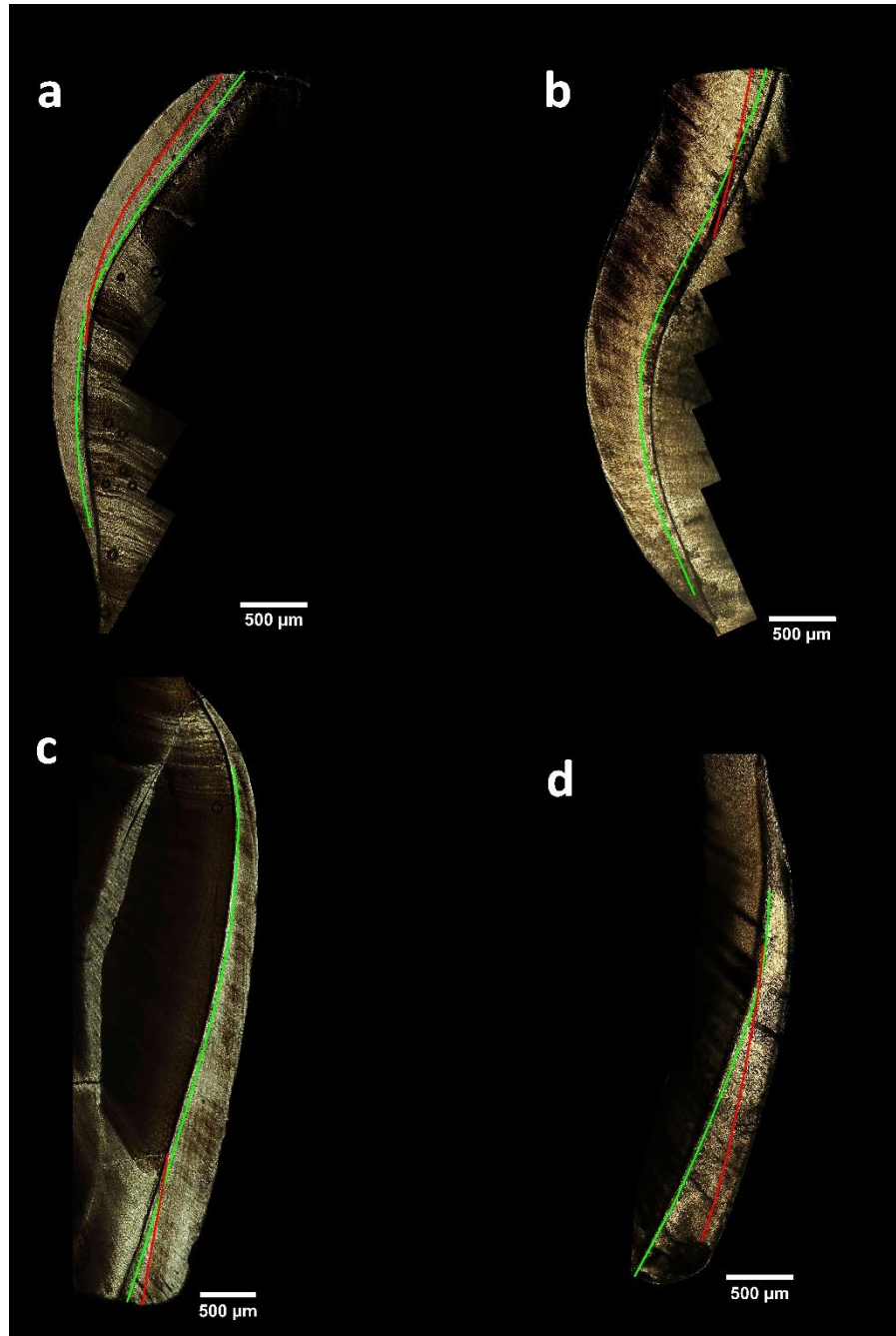
**Figure S1. Micrographs acquired at 100x magnification of the four exfoliated deciduous fossil teeth.** (a) Nadale 1, Neanderthal, lower right deciduous first molar, lingual aspect, the section pass through the metaconid; (b) Fumane 1, Neanderthal, lower left deciduous second molar, buccal aspect, he section pass through the hypoconid; (c) Riparo Broion 1, Neanderthal, upper left deciduous canine, buccal aspect; (d) Fumane 2, UPMH, upper right lateral deciduous incisor, buccal aspect. Red lines highlight the position of the Neonatal line marking birth event; green lines highlight the laser ablation paths.
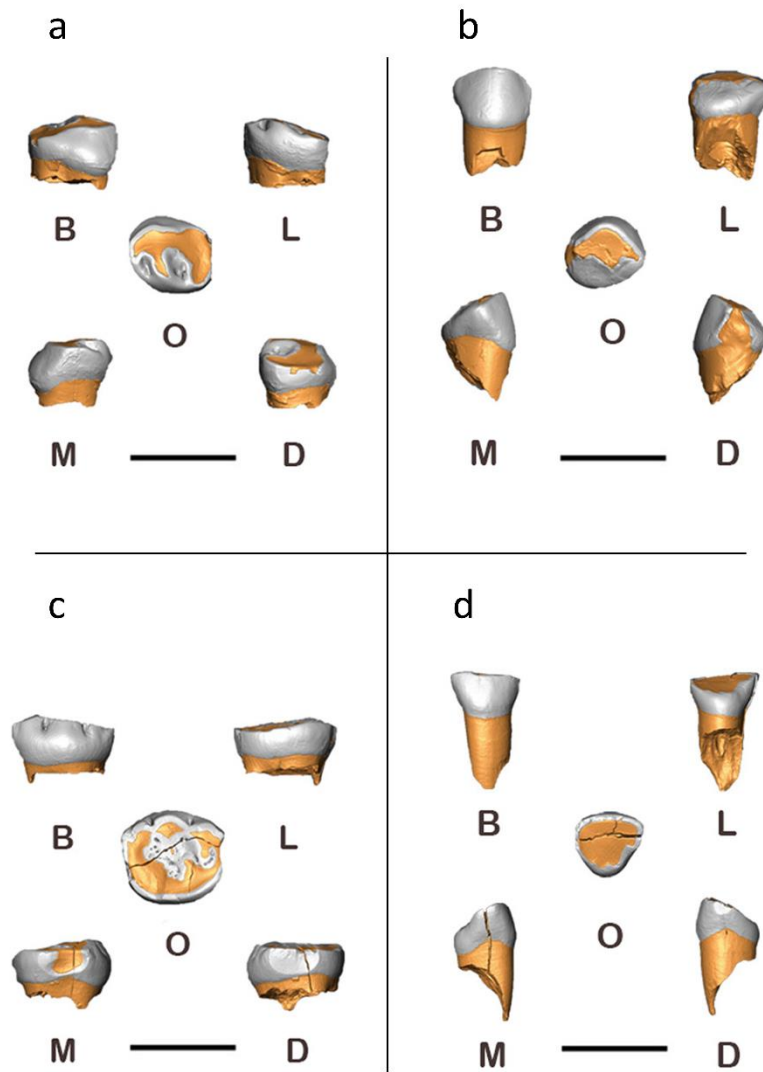
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**Figure S2. Three-dimensional digital models of the four exfoliated deciduous fossil teeth.