

### ARCHIVIO ISTITUZIONALE DELLA RICERCA

### Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Early life of Neanderthals

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Early life of Neanderthals / Nava, Alessia; Lugli, Federico; Romandini, Matteo; Badino, Federica; Evans, David; Helbling, Angela H; Oxilia, Gregorio; Arrighi, Simona; Bortolini, Eugenio; Delpiano, Davide; Duches, Rossella; Figus, Carla; Livraghi, Alessandra; Marciani, Giulia; Silvestrini, Sara; Cipriani, Anna; Giovanardi, Tommaso; Pini, Roberta; Tuniz, Claudio; Bernardini, Federico; Dori, Irene; Coppa, Alfredo; Cristiani, Emanuela; Dean, Christopher; Bondioli, Luca; Peresani, Marco; Müller, Wolfgang; Benazzi, Stefano. - In: ARGATERAYOGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. - ISSN PARTARIA IS ALTIMATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. - ISSN

Published:

DOI: http://doi.org/10.1073/pnas.2011765117

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/). When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Nava A, Lugli F, Romandini M, Badino F, Evans D, Helbling A, Oxilia G, Arrighi S, Bortolini E, Delpiano D, Duches R, Figus C, Livraghi A, Marciani G, Silvestrini S, Ci priani A, Giovanardi T, Pini R, Nannini N, Tuniz C, Bernardini F, Dori I, Coppa A, Cristiani E, Dean C, Bondioli L, Peresani M, Müller W, Benazzi S (2020) Early life of Neanderthals. Proceedings of the National Academy of Sciences 117 (46) 28719-28726.

The final published version is available online at: https://doi.org/10.1073/pnas.2011765117

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<u>https://cris.unibo.it/</u>)

When citing, please refer to the published version.

### **Main Manuscript for**

### **Early life of Neanderthals**

Alessia Nava<sup>a,b,c,1,2</sup>, Federico Lugli<sup>d,e,1,2</sup>, Matteo Romandini<sup>d,f</sup>, Federica Badino<sup>d,g</sup>, David Evans<sup>h,i</sup>, Angela H. Helbling<sup>h,i</sup>, Gregorio Oxilia<sup>d</sup>, Simona Arrighi<sup>d</sup>, Eugenio Bortolini<sup>d</sup>, Davide Delpiano<sup>j</sup>, Rossella Duches<sup>k</sup>, Carla Figus<sup>d</sup>, Alessandra Livraghi<sup>j,l</sup>, Giulia Marciani<sup>d</sup>, Sara Silvestrini<sup>d</sup>, Anna Cipriani<sup>e,m</sup>, Tommaso Giovanardi<sup>e</sup>, Roberta Pini<sup>g</sup>, Claudio Tuniz<sup>n,o,p</sup>, Federico Bernardini<sup>n,o</sup>, Irene Dori<sup>q,r</sup>, Alfredo Coppa<sup>s,t,u</sup>, Emanuela Cristiani<sup>a</sup>, Christopher Dean<sup>v,w</sup>, Luca Bondioli<sup>b,x</sup>, Marco Peresani<sup>j,1</sup>, Wolfgang Müller<sup>h,i,1</sup>, Stefano Benazzi<sup>d,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>Bioarchaeology Service, Museum of Civilization, Rome, Italy <sup>c</sup>Skeletal Biology Research Centre, School of Anthropology and Conservation, University of Kent, Canterbury, UK <sup>d</sup>Department of Cultural Heritage, University of Bologna, Ravenna, Italy <sup>e</sup>Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy <sup>f</sup>Pradis Cave Museum, Clauzetto, Italy gInstitute of Environmental Geology and Geoengineering - IGAG CNR hInstitute of Geosciences, Goethe University Frankfurt, Frankfurt am Main, Germany <sup>i</sup>Frankfurt Isotope and Element Research Center (FIERCE), Goethe University Frankfurt, Frankfurt am Main, Germany <sup>j</sup>Department of Humanities, University of Ferrara, Italy <sup>k</sup>Prehistory Section - MuSe, Museum of Science, Trento, Italy <sup>l</sup>University Rovira i Virgili, Tarragona, Spain mLamont-Doherty Earth Observatory of Columbia University, 61 Route 9W, Palisades NY 10964-1000 USA <sup>n</sup>Abdus Salam International Centre for Theoretical Physics, Trieste, Italy <sup>o</sup>Centro Fermi, Museo Storico della Fisica e Centro di Studi e Ricerche Enrico Fermi, Roma, Italy PCenter for Archaeological Science, University of Wollongong, Wollongong, NSW, Australia <sup>q</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>s</sup>Department of Environmental Biology, Sapienza University of Rome, Rome, Italy 'Department of Genetics, Harvard Medical School, Boston, MA 02115, USA "Department of Evolutionary Anthropology, University of Vienna, Vienna, Austria 'Department of Earth Sciences, Natural History Museum, London, UK "Department of Cell and Developmental Biology, University College London, London, UK \*Department of Cultural Heritage, University of Padua, Padua, Italy \*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.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

