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Main Manuscript for

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.C.I., A.H., D.E., F.L., S.S., T.G., W.M. produced chemical/isotopic data; F.B. and R.P. produced ecological framework; A.N., C.D., L.B. produced histology data; C.T., F.B.R. produced the microtomographic record; A.H., A.N., D.E., E.B.R., 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.C., C.F., E.B., 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.

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 and one
8 Upper Paleolithic modern human from Northeastern-Italy via spatially-resolved
9 chemical/isotopic analyses and histomorphometry of deciduous teeth. Our results reveal
10 that the modern human nursing strategy, with onset of weaning at 5-6 months, was
11 present among these Neanderthals. This evidence, combined with dental development
12 akin to modern humans, highlights their similar metabolic constraints during early life
13 and excludes late weaning as a factor contributing to Neanderthals' demise.

14 **Significance Statement**

15 The extent to which Neanderthals differ from us is the current focus of many studies in
16 human evolution. There is debate about their pace of growth and early life metabolic
17 constraints, both of which are still poorly understood. Here we use chemical and isotopic
18 patterns in tandem with enamel growth rates of three Neanderthal milk teeth from
19 Northeastern Italy to explore the early life of these individuals. Our study shows that
20 these Neanderthals started to wean children at 5-6 months akin to modern humans,
21 implying similar energy demands during early infancy. Dental growth rates confirm this
22 and follow trajectories comparable with modern humans. Contrary to previous evidence,
23 we suggest that differences in weaning age did not contribute to Neanderthals' demise.

24

25

26 **Main Text**

27

28 **Introduction**

29

30 Maternal physiology, breastfeeding and the first introduction of supplementary foods are
31 key determinants of human growth (1). The high nutritional demands of the human brain

32 during the first years of life has been identified as the main reason for the early weaning
33 onset in modern humans (2, 3). Indeed, supplementary food is needed when infant's
34 nutritional requirements exceed what the mother can provide through breastmilk only (4)
35 and this dietary development can introduce foods that are higher in protein, calories and
36 key micro-nutrients than maternal milk (4, 5). Weaning onset occurs in contemporary
37 non-industrial human societies at a modal age of 6 months (6).

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 limited. Previous work
40 based on permanent teeth from eight Neanderthal specimens reported that Neanderthal
41 tooth crowns tend to develop faster than in modern humans, suggesting infant growth was
42 generally accelerated (7). However, a permanent first molar and a second deciduous
43 molar from La Chaise (France, 127-116 ka and <163 ka respectively) placed rates of
44 Neanderthal tooth growth within the range of modern humans (8). Equally, the
45 association between dental and skeletal growth in a 7-year-old Neanderthal from El
46 Sidròn (Spain, 49 ka) indicated that Neanderthals and modern humans were similar in
47 terms of ontogenetic development, with only small-scale dissimilarities in acceleration or
48 deceleration of skeletal maturation (9). Finally, other work suggested that the early
49 growth of the Neanderthal brain was as fast as in modern humans with similar energetic
50 demands (10). Maps of Ba/Ca ratios of permanent tooth sections of two early
51 Neanderthals (Payre 6, 250 ka and Scladina, 120 ka) have been interpreted
52 (controversially, see below) as indicators of weaning onset at ~9 months (11) and 7
53 months (12) of age respectively, later than the modal age in contemporary humans (6).
54 Similarly, wear stage analyses of a large number of deciduous dentitions suggested that
55 introduction of solid food in Neanderthals was delayed by one year compared to modern
56 humans (13).

57 Here we investigate such key aspects of early life in Neanderthals by combining new data
58 on chemical detection of weaning onset with deciduous enamel growth rates. We utilize
59 dental histomorphometry (8, 14), spatially-resolved chemical (15) and isotopic profiles
60 (16, 17) of dental enamel to reconstruct growth rates (14), nursing practices (4) and
61 mobility (16) during the Middle and Upper Paleolithic at high time resolution (up to

62 weekly). We analyzed an unprecedented set of teeth ($n = 4$) (*SI Appendix*, Text S1) from
63 adjacent archaeological sites in Northeastern Italy (*SI Appendix*, Text S2), dated from the
64 Late Middle to the Early Upper Paleolithic, from Neanderthal-modern human contexts
65 (70-40 ka). These four exfoliated deciduous fossil teeth include three Neanderthals
66 (Nadale 1, a lower right deciduous first molar (19), ~70 ka; Fumane 1, a lower left
67 deciduous second molar (18), ~50 ka; Riparo Broion 1, an upper left deciduous canine
68 (20), ~50 ka) and one Early Upper Paleolithic modern human (UPMH) as comparative
69 specimen from the Fumane site (Fumane 2, an upper right deciduous second incisor (21),
70 Protoaurignacian, ~40 ka) (Fig. 1).