** (a) Nadale 1 (lower right first deciduous molar); (b) Fumane 1 (lower left second deciduous molar); (c) Riparo Broion 1 (upper right deciduous canine); (d) Fumane 2 (upper right lateral deciduous incisor). Scale bar 10 mm. B, buccal; D, distal; L, lingual; M, mesial; O, occlusal
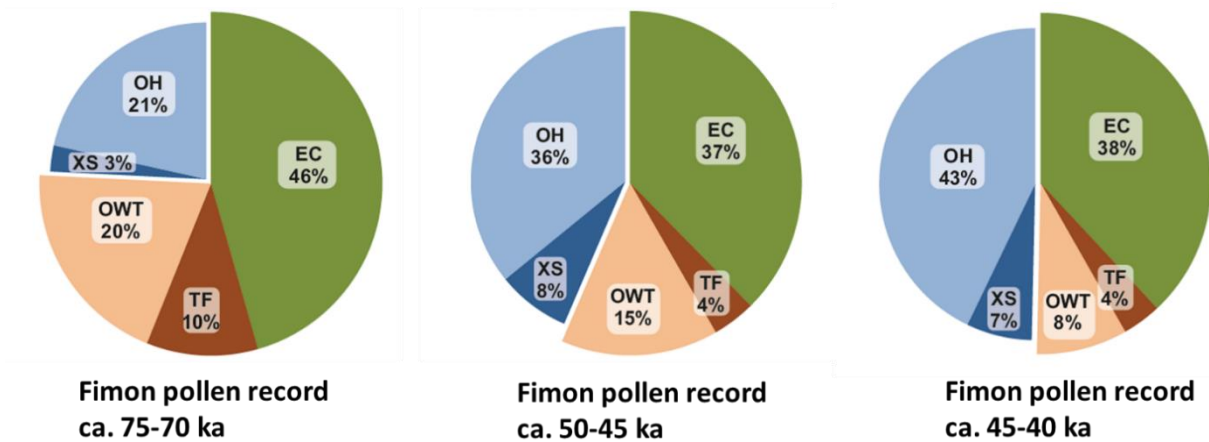
**Figure S3. Pollen record summary of different vegetation types during selected time-frames.** Pollen % are calculated based on the sum of terrestrial taxa and represent mean values over the selected time frame. Taxa are grouped according to their ecology and climatic preferences. Eurythermic conifers (EC): sum of *Pinus* and *Juniperus*; Temperate forest (TF): sum of deciduous *Quercus*, *Alnus glutinosa* type, *Fagus*, *Acer*, *Corylus, Carpinus, Fraxinus, Ulmus, Tilia* and *Salix*; Xerophytic steppe (XS): sum of *Artemisia* and Chenopodiaceae; other herbs (OH): sum of terrestrial herbs; other woody taxa (OWT) are also reported.