### This PDF file includes:

Main Text Figures 1 to 4 Supporting Information

### 1 Abstract

The early onset of weaning in modern humans has been linked to the high nutritional demand of brain development that is intimately connected with infant physiology and growth rate. In Neanderthals, ontogenetic patterns in early life are still debated, with some studies suggesting an accelerated development and others indicating only subtle differences to modern humans. Here we report the onset of weaning and rates of enamel growth using an unprecedented sample set of three late (~70-50 ka) Neanderthals 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 human evolution. There is debate about their pace of growth and early life metabolic 17 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 started to wean children at 5-6 months akin to modern humans, implying similar energy 21 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.

25

28

30

#### 26 27 Main Text

### 29 Introduction

Maternal physiology, breastfeeding and the first introduction of supplementary foods are key determinants of human growth (1)(1). The high nutritional demands of the human brain during the first years of life has been identified as the main reason for the early weaning onset in modern humans (2). Indeed, supplementary food is needed when an infant's nutritional requirements exceeds what the mother can provide through breastmilk only (3), an event that in contemporary non-industrial human societies occurs at a modal age of 6 months (4).

At present, our knowledge about the link between the pace of child growth, maternal behavior and the onset of weaning among Neanderthals is still scarce. Previous work reported that Neanderthal tooth crowns tend to develop faster than in modern humans,

suggesting infant growth was generally accelerated (5). Other earlier work suggested that 41 Neanderthal brain size was comparable to modern humans at birth, but that growth rates 42 in early infancy were higher (6). It has also been shown (7) that Neanderthals followed 43 modes of endocranial development largely similar to modern humans. However, a 44 permanent first molar and a second deciduous molar from La Chaise (France, 127-116 ka 45 and <163 ka respectively) placed rates of Neanderthal tooth growth within the range of 46 47 modern humans (8). Equally, the association between dental and skeletal growth in a 7year-old Neanderthal from El Sidròn (Spain, 49 ka) indicated that Neanderthals and 48 modern humans were similar in terms of ontogenetic development, with only small-scale 49 50 dissimilarities in acceleration or deceleration of skeletal maturation (9). Ba/Ca maps of 51 permanent tooth sections of two early Neanderthals have been interpreted 52 (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 53 modal age in modern humans today. Similarly, wear stage analyses of a large number of 54 55 deciduous dentitions suggested that introduction of solid food in Neanderthals was delayed by one year compared to modern humans (12). 56

Here we investigate such key aspects of early life in Neanderthals by combining new data 57 on chemical detection of weaning onset with deciduous enamel growth rates. We utilize 58 dental histomorphometry (8, 13), spatially-resolved chemical (14) and isotopic profiles 59 (15, 16) of dental enamel to reconstruct growth rates (13), nursing practices (3) and 60 mobility (15) during the Middle and Upper Paleolithic at high (up to weekly) time 61 resolution. We analyzed an unprecedented set of teeth (n = 4) (SI Appendix, Text S1) 62 from adjacent archaeological sites in Northeastern Italy (SI Appendix, Text S2), dated 63 from the Late Middle to the Early Upper Paleolithic, from Neanderthal-modern human 64 contexts (70-40 ka). These four exfoliated deciduous fossil teeth include three 65 Neanderthals (Fumane 1, a lower left deciduous second molar (17), ~50 ka; Nadale 1, a 66 67 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 68 comparative specimen from the Fumane site (Fumane 2, an upper right deciduous second 69 70 incisor (20), Protoaurignacian, ~40 ka) (Fig. 1).