71

72 **[Insert Figure 1 here]**

73 **Figure 1. Geographical, paleoecological and chronological framework.** (a) Oxygen
74 isotope curve from NGRIP (22), with Greenland Stadials 5-21 highlighted. Chronologies
75 of the human specimens are also reported (see Supplementary Information for details);
76 Fumane 2 is UPMH (green), while Nadale 1, Riparo Broion 1 and Fumane 1 are
77 Neanderthals (yellow). (b,c,d) Modelled Alpine glacier extent during the time intervals of
78 the teeth recovered at the sites of Fumane Cave (b,c), Riparo Broion (c) and Nadale (d);
79 location within Italy is shown in the inset. Simulations show a high temporal variability
80 in the total modelled ice volume during Marine Isotope Stages 4 (70 ka snapshot) and 3
81 (50, 40 ka snapshots) with glaciers flowing into the major valleys and possibly even onto
82 the foreland (23).

83

84 Exfoliated deciduous teeth derive from individuals who survived permanent tooth
85 replacement and were thus unaffected by any mortality-related bias (24). All teeth come
86 from the same geographic area within a ~55 km radius (Fig. 1), and Fumane 1 and 2 were
87 recovered from different archaeological layers in the same cave, thus allowing direct
88 comparisons in a well-constrained eco-geographical setting.

89 We quantified enamel incremental growth parameters such as postnatal crown formation
90 time and daily enamel secretion rates (25), and we detected the presence of the neonatal
91 line as birth marker (26) by optical light microscopy on thin sections of the deciduous
92 dental crowns. Weaning onset was investigated via Sr/Ca profiles on the same
93 histological sections along the enamel-dentine junction (EDJ) by laser-ablation

94 inductively-coupled-plasma mass spectrometry (LA-ICPMS) (15). In order to detect
95 mobility and/or potential non-local food sources in maternal diet, $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio
96 profiles were measured by LA-multi-collector-ICPMS (see Materials and Methods) (16,
97 17). Moreover, we evaluated elemental ratio profiles in teeth from children with known
98 life history (*SI Appendix*, Text S3, (15)).

99
100 **Results**

101
102 The neonatal lines marking birth were visible in all four archaeological specimens,
103 despite their worn crowns (*SI Appendix*, Fig. S1), allowing the precise estimation of
104 postnatal crown formation times (Fig. 2a). The deciduous first molar Nadale 1 and the
105 deciduous canine Riparo Broion 1 lie within the modern human variability (27-30), while
106 the second deciduous molar Fumane 1 shows a shorter postnatal crown formation time
107 compared with the known archaeological and modern human range (27). The UPMH
108 Fumane 2 deciduous lateral incisor postnatal crown formation time falls into the lower
109 limit of the modern human range (28, 30). Overall, the enamel growth rates and the time
110 to form postnatal enamel compares well with modern human data, regardless of
111 differences in their relative tissue volumes and morphologies (7-9).

112 Daily enamel secretion rates (DSRs) of all specimens, collected in the first 100 μm layer
113 along the EDJ where laser tracks were run, are reported in Figure 2b, compared with
114 range of variation (min., mean, max.) of modern humans (27-30). Neanderthal DSRs in
115 the first 100 μm of the enamel layer are slower than the corresponding modern human
116 range of variability. However, when the entire dental crown is considered, the
117 distributions of Neanderthal DSRs lie within the lower variability ranges of modern
118 humans (Fig. 2c). The UPMH Fumane 2 DSRs fit the lower portion of the modern human
119 ranges (Fig. 2b,c). The postnatal crown formation times in Neanderthals couple with
120 slower DSRs than in modern humans, as expected given the thinner enamel in
121 Neanderthals' permanent and deciduous teeth (31, 32).