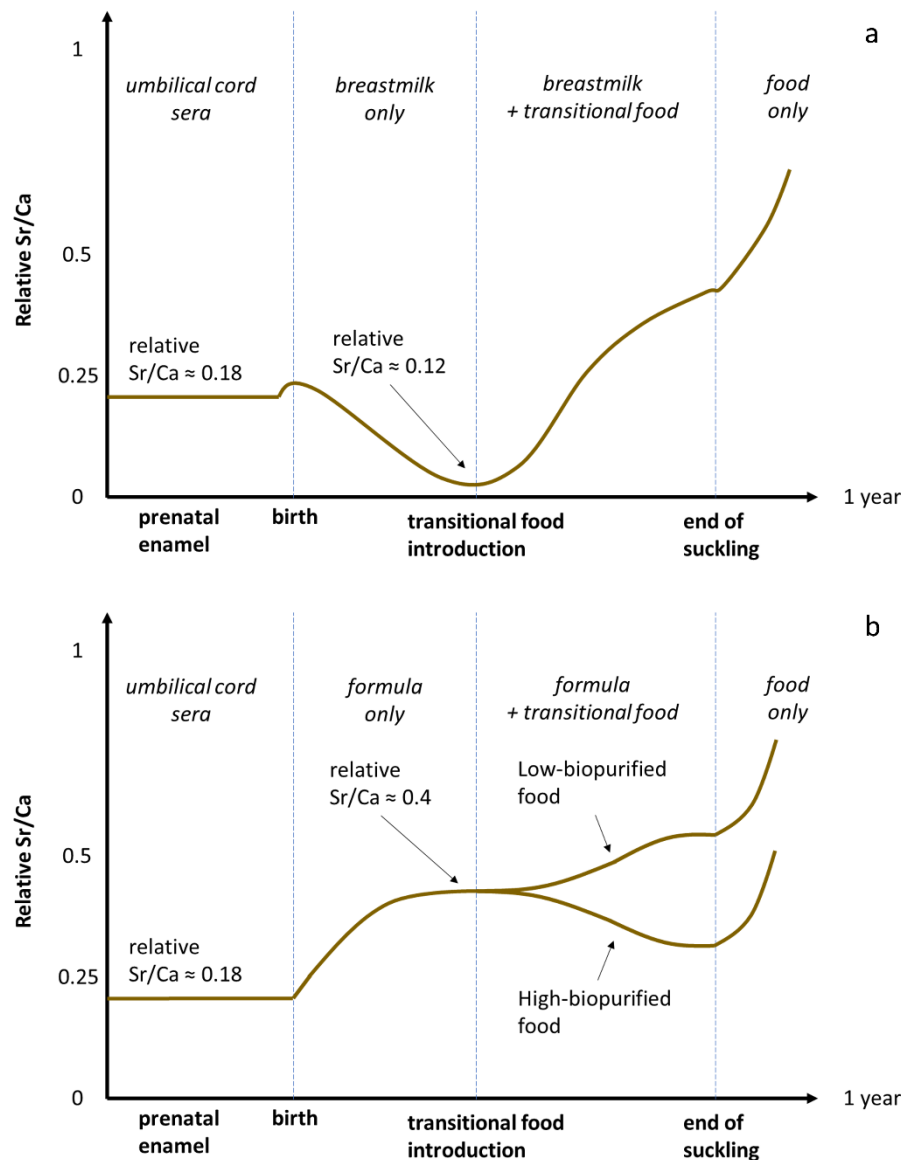
Figure S4. Sr/Ca models for (a) breast-fed infants and (b) formula fed-infants. These models assume a mother diet equal to 1. In this model, GIT function is ignored since it begins to significantly discriminate Sr over Ca at ~1 year of age in humans. A small peak in Sr/Ca signal is visible across birth in breast-fed infants (a); this has been observed empirically in our tooth samples and may relate to several factors, as e.g. high-metal content of colostrum (57) or potential changes in perinatal physiology (56). The same peak is probably masked in formula-fed infants (b) due to the rapid Sr/Ca increase.
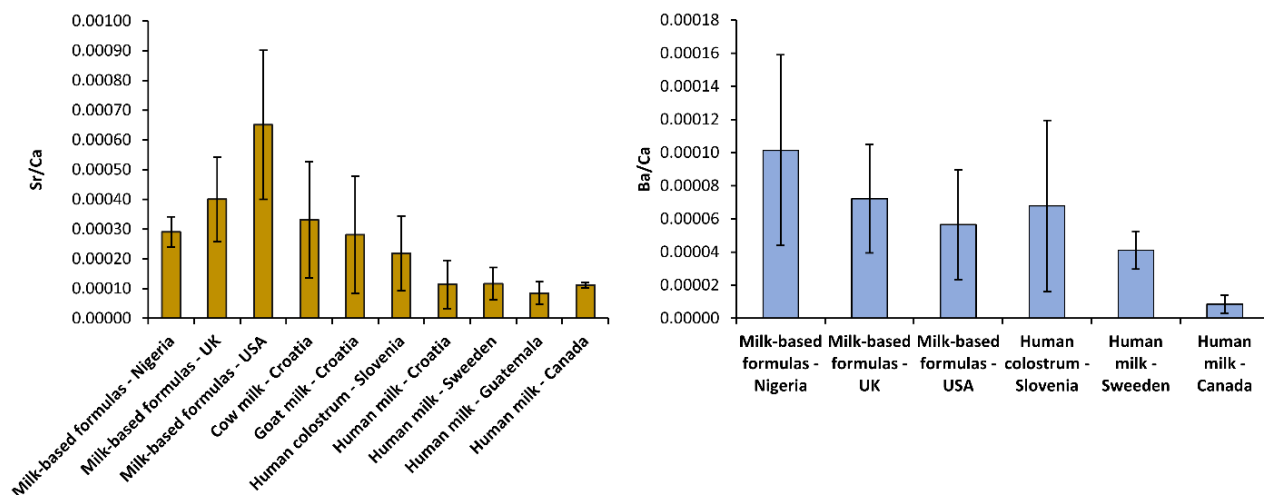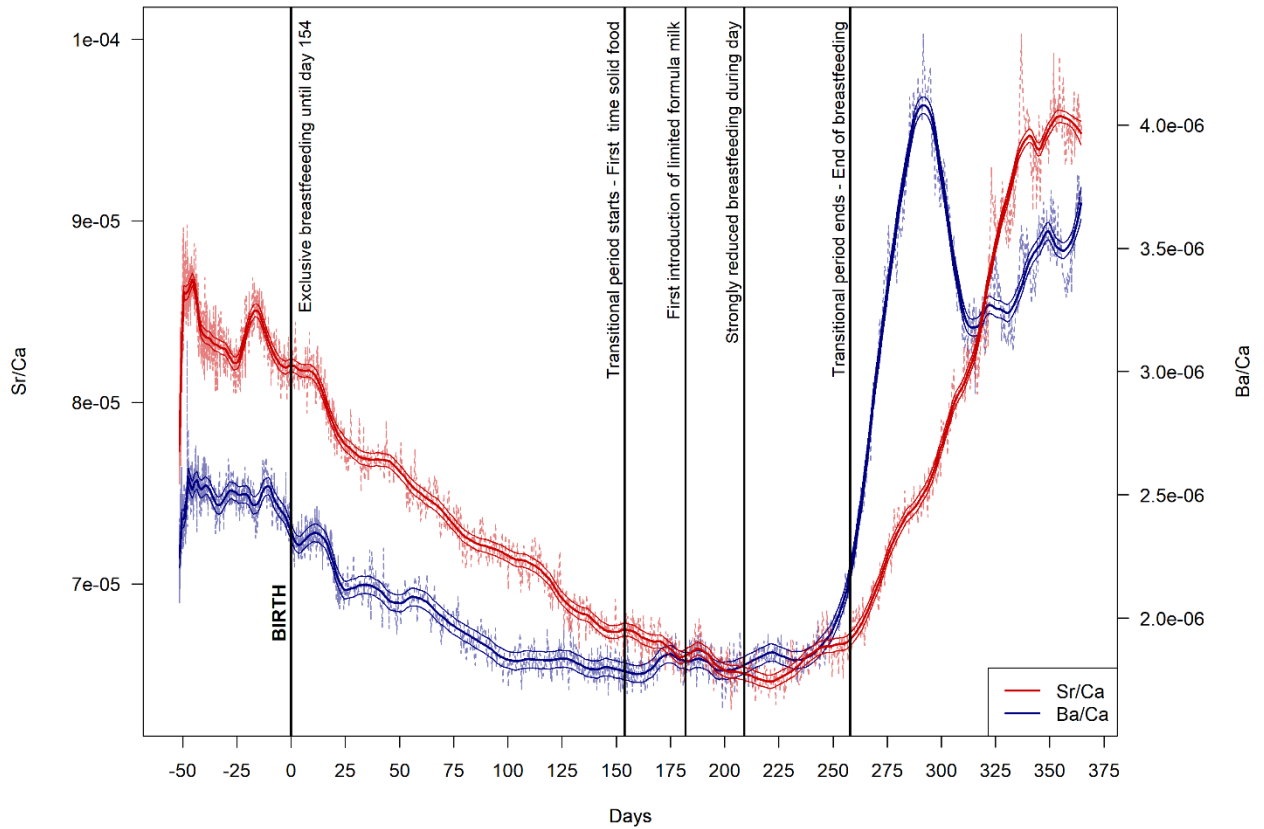
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1180 **Figure S5. Sr/Ca and Ba/Ca data of animal milks, human milks and formulas from**
1181 **literature.** Formulas are from Ikem et al. (72); cow and goat milks are from Bilandžić et
1182 al. (73); human colostrum is from Krachler et al. (55); human milks are from Bilandžić et
1183 al. (73), Björklund et al. (74), Li et al. (75) and Friel et al. (76). The geographical
1184 provenance of the samples is also reported. Error bars are standard deviations.
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**Figure S6. Time-resolved Sr/Ca and Ba/Ca profiles in modern reference deciduous teeth of the exclusively breastfed individual MCS1**. Deciduous second molar dm2; The elemental profiles were analyzed within enamel closest to the enamel-dentine-junction (EDJ).