71

### 72 [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 78 time and daily enamel secretion rates (24), and we detected the presence of the neonatal 79 line as birth marker (25) by optical light microscopy on thin sections of the deciduous 80 dental crowns. Chemical weaning was investigated via Element/Ca profiles on the same 81 82 histological sections along the enamel-dentine junction (EDJ) by laser-ablation inductively-coupled-plasma mass spectrometry (LA-ICPMS) (14). In order to detect 83 mobility and/or potential non-local food sources in maternal diet, <sup>87</sup>Sr/<sup>86</sup>Sr isotope ratio 84 profiles were measured by LA-multi-collector-ICPMS (see Materials and Methods) (15, 85 86 16).

- 87 Results
- 88

89 Weaning onset was determined using the topographical variation of the Sr/Ca ratio along 90 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 91 human milk is highly enriched in Ca, i.e. Ca is selectively transferred, compared to Sr, 92 93 across the mammary glands and the placenta (28, 29). Such behavior is confirmed by 94 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 95 96 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 97 98 breastfeeding and changes in the slope of the profile across the major dietary transitions 99 (i.e. introduction of solid food and end of weaning) (27). This model has been tested 100 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). 101 102 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 103 104 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, 105 106 33-35).

107 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 108 postnatal crown formation times (Fig. 2a). The deciduous first molar Nadale 1 and the 109 deciduous canine Riparo Broion 1 lie within the modern human variability (36-39), while 110 111 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 112 Fumane 2 deciduous lateral incisor postnatal crown formation time falls instead in the 113 lower limit of the modern human range (37, 39). Overall, the enamel growth rates and the 114 time to form postnatal enamel compares well with modern human data, regardless of 115 differences in their relative tissue volumes and morphologies (5, 8, 9). 116

Daily enamel secretion rates (DSRs) of all specimens, collected in the 100 µm thickness 117 along the enamel dentine junction where laser tracks were run, are reported in Figure 2b, 118 compared with range of variation (min., mean, max.) of modern humans (36-39). 119 Neanderthal DSRs in the first 100 µm of enamel thickness are slower than the 120 corresponding modern human range of variability. However, when the entire dental 121 crown is considered, the distributions of Neanderthal DSRs lie within the lower 122 123 variability ranges of modern humans (Fig. 2c). The UPMH Fumane 2 DSRs fit the lower portion of the modern human ranges (Fig. 2b,c). The postnatal crown formation times in 124 Neanderthals couple with slower DSRs than in modern humans, as expected given the 125 126 thinner enamel in Neanderthals' permanent and deciduous teeth (40, 41).

127

128 [Insert Figure 2 here]

129

Nadale 1, Fumane 1 & 2 are sufficiently well-preserved from a geochemical point of 130 131 view, Riparo Broion 1 instead shows some diagenetic overprint (overall Ba is far more affected than Sr; see SI Appendix, Text S4 for our diagenesis assessment strategy), but the 132 overall primary elemental signature can still be discerned. Two out of the three 133 Neanderthals, Fumane 1 and Riparo Broion 1, clearly show breastfeeding signals and a 134 135 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 months; Fig. 3). An even stronger signal of transitional food intake is visible in Fumane 1 137 at 200 days (6.6 months) in the form of a steep increase in Sr/Ca ratio. For the oldest 138 Neanderthal specimen Nadale 1, following a marked variability before birth, the Sr/Ca 139 140 profile slightly decreases until 140 days (4.7 months). We cannot determine the weaning onset for this individual, who was still being exclusively breastfed by ~5 months of life. 141 The UPMH Fumane 2 has a substantial portion of the prenatal enamel preserved and only 142 a short postnatal enamel growth record (~85 days vs ~55 days respectively). This 143 144 precludes the chemical detection of the onset of weaning, although the Sr/Ca drop at birth clearly indicates breast-feeding. 145