122

123 **[Insert Figure 2 here]**

124 **Fig. 2. Dental crown growth parameters.** (a) Postnatal crown formation time in days
125 from birth for the four investigated fossil deciduous teeth relative to the range of
126 variability reported in literature for modern and archaeological individuals (red, blue,
127 green lines). (b) Boxplot of the daily secretion rate (DSR) variation *in the first 100 μm*
128 from the enamel-dentine-junction (min., second quartile, median, third quartile, max.) in
129 comparison to the corresponding variability (min., mean, max.) of modern humans (MH),
130 re-assessed from (27-30). (c) Boxplot of the daily secretion rate variation *across the*
131 *whole crown* (min., second quartile, median, third quartile, max.) and range of variation
132 (min., mean, max.) of modern humans (MH), re-assessed from (27-30). Ldm1 = lower
133 deciduous first molar; Ldm2 = lower deciduous second molar; Udc = upper deciduous
134 canine; Ldi2 = lower deciduous later incisor.

137 Weaning onset was determined using the topographical variation of the Sr/Ca ratio along
138 the EDJ (15) (Fig. 3a, *SI Appendix*, Text S3). In exclusively breastfed newborns, the
139 enamel Sr/Ca ratio is markedly lower relative to their prenatal levels (15, 33, 34). This is
140 because human milk is highly enriched in Ca, i.e. Ca is selectively transferred, compared
141 to Sr, across the mammary glands and the placenta (35, 36). Such behavior is confirmed
142 by analyses of breastmilk and infant sera (37). In comparison to human, herbivore milk
143 (and derived formula) is characterized by higher Sr/Ca levels, due to the lower initial
144 trophic position (38). Our dietary model for early life (Fig. 3a, *SI Appendix*, Text S3)
145 agrees with the expected Sr behavior (15, 34, 39), showing a decrease in Sr/Ca during
146 exclusive breastfeeding and changes in the slope of the profile across the major dietary
147 transitions (i.e. introduction of solid food and end of weaning; for additional discussion
148 see *SI Appendix*, Text S3) (34). This model has been tested successfully in this study on a
149 set of contemporary children's teeth with known dietary histories, including their
150 mothers' eating habits (*SI Appendix*, Text S3 and Fig. S6-S8). Alternative literature
151 models for Ba/Ca point to an increase of Ba/Ca in postnatal enamel during breastfeeding
152 (11, 12); yet, due to even stronger discrimination across biological membranes, Ba/Ca
153 behavior is expected to be similar to Sr/Ca (34), as indeed unequivocally observed here
154 (*SI Appendix*, Text S3 and Fig. S6-S8) and elsewhere (15, 40-42).

155

156 **[Insert Figure 3 here]**

157 **Fig. 3. Nursing histories from time-resolved Sr/Ca variation in Middle-Upper**
158 **Paleolithic deciduous teeth.** NEA = Neanderthal; UPMH = Upper Paleolithic modern
159 human. The elemental profiles (Sr/Ca; Ba/Ca for comparison) were analyzed within
160 enamel closest to the enamel-dentine junction (EDJ); [U] is reported as diagenetic
161 alteration proxy for all fossil specimens (15) (see *SI Appendix*, Text S4, Fig. S13);
162 diagenetically-affected sections are greyed-out. All are plotted relative to secretion time
163 (in days); the birth event is highlighted by a vertical line in each plot. Elemental ratios are
164 reported mass (weight)-based, not as mol/mol (15). The compositional profiles were
165 smoothed with a locally weighted polynomial regression fit (LOWESS), with its
166 associated standard error range (± 3 S.E.) for each predicted value. (a) Comparison
167 between two contemporary individuals with known feeding histories, MCS1 (exclusively
168 breastfed) and MCS2 (exclusively formula-fed); t1=transitional period, i.e. first time
169 solid food starts; t2=progressively reduced breastfeeding during day; t3=transitional
170 period ends, end of breastfeeding. (b) Nadale 1: the slight decrease of Sr/Ca indicates
171 exclusive breastfeeding until the end of crown formation (4.7 months). (c) Fumane 1:
172 Sr/Ca variation indicates breastfeeding until 4 months of age (fully comparable with
173 MCS1 sample, see Supplementary Figure S6). (d) Riparo Broion 1: Sr/Ca profile
174 indicates exclusive breastfeeding until 5 months of age. (e) Fumane 2: 55 days of
175 available postnatal enamel shows exclusive breastfeeding. (f) Comparative Sr/Ca profiles
176 of all fossil specimens adjusted to the birth event; the interpolated modelled profiles were
177 calculated based on those portions unaffected by diagenesis ([U]< 0.05 ppm), with strong
178 smoothing parameters to reveal the biogenic signal. Riparo Broion 1, the specimen most
179 affected by diagenesis, retains the overall outline of a breastfeeding signal (see panel a).
180 See Material and Methods section for details.