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**Figure S7. Time-resolved Sr/Ca and Ba/Ca profiles in modern reference deciduous teeth of the exclusively formula-fed individual MCS2**. Deciduous canine dc. The elemental profiles were analyzed within enamel closest to the enamel-dentine-junction (EDJ).

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**Figure S8. Time-resolved Sr/Ca and Ba/Ca profiles in modern reference deciduous teeth of the** mixed breast- formula-fed individual **individual MCS3.** deciduous canine dc. The elemental profiles were analyzed within enamel closest to the enamel-dentine-junction (EDJ).

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**Figure S9. Time-resolved Sr/Ca, Ba/Ca, Mg/Ca and [U] profiles Nadale 1 deciduous teeth.** The elemental profiles were analyzed within enamel closest to the enamel-dentine-junction (EDJ).

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**Figure S10. Time-resolved Sr/Ca, Ba/Ca, Mg/Ca and [U] profiles Fumane 1 deciduous teeth.** The elemental profiles were analyzed within enamel closest to the enamel-dentine-junction (EDJ); While Sr seems only partly affected by this overprint, Ba tends to precisely resemble the small-scale chemical fluctuations of the diagenetic proxies (i.e. U). The anticorrelation between U and Mg/Ca indicates a loss Mg during the post-burial history, and the likely precipitation of low-Mg phases. Black arrows highlight the worst diagenetically-affected domains of the enamel.
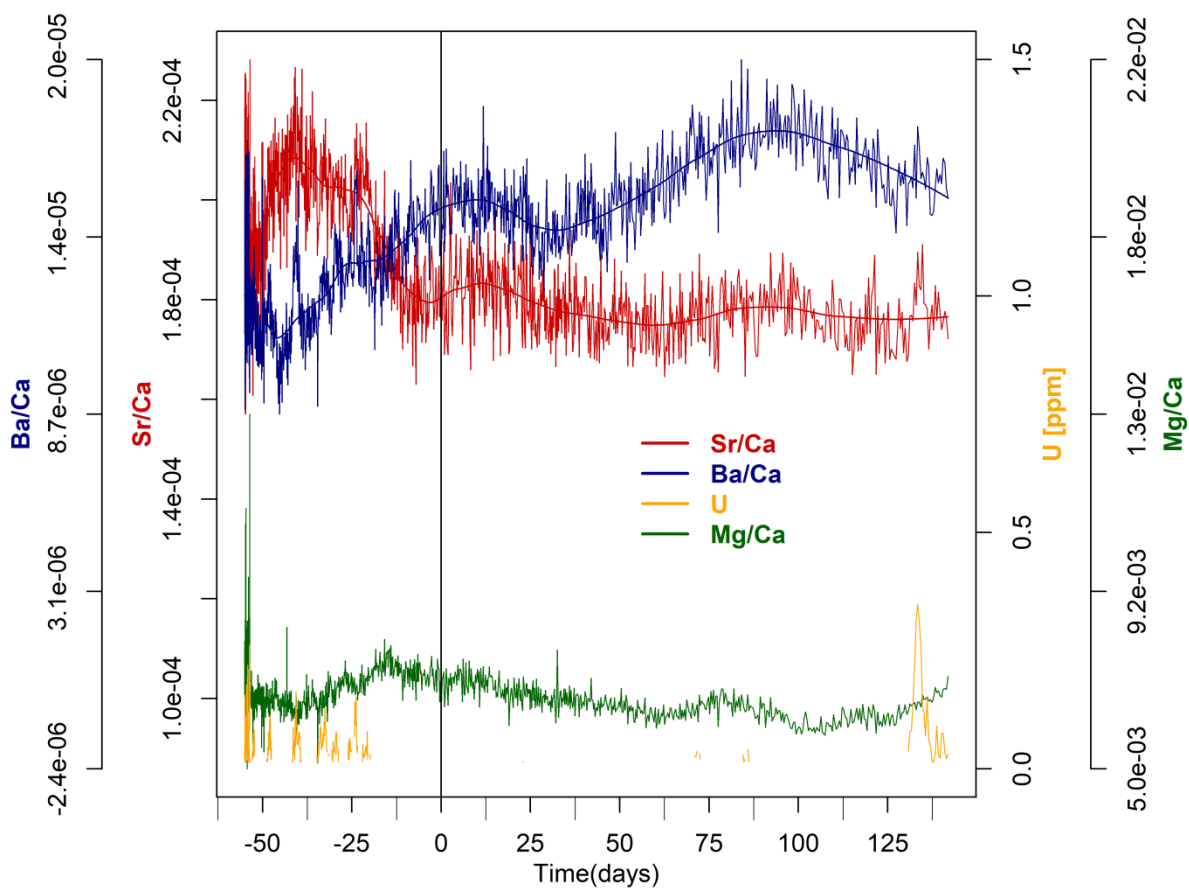
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**Figure S11. Time-resolved Sr/Ca, Ba/Ca, Mg/Ca and [U] profiles Riparo Broion 1