147 [Insert Figure 3 here]

148

149 The Sr isotope profiles of all investigated teeth show very limited intra-sample variability, confirming that Sr/Ca variations likely relate to changes in dietary end-150 members rather than diverse geographical provenance of food sources (Fig. 4). These 151 data also give insights in Neanderthal mobility and resource gathering. The <sup>87</sup>Sr/<sup>86</sup>Sr 152 ratios of all Neanderthal teeth overlap with the respective local baselines, defined through 153 archaeological micromammals (42). This suggests that the mothers mostly exploited local 154 food resources. Fumane 1 and Fumane 2, both from the same archaeological site, are 155 characterized by contrasting <sup>87</sup>Sr/<sup>86</sup>Sr ratios (0.7094 vs 0.7087), indicative of a different 156 use of resources between Neanderthal (local resources) and early UPMH (non-local 157 resources). Such behavior might have been driven by climatic fluctuations, suggesting 158 colder conditions at  $\sim 40$  ka, dominated by steppe and Alpine meadows (43). 159

160

### 161 [Insert Figure 4 here]

162

### 163 Discussion

164

Nursing strategies are strictly linked to fertility rates, maternal energetic investment, 165 166 immune development and infant mortality (44). All of these ultimately contribute to demographic changes of a specific population, with key relevance to the study of human 167 evolution. Prolonged exclusive breastfeeding has a positive impact on an infant's immune 168 system; however, longer breastfeeding negatively influences women's fertility via 169 170 lactational amenorrhea and thus inter-birth intervals (45). It has been shown that the age peak for weaning onset is reached at around 2.1 times birth weight (46), implying that 171 infants who grow more rapidly need to be weaned earlier than those with a slower pace of 172 growth. Based on modern models, a sustainable timing for infant weaning onset would 173 thus range between 3 and 5 months of age (3). However, contemporary non-industrial 174

175 societies start weaning their children at a modal age of 6 months (4). Similarly, the World Health Organization recommends exclusive breastfeeding for the first six months of an 176 infant's life (47). This time frame broadly corresponds to the age at which the masticatory 177 apparatus develops, favoring the chewing of first solid foods (3). Such evidence suggests 178 that both skeletal development and infant energy demand contribute to the beginning of 179 the weaning transition. Introduction of non-breastmilk foods is also crucial in reducing 180 181 the energetic burden of lactation for the mother (4). Breastfeeding represents a substantial investment of energy resources (total caloric content of modern human breastmilk =~60 182 kcal/100 mL) (48), entailing an optimal energy allocation between baby feeding and other 183 184 subsistence-related activities.

185 Our time-resolved chemical data point to an introduction of non-breastmilk foods at ~5-6 months in the infant diet of two Neanderthals, sooner than previously observed (10, 11) 186 187 and fully within the modern human pre-industrial figures (4). This evidence, combined with deciduous dental growth akin to modern humans, indicates similar metabolic 188 189 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 early UPMHs, as seen in Sr isotope profiles. The Fumane 2 mother spent the end of her 192 193 pregnancy and the first 55 days after delivery away from the site and was consuming lowbiopurified non-local foodstuff with elevated Sr/Ca. Conversely, all Neanderthal mothers 194 195 spent the last part of their pregnancies and the lactation periods locally and were consuming high-biopurified local food due to the low Sr/Ca-values (see Fig. 3e). 196

197 The introduction of non-breastmilk food at ~5-6 months implies relatively short interbirth intervals for Neanderthals due to an earlier resumption of post-partum ovulation 198 (49). Moreover, considering the birth weight model (46), we hypothesize that 199 200 Neanderthal newborns were of similar weight to modern human neonates, pointing to a likely similar gestational history and early-life ontogeny. In a broader context, our results 201 suggest that nursing mode and time among Late Pleistocene humans in Europe were 202 203 likely not influenced by taxonomic differences in physiology. Therefore, our findings do not support the hypothesis that long postpartum infertility was a contributing factor to the 204

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).