181
182

183 Nadale 1 (Fig. 3b), Fumane 1 (Fig. 3c) and Fumane 2 (Fi. 3e) are sufficiently well-
184 preserved from a geochemical point of view. Riparo Broion 1 (Fig. 3d) in contrast shows
185 some diagenetic overprint, but the overall biogenic elemental pattern can still be
186 discerned (Fig. 3f, where only the portions with [U]<0.05 ppm are included in the
187 interpolated profiles). Overall, Ba is more diagenetically affected than Sr (see *SI*
188 *Appendix*, Text S4 for our diagenesis assessment strategy and detailed description of the
189 diagenetic overprints).

190 Two out of the three Neanderthals, Fumane 1 and Riparo Broion 1, clearly show a
191 decreasing trend in Sr/Ca ratio immediately post-birth, followed by slope changes with
192 the first introduction of non-breastmilk food at 115 days (3.8 months) and 160 days (5.3
193 months; Fig. 3c,d), respectively. An even stronger signal of transitional food intake is
194 visible in Fumane 1 at 200 days (6.6 months) in the form of a steep increase in Sr/Ca

195 ratio. For the oldest Neanderthal specimen Nadale 1, following a marked variability
196 before birth, the Sr/Ca profile slightly decreases until 140 days (4.7 months, Fig. 3b). We
197 cannot determine the weaning onset for this individual, who was still being exclusively
198 breastfed by ~5 months of life. The UPMH Fumane 2 has a substantial portion of the
199 prenatal enamel preserved and only a short postnatal enamel growth record (~85 days vs
200 ~55 days respectively, Fig. 3e). This precludes the chemical detection of the onset of
201 weaning, although the Sr/Ca drop at birth clearly indicates breastfeeding. The prenatal
202 Sr/Ca increase in Fumane 2 could be related to changing dietary habits of the mother
203 during pregnancy. A similar trend in prenatal enamel is observable in MCS2 (Figure 3a),
204 whose mother followed a diet poor in meat during pregnancy. The Sr isotope profiles of
205 all investigated teeth show very limited intra-sample variability, confirming that Sr/Ca
206 variations likely relate to changes in dietary end-members rather than diverse
207 geographical provenance of food sources (Fig. 4). These data also give insights in
208 Neanderthal mobility and resource gathering. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of all Neanderthal teeth
209 mostly overlap with the respective local baselines, defined through archaeological
210 micromammals (43). This suggests that the mothers mostly exploited local food
211 resources. Fumane 1 and Fumane 2, both from the same archaeological site, are
212 characterized by contrasting mean $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (0.7093 vs 0.7088), indicative of a
213 different use of resources between Neanderthal (local resources) and early UPMH (non-
214 local resources). Such behavior might have been driven by climatic fluctuations,
215 suggesting colder conditions at ~40 ka, dominated by steppe and Alpine meadows (44).

216

217 **[Insert Figure 4 here]**

218 **Fig. 4. Mobility of the Middle-Upper Paleolithic infants via time-resolved $^{87}\text{Sr}/^{86}\text{Sr}$**
219 **profiles of their deciduous teeth.** Grey horizontal bands represent the local Sr isotopic
220 baselines defined via the Sr isotopic composition of archaeological rodent enamel (*SI*
221 *Appendix*, Table S1). The birth event is indicated by a vertical line. (a,b) Nadale 1 /
222 Fumane 1: exploitation of local food resources through the entire period; (c) Riparo
223 Broion 1: possible limited seasonal mobility (non-local values between c. -45 and 85
224 days, ~4 months); (d) Fumane 2: exploitation of non-local food resources through the
225 entire period.