deciduous teeth.** The elemental profiles were analyzed within enamel closest to the
enamel-dentine-junction (EDJ); While Sr seems only partly affected by this overprint, Ba
tends to precisely resemble the small-scale chemical fluctuations of the diagenetic proxies
(i.e. U). The anticorrelation between U and Mg/Ca indicates a loss Mg during the post-
burial history, and the likely precipitation of low-Mg phases. Black arrows highlight the
worst diagenetically-affected domains of the enamel.
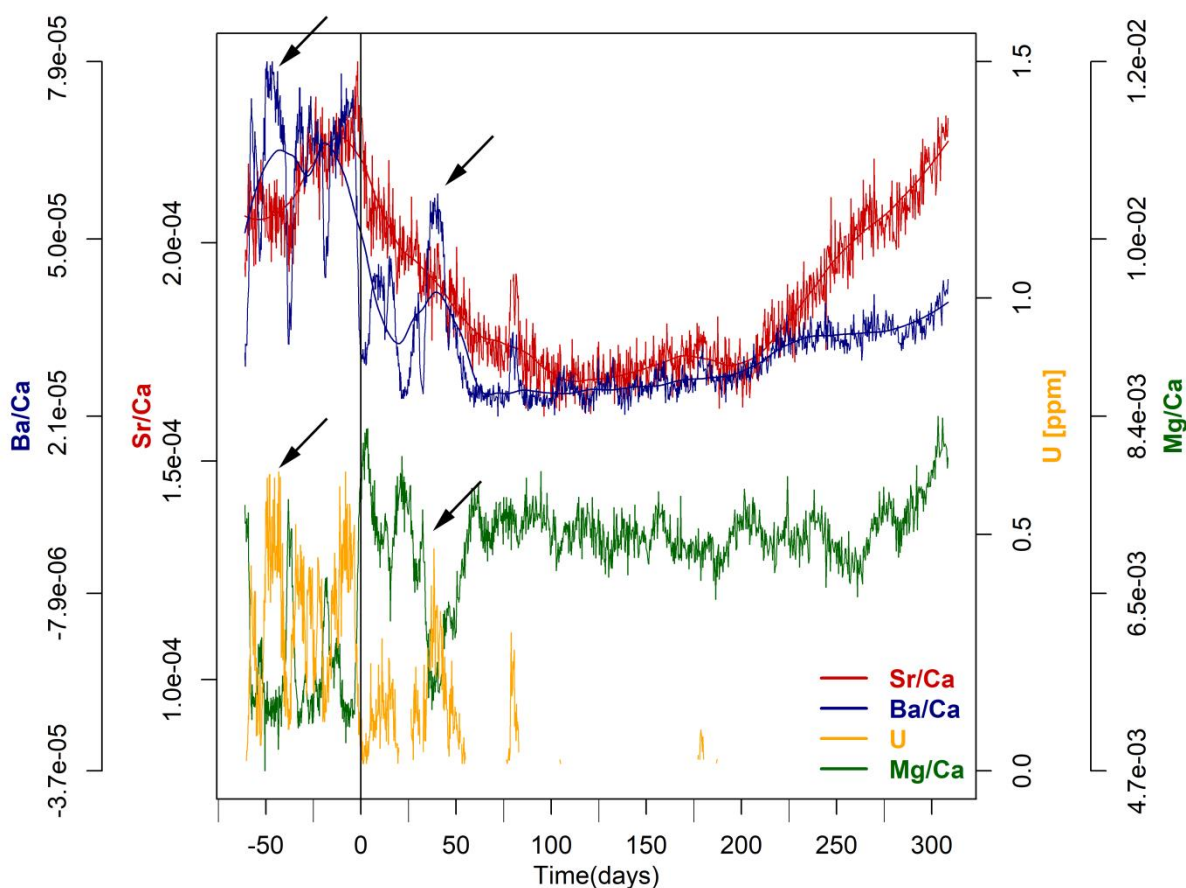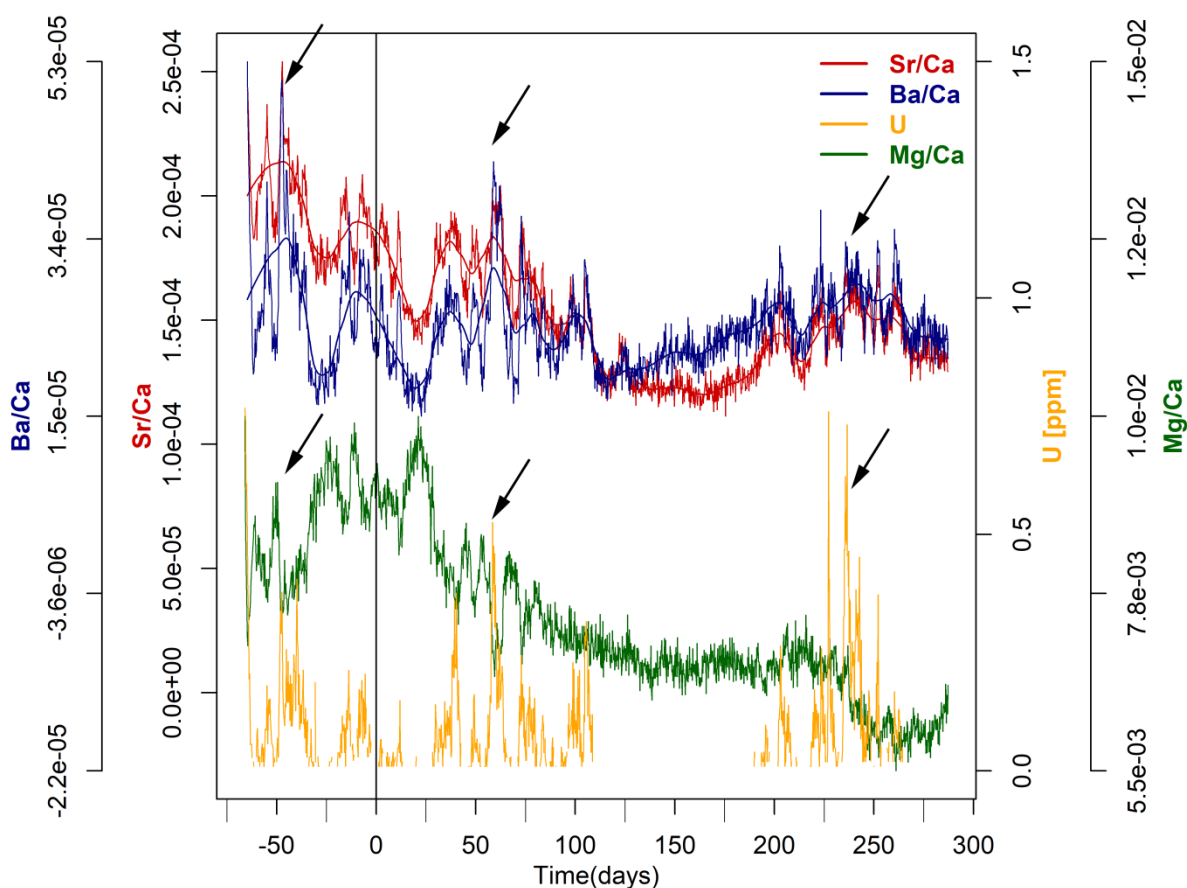
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**Figure S12. Time-resolved Sr/Ca, Ba/Ca, Mg/Ca and [U] profiles Fumane 2 deciduous tooth.** The elemental profiles were analyzed within enamel closest to the enamel-dentine-junction (EDJ).