210 211

### 212 Materials and Methods213

### 214 Thin slices of teeth preparation

215 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 216 217 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 218 makes use of dental adhesives, composite resins, and embedding resins. In order to be 219 able to remove the crown from the resin block after sectioning and to restore the dental 220 221 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 222 223 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 224 procedure. The sample was cured for 24 hours at room temperature. Teeth were sectioned 225 using an IsoMet low speed diamond blade microtome (Buehler Ltd). After the first cut, a 226 microscope slide previously treated with liquid silane (3 M RelyX Ceramic Primer) was 227 attached on the exposed surface using a light curing adhesive (3M Scotchbond Multi-228 229 Purpose Adhesive) to prevent cracks and any damage during the cutting procedure. A 230 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 231 232 resistant abrasive paper of different grits (Carbimet, Buehler Ltd). Finally, the sections 233 were polished with a micro-tissue (Buehler Ltd) and diamond paste with 1 µm size (DB-234 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

- magnifications (40x, 100x, 400x). Overlapping pictures of the dental crown were
  assembled in a single micrograph using the software ICE 2.0 (Image Composite Editor,
  Microsoft Research Computational Photography Group) (*SI Appendix*, Fig. S1).
- After sectioning, the crowns were released from the epoxy block using the Crystalbond
  cleaning agent and reconstructed using light curing dental restoration resin (Heraeus
  Charisma Dental Composite Materials).

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

To determine local Sr isotope baselines we analyzed archaeological rodent teeth (*SI Appendix*, Table S1). Samples were prepared at the Department of Chemical and Geological Sciences of the University of Modena and Reggio Emilia, following protocols described elsewhere (15, 55) and briefly summarized here.

From each archaeological site we selected several rodent tooth specimens, according to the stratigraphic distribution of human samples. Enamel from micromammal incisors was manually removed using a scalpel. Few teeth were also analyzed as whole (dentine + enamel). Before the actual digestion with 3M HNO<sub>3</sub>, samples (1-5 mg in mass) were washed with MilliQ (ultrasonic bath) and leached with ~0.5 M HNO<sub>3</sub>. Sr of the digested specimens was separated from the matrix using 30  $\mu$ l columns and Triskem Sr-Spec resin.

255 Sr isotope ratios were measured using a Neptune (ThermoFisher) multi-collector inductively-coupled-plasma mass spectrometer (MC-ICPMS) housed at the Centro 256 257 Interdipartimentale Grandi Strumenti (UNIMORE) during different analytical sessions. Seven Faraday detectors were used to collect signals of the following masses: <sup>82</sup>Kr, <sup>83</sup>Kr, 258 259 <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 Neptune through an APEX desolvating system. Corrections for Kr and Rb interferences 260 follow previous works (15). Mass bias corrections used an exponential law and a  ${}^{88}$ Sr/ ${}^{86}$ Sr 261 ratio of 8.375209 (56). The Sr ratios of samples were reported to a SRM987 value of 262 0.710248 (57). During one session, SRM987 yielded an average <sup>87</sup>Sr/<sup>86</sup>Sr ratio of 263  $0.710243 \pm 0.000018$  (2 S.D., n = 8). Total laboratory Sr blanks did not exceed 100 pg. 264 Spatially-resolved Sr isotopic analysis by laser-ablation plasma mass spectrometry 265

266 (LA-MC-ICPMS)

267 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 268 analytical protocols described by Müller & Anczkiewicz (2016) (16); only a brief 269 270 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 271 Inc.) equipped with a two-volume LA cell (Laurin Technic) was connected to a 272 NeptunePlus (ThermoFisher) MC-ICPMS using nylon6-tubing and a 'squid' signal-273 smoothing device (58). Ablation took place in a He atmosphere (300 ml/min), with  $\sim 1000$ 274 ml/min Ar added at the funnel of the two-volume LA cell and 3.5 ml/min N<sub>2</sub> before the 275 squid. Laser fluence on target was  $\sim 5 \text{ J/cm}^2$ . 276