226

227 **Discussion**

228

229 Nursing strategies are strictly linked to fertility rates, maternal energetic investment,
230 immune development and infant mortality (45). All of these ultimately contribute to
231 demographic changes of a specific population, with key relevance to the study of human
232 evolution. Prolonged exclusive breastfeeding has a positive impact on an infant's immune
233 system; however, longer breastfeeding negatively influences women's fertility via
234 lactational amenorrhea and thus inter-birth intervals (46). It has been shown that the age
235 peak for weaning onset is reached at around 2.1 times birth weight (47), implying that
236 infants who grow more rapidly need to be weaned earlier than those with a slower pace of
237 growth. Based on modern models, a sustainable timing for infant weaning onset would
238 thus range between 3 and 5 months of age (4). However, contemporary non-industrial
239 societies start weaning their children at a modal age of 6 months (6). Similarly, the World
240 Health Organization recommends exclusive breastfeeding for the first six months of an
241 infant's life (48). This time frame broadly corresponds to the age at which the masticatory
242 apparatus develops, favoring the chewing of first solid foods (4). Such evidence suggests
243 that both skeletal development and infant energy demand contribute to the beginning of
244 the weaning transition. Introduction of non-breastmilk foods is also crucial in reducing
245 the energetic burden of lactation for the mother (6). Breastfeeding represents a substantial
246 investment of energy resources (total caloric content of modern human breastmilk \approx 60
247 kcal/100 mL) (49), entailing an optimal energy allocation between baby feeding and other
248 subsistence-related activities.

249 Our time-resolved chemical data point to an introduction of non-breastmilk foods at \sim 5-6
250 months in the infant diet of two Neanderthals, sooner than previously observed (11, 12)
251 and fully within the modern human pre-industrial figures (6). Neanderthals, therefore,
252 were capable of being weaned at least from the fifth post-natal month in terms of
253 supplementing the nutritional requirements of an infant that is growing a large brain with
254 high energy requirements. This evidence, combined with deciduous dental growth akin to
255 modern humans, indicates similar metabolic constraints for the two taxa during early life.
256 The differential food exploitation of Fumane 1 and Fumane 2 mothers suggests a
257 different human-environment interaction between Neanderthals and early UPMHs, as

258 seen in Sr isotope profiles. The UPMH Fumane 2 mother was consuming low-biopurified
259 non-local foodstuff with elevated Sr/Ca and possibly spent the end of her pregnancy and
260 the first 55 days after delivery away from the Fumane site. The most parsimonious
261 interpretation is that mother and child of Fumane 2 likely lived away from Fumane Cave
262 and that, many years after, the UPMH child lost his tooth at Fumane Cave, away from his
263 original birthplace. Conversely, all Neanderthal mothers spent the last part of their
264 pregnancies and the lactation periods locally and were consuming high-biopurified local
265 food (see low Sr/Ca-values in Fig. 3f). Such evidence of a seeming limited mobility for
266 these Neanderthals women counters previous hypotheses of a large home-range of
267 Neanderthals (50, 51).

268 The introduction of non-breastmilk food at ~5-6 months implies relatively short inter-
269 birth intervals for Neanderthals due to an earlier resumption of post-partum ovulation
270 (52). Moreover, considering the birth weight model (47), we hypothesize that
271 Neanderthal newborns were of similar weight to modern human neonates, pointing to a
272 likely similar gestational history and early-life ontogeny. In a broader context, our results
273 suggest that nursing mode and time among Late Pleistocene humans in Europe were
274 likely not influenced by taxonomic differences in physiology. Therefore, our findings do
275 not support the hypothesis that long postpartum infertility was a contributing factor to the
276 demise of Neanderthals (13). On the other hand, genetic evidence indicates that
277 Neanderthal groups were limited in size (53), which is not in agreement with the shorter
278 inter-birth interval proposed here. Thus, other factors such as e.g. cultural behavior,
279 shorter life-span and high juvenile mortality might have played a focal role in limiting
280 Neanderthal's group size (54, 55).