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**Fig. S13: Scatter plots of U vs. the residuals of Sr, Ba or Mg variation for the diagenetically most affected segments in Nadale 1 (start till -10days), Fumane 1 (start till 100 days), Riparo Broion 1(start till 125 days) and Fumane 2 (start till -50 days).** Residuals were derived from the smoothed elemental profiles of Fig. 3e, calculated with a local polynomial regression fitting - LOWESS (77) - on the laser path portions with U≤LOD. The residual Sr, Ba, Mg variability rather all data were used as we wanted as much as possible remove biological variation overprint any diagenesis signal.

**Table S1:** Sr isotopes of local rodent teeth by MC-ICPMS

| Site | Local geology | Rodent species | Sample type | $^{87}Sr/^{87}Sr$ | 2 S.E. |
|---|---|---|---|---|---|
| Nadale | Eocene limestone | *Microtinae* indet. | enamel | 0.70847 | 0.00001 |
| | | | enamel | 0.70843 | 0.00001 |
| | | | enamel | 0.70825 | 0.00003 |
| | | | enamel | 0.70864 | 0.00001 |
| | | | enamel | 0.70857 | 0.00001 |
| | | | **mean (± 2 S.D.)** | **0.70847** | **0.00030** |
| Riparo Broion | Eocene Oligocene limestone | *Microtinae* indet. | whole tooth | 0.70826 | 0.00001 |
| | | | whole tooth | 0.70820 | 0.00001 |
| | | | whole tooth | 0.70814 | 0.00001 |
| | | | whole tooth | 0.70827 | 0.00001 |
| | | | whole tooth | 0.70838 | 0.00001 |
| | | | **mean (± 2 S.D.)** | **0.70825** | **0.00018** |
| Fumane Cave | Jurassic-Cretaceous limestone and marl | *Microtinae* indet. | enamel | 0.70948 | 0.00001 |
| | | | enamel | 0.70937 | 0.00001 |
| | | | enamel | 0.70947 | 0.00001 |
| | | | enamel | 0.70940 | 0.00001 |
| | | | enamel | 0.70962 | 0.00001 |
| | | | enamel | 0.70958 | 0.00001 |
| | | | **mean (± 2 S.D.)** | **0.70948** | **0.00020** |

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1244 **Table S2.** Discrimination factors of Sr over Ca within mother and infant bodies; fluxes
1245 through different tissues are reported in brackets; a Sr/Ca relative to a mother diet equal
1246 to 1 has been calculated for each end-member; the different enamel portions where a
1247 specific signal is fixed are also reported.

| End-member (flux) | (Sr-over-Ca discrimination factor) | Relative Sr/Ca | Reference | Enamel |
|---|---|---|---|---|
| **Diet** | - | **1** | - | - |
| **Mother sera (diet-blood)** | 0.30 ± 0.08* | **0.3** | Balter, 2004 | - |
| **Umbilical cord sera (mother sera - placenta)** | 0.6 | **0.18** | ICRP, 2004 | prenatal |
| **Breastmilk (mother sera - mammary gland)** | 0.4 | **0.12** | ICRP, 2004 | postnatal, breast-fed infant |
| **Animal milk** | One throphic level lower than human breastmilk (Sr/Ca ~3.3-fold higher than human milk) | **0.40** | Balter, 2004; see text | postnatal, formula-fed infant |

*this value is relative to the difference between mammals' muscle (or bone) tissue and their diet, based on a large trophic chain study; for simplicity any eventual discrimination between blood and muscles (or bones) is ignored.

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1250    **Table S3**. Ba, Sr, Ca, Ba/Ca and Sr/Ca values of umbilical cord sera, breast-fed infant

1251    sera and formula-fed infant sera from (52, 55). Values are reported as mean ± sd.

| Elemental contents and ratios | Maternal sera[a] | Umbilical cord sera[b] | Umbilical cord sera[a] | Breast-fed infant (ca. 3 months) sera[b] | Formula-fed infant (ca. 3 months) sera[b] | Colostrum[a] |
|---|---|---|---|---|---|---|
| **Ba (µg/L)** | 6 ± 7.8 | 0.8 ± 0.8 | 1.5 ± 1.7 | 1.9 ± 0.4 | 3.8 ± 1.4 | 10.6 ± 8.7 |
| **Sr (µg/L)** | 22.3 ± 8.9 | 20 ± 9 | 19.6 ± 7.2 | 12 ± 3 | 40 ± 25 | 37 ± 18 |
| **Ca (mg/L)** | 92 ± 16 | 95 ± 13 | 104 ± 16 | 112 ± 4 | 116 ± 8 | 210 ± 60 |
| **Ba/Ca*10³** | 0.082 ± 0.093 | 0.010 ± 0.009 | 0.017 ± 0.018 | 0.017 ± 0.004 | 0.034 ± 0.014 | 0.068 ± 0.052 |
| **Sr/Ca*10³** | 0.267 ± 0.135 | 0.228 ± 0.121 | 0.204 ± 0.096 | 0.108 ± 0.031 | 0.361 ± 0.238 | 0.218 ± 0.126 |

[a]Krachler et al. (1999, *European Journal of Clinical Nutrition*); [b]Krachler et al. (1999, *Biological Trace Element Research*)

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**Legends for Datasets**

**Dataset S1**. $^{87}Sr/^{86}Sr$, $^{84}Sr/^{86}Sr$ and $^{85}Rb/^{86}Sr$ data of Middle-Upper Paleolithic deciduous teeth (baseline, interference, mass-bias/elemental-fractionation-corrected (see text); very minor offset of $^{84}Sr/^{86}Sr$ from 0.0565 is due to residual variability of $^{84}Kr$-backgrounds for protracted profile analyses).

**Dataset S2**. Sr/Ca and Ba/Ca data of modern reference deciduous teeth.

**Dataset S3**. Sr/Ca, Ba/Ca, Mg/Ca and [U] data of Middle-Upper Paleolithic deciduous teeth (LOD indicates that [U]<limit of detection).

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