277 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 278 279 below), in continuous profiling mode following the enamel-dentine-junction (EDJ) from 280 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 (<sup>88</sup>Sr) while maintaining robust plasma 281 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. 282 283 In view of the low Sr concentrations in these human enamel samples (~60-100  $\mu$ g/g), we utilized 130  $\mu$ m spots, a scan speed of 5  $\mu$ m/s and a repetition rate of 20 Hz to maintain 284 <sup>88</sup>Sr ion currents of ~2-3.5 x  $10^{-11}$  A. Nine Faraday detectors were used to collect the ion 285 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, 286 287 <sup>90</sup>Zr. Baseline, interference and mass bias corrections follow (16). The isotopicallyhomogenous (Sr) enameloid of a modern shark was used to assess accuracy of the Sr-288 isotopic analysis and yielded  ${}^{87}$ Sr/ ${}^{86}$ Sr = 0.70916 ± 2 and  ${}^{84}$ Sr/ ${}^{86}$ Sr = 0.0565 ± 1 (2 S.D.). 289 Raw data are reported in Dataset S1. 290

## 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, 302 303 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 304 well as a scan speed of 5 µm/s and a repetition rate of 15 Hz; prior to acquisition, samples 305 306 were pre-cleaned using slightly larger spot sizes (22 - 57 µm), 20 Hz and faster scan speeds (25 - 50  $\mu$ m/s); laser fluence was ~5 J/cm<sup>2</sup>. The following isotopes (*m/z*) were 307 analyzed: <sup>25</sup>Mg, <sup>27</sup>Al, <sup>43</sup>Ca, (<sup>44</sup>Ca), <sup>55</sup>Mn, <sup>66</sup>Zn, <sup>85</sup>Rb, (<sup>86</sup>Sr), <sup>88</sup>Sr, <sup>89</sup>Y, <sup>138</sup>Ba, <sup>140</sup>Ce, (<sup>166</sup>Er, 308 <sup>172</sup>Yb), <sup>208</sup>Pb, <sup>238</sup>U. The total sweep times for the Element XR and the 8900 ICP-MS/MS 309 were  $\sim 0.8$  and 0.4-0.5 s, respectively; however, because of the slow scan speeds, this 310 311 small difference has no effect on the compositional profiles presented here. Primary standardization was achieved using NIST SRM612. Ca was employed as internal 312 standard (<sup>43</sup>Ca); [Ca] at 37 %m/m was used to calculate concentrations for unknown 313 bioapatites, although not required for X/Ca ratios. Accuracy and reproducibility were 314 315 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 316 interest) here are  $1.8 \pm 6.6\%$  and  $-0.2 \pm 6.0\%$  (%bias  $\pm 2$  S.D. (%)); this compares well 317 with the long-term reproducibility for these analytes reported previously (60). Raw data 318 319 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 lasertracks

326 Dental enamel is capable of recording, at microscopic level during its formation, regular physiological and rhythmic growth markers (63-65). These incremental markings are 327 visible under transmitted light in longitudinal histological thin sections of dental crowns. 328 329 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 330 enamel is expressed in humans at two different levels: a circadian rhythm that produces 331 332 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 333 individual during tooth growth cause a disruption of the enamel matrix secretion and 334 335 mark the corresponding position of the secretory ameloblast front, producing Accentuated 336 (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, 337 338 namely the Neonatal Line (NL) (25, 71, 72).

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

A prism segment starting from the most apical available point on the enamel dentine 341 342 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 343 344 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 345 to the sum of the single prism segments. To obtain time (in days) from the prism length 346 measurements, local daily secretion rates (24) (DSR) were calculated around the prism 347 348 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 349 350 accentuated lines (ALs) in the modern sample closely match the timing of known 351 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). 352

353 DSRs were collected across the whole crown on spots chosen randomly in order to get 354 the DSRs distribution. Groups of cross striations ranging from 3 to 7 were measured. For 355 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.