281

282

283 **Materials and Methods**

284

285 **Thin slices of teeth preparation**

286 Prior to sectioning, a photographic record of the samples was collected. Thin sections of
287 the dental crowns were obtained using the standard method in dental histology described
288 in (56, 57) and prepared at the Service of Bioarchaeology of the Museo delle Civiltà in
289 Rome. The sectioning protocol consists of a detailed embedding-cutting-mounting

290 procedure that makes use of dental adhesives, composite resins, and embedding resins. In
291 order to be able to remove the crown from the resin block after sectioning and to restore
292 the dental crowns, the teeth were initially embedded with a reversible resin (Crystalbond
293 509, SPI Supplies) that does not contaminate chemically the dental tissues and is soluble
294 in Crystalbond cleaning agent (Aramco Products, Inc.). A second embedding in epoxy
295 resin (EpoThin 2, Buehler Ltd) guarantees the stability of the sample during the cutting
296 procedure. The sample was cured for 24 hours at room temperature. Teeth were sectioned
297 using an IsoMet low speed diamond blade microtome (Buehler Ltd). After the first cut, a
298 microscope slide previously treated with liquid silane (3 M RelyX Ceramic Primer) was
299 attached on the exposed surface using a light curing adhesive (3M Scotchbond Multi-
300 Purpose Adhesive) to prevent cracks and any damage during the cutting procedure. A
301 single longitudinal bucco-lingual thin section, averaging 250 μm thick, was cut from each
302 specimen. Each ground section was reduced to a thickness of ~ 150 μm using water
303 resistant abrasive paper of different grits (Carbimet, Buehler Ltd). Finally, the sections
304 were polished with a micro-tissue (Buehler Ltd) and diamond paste with 1 μm size (DB-
305 Suspension, M, Struers).

306 Each thin section was digitally recorded through a camera (Nikon DSFI3) paired with a
307 transmitted light microscope (Olympus BX 60) under polarized light, with different
308 magnifications (40x, 100x, 400x, including the ocular magnifications). Overlapping
309 pictures of the dental crown were assembled in a single micrograph using the software
310 ICE 2.0 (Image Composite Editor, Microsoft Research Computational Photography
311 Group) (*SI Appendix*, Fig. S1).

312 After sectioning, the crowns were released from the epoxy block using the Crystalbond
313 cleaning agent and reconstructed using light curing dental restoration resin (Heraeus
314 Charisma Dental Composite Materials).

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

316 To determine local Sr isotope baselines we analyzed archaeological rodent teeth from the
317 same sites where the human teeth were found (*SI Appendix*, Table S1). Samples were
318 prepared at the Department of Chemical and Geological Sciences of the University of

319 Modena and Reggio Emilia, following protocols described elsewhere (16, 58) and briefly
320 summarized here.

321 From each archaeological site we selected several rodent teeth, according to the
322 stratigraphic distribution of human samples. Enamel from micromammal incisors was
323 manually removed using a scalpel. Few teeth were also analyzed as whole (dentine +
324 enamel). Before the actual dissolution with 3M HNO₃, samples (1-5 mg in mass) were
325 washed with MilliQ (ultrasonic bath) and leached with ~0.5 M HNO₃. Sr of the dissolved
326 specimens was separated from the matrix using 30 µl columns and Eichrom Sr-Spec
327 resin.

328 Sr isotope ratios were measured using a Neptune (ThermoFisher) multi-collector
329 inductively-coupled-plasma mass spectrometer (MC-ICPMS) housed at the Centro
330 Interdipartimentale Grandi Strumenti (UNIMORE) during different analytical sessions.
331 Seven Faraday detectors were used to collect signals of the following masses: ⁸²Kr, ⁸³Kr,
332 ⁸⁴Sr, ⁸⁵Rb, ⁸⁶Sr, ⁸⁷Sr, ⁸⁸Sr. Sr solutions were diluted to ~50 ppb and introduced into the
333 Neptune through an APEX desolvating system. Corrections for Kr and Rb interferences
334 follow previous works (16). Mass bias corrections used an exponential law and a ⁸⁸Sr/⁸⁶Sr
335 ratio of 8.375209 (59). The Sr ratios of samples were reported to a SRM987 value of
336 0.710248 (60). During one session, SRM987 yielded an average ⁸⁷Sr/⁸⁶Sr ratio of
337 0.710243 ± 0.000018 (2 S.D., n = 8). Total laboratory Sr blanks did not exceed 100 pg.

