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## Main Manuscript for

### Early life of Neanderthals

Alessia Nava<sup>a,b,1,2</sup>, Federico Lugli<sup>c,d,1,2</sup>, Matteo Romandini<sup>c,e</sup>, Federica Badino<sup>c,f</sup>, David Evans<sup>g,h</sup>, Angela H. Helbling<sup>g,h</sup>, Gregorio Oxilia<sup>c</sup>, Simona Arrighi<sup>c</sup>, Eugenio Bortolini<sup>c</sup>, Davide Delpiano<sup>i</sup>, Rossella Duches<sup>j</sup>, Carla Figus<sup>c</sup>, Alessandra Livraghi<sup>i,k</sup>, Giulia Marciani<sup>c</sup>, Sara Silvestrini<sup>c</sup>, Anna Cipriani<sup>d,l</sup>, Tommaso Giovanardi<sup>d</sup>, Roberta Pini<sup>f</sup>, Claudio Tuniz<sup>m,n,o</sup>, Federico Bernardini<sup>m,n</sup>, Irene Dori<sup>p,q</sup>, Alfredo Coppa<sup>r,s,t</sup>, Emanuela Cristiani<sup>a</sup>, Christopher Dean<sup>u,v</sup>, Luca Bondioli<sup>w,x</sup>, Marco Peresani<sup>f,i,1</sup>, Wolfgang Müller<sup>g,h,1</sup>, Stefano Benazzi<sup>c,y,1</sup>

<sup>a</sup>DANTE - Diet and ANcient TEchnology Laboratory, Department of Maxillo-Facial Sciences, Sapienza University of Rome, Rome, Italy <sup>b</sup>Skeletal Biology Research Centre, School of Anthropology and Conservation, University of Kent, Canterbury, UK <sup>c</sup>Department of Cultural Heritage, University of Bologna, Ravenna, Italy <sup>d</sup>Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy <sup>e</sup>Pradis Cave Museum, Clauzetto, Italy <sup>f</sup>Institute of Environmental Geology and Geoengineering - IGAG CNR <sup>g</sup>Institute of Geosciences, Goethe University Frankfurt, Frankfurt am Main, Germany <sup>h</sup>Frankfurt Isotope and Element Research Center (FIERCE), Goethe University Frankfurt, Frankfurt am Main, Germany <sup>i</sup>Department of Humanities, University of Ferrara, Italy <sup>j</sup>Prehistory Section - MuSe, Museum of Science, Trento, Italy <sup>k</sup>University Rovira i Virgili, Tarragona, Spain <sup>l</sup>Lamont-Doherty Earth Observatory of Columbia University, 61 Route 9W, Palisades NY 10964-1000 USA <sup>m</sup>Abdus Salam International Centre for Theoretical Physics, Trieste, Italy <sup>n</sup>Centro Fermi, Museo Storico della Fisica e Centro di Studi e Ricerche Enrico Fermi, Roma, Italy <sup>o</sup>Center for Archaeological Science, University of Wollongong, Wollongong, NSW, Australia <sup>p</sup>Soprintendenza Archeologia, Belle Arti e Paesaggio per le province di Verona, Rovigo e Vicenza, Italy <sup>q</sup>Department of Biology, Laboratory of Anthropology, University of Florence, Florence, Italy <sup>r</sup>Department of Environmental Biology, Sapienza University of Rome, Rome, Italy <sup>s</sup>Department of Genetics, Harvard Medical School, Boston, MA 02115, USA <sup>t</sup>Department of Evolutionary Anthropology, University of Vienna, Vienna, Austria <sup>u</sup>Department of Earth Sciences, Natural History Museum, London, UK <sup>v</sup>Department of Cell and Developmental Biology, University College London, London, UK <sup>w</sup>Bioarchaeology Service, Museum of Civilization, Rome, Italy <sup>x</sup>Department of Cultural Heritage, University of Padua, Padua, Italy <sup>y</sup>Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

<sup>1</sup>To whom correspondence may be addressed. Email: alessia.nava@uniroma1.it; federico.lugli6@unibo.it; marco.peresani@unife.it; w.muller@em.uni-frankfurt.de; stefano.benazzi@unibo.it

<sup>2</sup>These authors contributed equally to this work.

## Classification

Biological Sciences, Anthropology

Physical Sciences, Geology

## **Keywords**

Neanderthal ontogeny, nursing strategy, dental histology, spatially-resolved chemical analyses, life histories, Sr/Ca.

## **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  $\mu\text{m}$  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  $\mu\text{m}$  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  $\mu\text{m}$*   
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.  
135  
136

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 ( $\pm 3$  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  $\mu\text{m}$  thick, was cut from each  
302 specimen. Each ground section was reduced to a thickness of  $\sim 150 \mu\text{m}$  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  $\mu\text{m}$  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 HNO<sub>3</sub>, samples (1-5 mg in mass) were  
325 washed with MilliQ (ultrasonic bath) and leached with ~0.5 M HNO<sub>3</sub>. 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: <sup>82</sup>Kr, <sup>83</sup>Kr,  
332 <sup>84</sup>Sr, <sup>85</sup>Rb, <sup>86</sup>Sr, <sup>87</sup>Sr, <sup>88</sup>Sr. 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 <sup>88</sup>Sr/<sup>86</sup>Sr  
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 <sup>87</sup>Sr/<sup>86</sup>Sr 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 N<sub>2</sub> before the  
349 squid. Laser fluence on target was ~5 J/cm<sup>2</sup>.

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 (<sup>88</sup>Sr) while maintaining robust plasma  
355 conditions, i.e. <sup>232</sup>Th<sup>16</sup>O/<sup>232</sup>Th <0.5% and <sup>232</sup>Th/<sup>238</sup>U>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 <sup>88</sup>Sr ion currents of ~2-3.5 x 10<sup>-11</sup> A. Nine Faraday detectors were used to collect the ion  
359 currents of the following masses (m/z): <sup>83</sup>Kr, ~83.5, <sup>84</sup>Sr, <sup>85</sup>Rb, <sup>86</sup>Sr, ~86.5, <sup>87</sup>Sr, <sup>88</sup>Sr,  
360 <sup>90</sup>Zr. 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 <sup>87</sup>Sr/<sup>86</sup>Sr = 0.70916 ± 2 and <sup>84</sup>Sr/<sup>86</sup>Sr = 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  $\mu\text{m}$ ), 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:  $^{25}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{43}\text{Ca}$ , ( $^{44}\text{Ca}$ ),  $^{55}\text{Mn}$ ,  $^{66}\text{Zn}$ ,  $^{85}\text{Rb}$ , ( $^{86}\text{Sr}$ ),  $^{88}\text{Sr}$ ,  $^{89}\text{Y}$ ,  $^{138}\text{Ba}$ ,  $^{140}\text{Ce}$ , ( $^{166}\text{Er}$ ,  
382  $^{172}\text{Yb}$ ),  $^{208}\text{Pb}$ ,  $^{238}\text{U}$ . The total sweep times for the Element XR and the 8900 ICP-MS/MS  
383 were  $\sim 0.8$  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 ( $^{43}\text{Ca}$ ); [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  $\pm 2$  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 ( $\pm 3$  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  $\mu\text{m}$  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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