### 362 Acknowledgments

361

363

Archaeological excavations at Fumane and De Nadale are coordinated by University of 364 365 Ferrara and supported by public institutions (Fumane: Lessinia Mountain Community, Fumane Municipality, BIMAdige; De Nadale: Zovencedo Municipality) and private 366 associations and companies (De Nadale: RAASM). Archaeological excavations at Riparo 367 Broion are coordinated by University of Bologna and University of Ferrara and supported 368 by H2020 grant 724046 – SUCCESS. Superintendency SAPAB-VR provided access to 369 370 the samples of Nadale 1, Riparo Broion 1, Fumane 1 and Fumane 2. We thank the parents and the children who donated deciduous teeth and who carefully recorded the dietary 371 events of their children. Michael P. Richards and Marcello Mannino are thanked for 372 stimulating discussions and for having initiated isotopic studies of the specimens at 373 374 Fumane. This project was funded by the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme (grant agreement 375 No 724046 - SUCCESS awarded to Stefano Benazzi - erc-success.eu and grant 376 agreement No 639286 - HIDDEN FOODS awarded to Emanuela Cristiani -377 www.hiddenfoods.org). FIERCE is financially supported by the Wilhelm and Else 378 Heraeus Foundation and by the Deutsche Forschungsgemeinschaft (DFG, INST 161/921-379 1 FUGG and INST 161/923-1 FUGG), which is gratefully acknowledged. LA-ICPMS 380 analyses at Royal Holloway University of London, used for early comparative samples 381 shown in the supporting material, was supported by NERC equipment funding (NERC 382 383 CC073).

- 386
- 387 388
- 389

390 391 392	Refere	nces
393 394	1.	Sellen DW (2007) Evolution of infant and young child feeding: implications for contemporary public health <i>Annu Rev. Nutr.</i> 27:123-148
395	2	Kennedy GE (2005) From the ane's dilemma to the wearling's dilemma: early
396	2.	wearing and its evolutionary context <i>Journal of human evolution</i> 48(2):123-145
397	3.	Humphrey LT (2010) Weaning behaviour in human evolution. Seminars in Cell
398		and Developmental Biology 21(4):453-461.
399	4.	Sellen DW (2001) Comparison of infant feeding patterns reported for
400		nonindustrial populations with current recommendations. <i>The Journal of nutrition</i>
401	-	131(10):2707-2715.
402	5.	Smith TM, et al. (2010) Dental evidence for ontogenetic differences between
403 404		modern humans and Neanderthals. Proceedings of the National Academy of Sciences of the United States of America 107(49):20923-20928
405	6	Gunz P. Neubauer S. Maureille B. & Hublin I-I (2010) Brain development after
405	0.	birth differs between Neanderthals and modern humans <i>Current biology</i>
407		20(21)·R921-R922
407	7	de León MSP Bienvenu T Akazawa T & Zollikofer CP (2016) Brain
408	7.	development is similar in Neanderthals and modern humans. Current Biology
405		26(14):R665-R666
410 A11	8	Macchiarelli R et al. (2006) How Neanderthal molar teeth grew Nature
412	0.	444(7120):748.
413	9.	Rosas A, et al. (2017) The growth pattern of Neandertals, reconstructed from a
414		juvenile skeleton from El Sidrón (Spain). Science 357(6357):1282-1287.
415	10.	Smith TM, et al. (2018) Wintertime stress, nursing, and lead exposure in
416		Neanderthal children. Science Advances 4(10):eaau9483.
417	11.	Austin C, et al. (2013) Barium distributions in teeth reveal early-life dietary
418		transitions in primates. Nature 498(7453):216-219.
419	12.	Skinner M (1997) Dental wear in immature Late Pleistocene European hominines.
420		J. Archaeol. Sci. 24(8):677-700.
421	13.	Dean MC (2010) Retrieving chronological age from dental remains of early fossil
422		hominins to reconstruct human growth in the past. Philosophical Transactions of
423		the Royal Society of London B: Biological Sciences 365(1556):3397-3410.
424	14.	Müller W, et al. (2019) Enamel mineralization and compositional time-resolution
425		in human teeth evaluated via histologically-defined LA-ICPMS profiles.
426		Geochimica et Cosmochimica Acta 255:105-126.
427	15.	Lugli F, et al. (2019) Strontium and stable isotope evidence of human mobility
428		strategies across the Last Glacial Maximum in southern Italy. Nature ecology &
429		evolution 3(6):905-911.
430	16.	Müller W & Anczkiewicz R (2016) Accuracy of laser-ablation (LA)-MC-ICPMS
431		Sr isotope analysis of (bio) apatite-a problem reassessed. Journal of Analytical
432		Atomic Spectrometry 31(1):259-269.
433	17.	Benazzi S, et al. (2014) Middle Paleolithic and Uluzzian human remains from
434		Fumane Cave, Italy. Journal of Human Evolution 70:61-68.