338 **Spatially-resolved Sr isotopic analysis by laser-ablation plasma mass spectrometry**
339 **(LA-MC-ICPMS)**

340 LA-MC-ICPMS analyses were conducted at the Frankfurt Isotope and Element Research
341 Center (FIERCE) at Goethe University, Frankfurt am Main (Germany) and closely follow
342 analytical protocols described by Müller & Anczkiewicz (2016) (17); only a brief
343 summary is provided here aiming at highlighting project-specific differences. A 193 nm
344 ArF excimer laser (RESOLUTION S-155, formerly Resonetics, ASI, now Applied Spectra
345 Inc.) equipped with a two-volume LA cell (Laurin Technik) was connected to a
346 NeptunePlus (ThermoFisher) MC-ICPMS using nylon6-tubing and a 'squid' signal-
347 smoothing device (61). Ablation took place in a He atmosphere (300 ml/min), with ~1000

348 ml/min Ar added at the funnel of the two-volume LA cell and 3.5 ml/min N₂ before the
349 squid. Laser fluence on target was ~5 J/cm².

350 Spatially-resolved Sr isotopic analyses of dental enamel were performed on the thin
351 sections (100-150 μm thick) used for enamel histology and trace element analysis (see
352 below), in continuous profiling mode following the enamel-dentine-junction (EDJ) from
353 apex to cervix (14), less than 100 μm away from the EDJ. Tuning of the LA-MC-ICPMS
354 used NIST 616 glass for best sensitivity (⁸⁸Sr) while maintaining robust plasma
355 conditions, i.e. ²³²Th¹⁶O/²³²Th <0.5% and ²³²Th/²³⁸U>0.95 with RF-power of ~1360 W.
356 In view of the low Sr concentrations in these human enamel samples (~60-100 μg/g), we
357 utilized 130 μm spots, a scan speed of 5 μm/s and a repetition rate of 20 Hz to maintain
358 ⁸⁸Sr ion currents of ~2-3.5 x 10⁻¹¹ A. Nine Faraday detectors were used to collect the ion
359 currents of the following masses (m/z): ⁸³Kr, ~83.5, ⁸⁴Sr, ⁸⁵Rb, ⁸⁶Sr, ~86.5, ⁸⁷Sr, ⁸⁸Sr,
360 ⁹⁰Zr. Baseline, interference and mass bias corrections follow (17). The isotopically-
361 homogenous (Sr) enameloid of a modern shark was used to assess accuracy of the Sr-
362 isotopic analysis and yielded ⁸⁷Sr/⁸⁶Sr = 0.70916 ± 2 and ⁸⁴Sr/⁸⁶Sr = 0.0565 ± 1 (2 S.D.).
363 Raw data are reported in Dataset S1.

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

366 All LA-ICPMS analyses of archaeological samples were conducted at the Frankfurt
367 Isotope and Element Research Center (FIERCE) at Goethe University, Frankfurt am
368 Main (Germany), using the same LA system described above, but connected via a squid
369 smoothing-device to an Element XR ICPMS. Analytical protocols follow those by Müller
370 et al (2019) (15); and only a brief summary is provided here aimed at highlighting
371 differences. LA-ICPMS trace element ratios/concentrations of the comparative
372 contemporary teeth were obtained at Royal Holloway University of London (RHUL)
373 using the RESOLUTION M-50 prototype LA system featuring a Laurin two-volume LA cell
374 (58), coupled to an Agilent 8900 triple-quadrupole-ICPMS (ICP-QQQ or ICP-MS/MS).
375 Compositional profiles were analyzed parallel and as close as possible to the EDJ,
376 following the same tracks used for Sr isotope analyses. We employed 15 μm spot sizes
377 (FIERCE) or 6 μm (MCS3, RHUL) and 34 μm (MCS1 and 2, RHUL), respectively, as

378 well as a scan speed of 5 $\mu\text{m/s}$ and a repetition rate of 15 Hz; prior to acquisition, samples
379 were pre-cleaned using slightly larger spot sizes (22 - 57 μm), 20 Hz and faster scan
380 speeds (25 - 50 $\mu\text{m/s}$); laser fluence was $\sim 5 \text{ J/cm}^2$. The following isotopes (m/z) were
381 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 ,
382 ^{172}Yb), ^{208}Pb , ^{238}U . The total sweep times for the Element XR and the 8900 ICP-MS/MS
383 were ~ 0.8 and 0.4-0.5 s, respectively; however, because of the slow scan speeds, this
384 small difference has no effect on the compositional profiles presented here. Primary
385 standardization was achieved using NIST SRM612. Ca was employed as internal
386 standard (^{43}Ca); [Ca] at 37 %m/m was used to calculate concentrations for unknown
387 bioapatites, although not required for X/Ca ratios. Accuracy and reproducibility were
388 assessed using repeated analyses of the STDP-X-glasses (62) as secondary reference
389 materials; the respective values for Sr/Ca and Ba/Ca (the element/Ca ratios of principal
390 interest) here are $1.8 \pm 6.6\%$ and $-0.2 \pm 6.0\%$ (%bias ± 2 S.D. (%)); this compares well
391 with the long-term reproducibility for these analytes reported previously (63). Raw data
392 are reported in Dataset S2 and S3.

393 The compositional/isotopic profiles were smoothed with a locally weighted polynomial
394 regression fit (LOWESS), with its associated standard error range (± 3 S.E.) for each
395 predicted value (64). The statistical package R (ver. 44.0.0) (65) was used for all
396 statistical computations and generation of graphs.

397 **Assessment of the enamel growth parameters and of the chronologies along the laser** 398 **tracks**

399 Dental enamel is capable of recording, at microscopic level during its formation, regular
400 physiological and rhythmic growth markers (66-68). These incremental markings are
401 visible under transmitted light in longitudinal histological thin sections of dental crowns.
402 Enamel forms in a rhythmic manner, reflecting the regular incremental secretion of the
403 matrix by the ameloblasts (i.e. the enamel forming cells). The rhythmical growth of
404 enamel is expressed in humans at two different levels: a circadian rhythm that produces
405 the daily cross striations(69, 70) and a longer period rhythmic marking (near- weekly in
406 humans) that give rise to the Retzius lines (71). Physiological stresses affecting the
407 individual during tooth growth cause a disruption of the enamel matrix secretion and

408 mark the corresponding position of the secretory ameloblast front, producing Accentuated
409 (Retzius) Lines (ALs) (72, 73). The birth event is recorded in the forming enamel of
410 individuals surviving the perinatal stage, and leaves - usually the first - Accentuated Line,
411 namely the Neonatal Line (NL) (26, 74, 75).

412 The time taken to form the dental crown after birth was measured on each thin section
413 adapting the methods described in literature (30, 76).

414 A prism segment starting from the most apical available point on the enamel dentine
415 junction (EDJ) and extending from this point to an isochronous incremental line (i.e. the
416 NL, an AL or a Retzius line) was measured. The incremental line was followed back to
417 the EDJ and a second prism segment was measured in the same way. The process was
418 repeated until the most cervical enamel was reached. The crown formation time is equal
419 to the sum of the single prism segments. To obtain time (in days) from the prism length
420 measurements, local daily secretion rates (25) (DSR) were calculated around the prism
421 segments and within 100 μm from the EDJ, by counting visible consecutive cross
422 striations and dividing it by the corresponding prism length. The chronologies of
423 accentuated lines (ALs) in the modern sample closely match the timing of known
424 disruptive life history events in the mother (illness, surgery) and infant, and so are well
425 within the range or error (1.2-4.4%) observed for this histological ageing method (67).

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

429 After LA-ICPMS analyses, a micrograph highlighting the laser tracks was acquired at
430 50x magnification. This was superimposed to a second micrograph of the same thin
431 section at 100x magnification, to gain better visibility of the enamel microstructural
432 features. The chronologies along the laser tracks were obtained matching the tracks with
433 the isochronous lines.

434

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436

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