435	18.	Arnaud J, et al. (2017) A Neanderthal deciduous human molar with incipient
436		carious infection from the Middle Palaeolithic De Nadale cave, Italy. American
437		journal of physical anthropology 162(2):370-376.
438	19.	Romandini M, et al. (in review) A late Neanderthal tooth from northeastern Italy.
439		Journal of Human Evolution.
440	20.	Benazzi S, et al. (2015) The makers of the Protoaurignacian and implications for
441		Neandertal extinction. Science 348(6236):793-796.
442	21.	Rasmussen SO, et al. (2014) A stratigraphic framework for abrupt climatic
443		changes during the Last Glacial period based on three synchronized Greenland
444		ice-core records: refining and extending the INTIMATE event stratigraphy.
445		<i>Ouaternary Science Reviews</i> 106:14-28.
446	22.	Seguinot J. <i>et al.</i> (2018) Modelling last glacial cycle ice dynamics in the Alps.
447		<i>The Cryosphere</i> 12(10):3265-3285.
448	23.	Wood JW. <i>et al.</i> (1992) The osteological paradox: problems of inferring
449		prehistoric health from skeletal samples [and comments and reply]. Current
450		Anthropology 33(4):343-370.
451	24.	Nava A, et al. (2017) New Regression Formula to Estimate the Prenatal Crown
452		Formation Time of Human Deciduous Central Incisors Derived from a Roman
453		Imperial Sample (Velia, Salerno, I-II cent. CE). <i>PloS ONE</i> 12(7):e0180104.
454	25.	Dean MC. Spiers KM. Garrevoet J. & Le Cabec A (2019) Synchrotron X-ray
455		fluorescence mapping of Ca. Sr and Zn at the neonatal line in human deciduous
456		teeth reflects changing perinatal physiology. Archives of oral biology 104:90-102.
457	26.	Humphrey LT. Dean MC. Jeffries TE. & Penn M (2008) Unlocking evidence of
458		early diet from tooth enamel. <i>Proceedings of the National Academy of Sciences of</i>
459		the United States of America 105(19):6834-6839.
460	27.	Humphrey LT (2014) Isotopic and trace element evidence of dietary transitions in
461		early life. Annals of Human Biology 41(4):348-357.
462	28.	Humphrey LT, Dirks W, Dean MC, & Jeffries TE (2008) Tracking dietary
463		transitions in weanling baboons (Papio hamadryas anubis) using
464		strontium/calcium ratios in enamel. <i>Folia Primatologica</i> 79(4):197-212.
465	29.	Rossipal E. Krachler M. Li F. & Micetic-Turk D (2000) Investigation of the
466	_,.	transport of trace elements across barriers in humans: studies of placental and
467		mammary transfer. Acta Paediatrica 89(10):1190-1195.
468	30.	Krachler M. Rossinal E. & Micetic-Turk D (1999) Concentrations of trace
469	001	elements in sera of newborns, young infants, and adults, <i>Biological trace element</i>
470		research 68(2):121
471	31.	Burton JH. Price TD. & Middleton WD (1999) Correlation of bone Ba/Ca and
472	011	Sr/Ca due to biological purification of calcium. J. Archaeol. Sci. 26(6):609-616.
473	32.	Tsutava T & Yoneda M (2015) Reconstruction of breastfeeding and weaning
474	52.	practices using stable isotope and trace element analyses: a review <i>American</i>
475		Journal of Physical Anthropology 156(S59):2-21
476	33	Peek S & Clementz MT (2012) Sr/Ca and Ba/Ca variations in environmental and
477		biological sources: a survey of marine and terrestrial systems. <i>Geochimica et</i>
478		Cosmochimica Acta 95:36-52.

479	34.	Metcalfe JZ, Longstaffe FJ, & Zazula GD (2010) Nursing, weaning, and tooth
480		development in woolly mammoths from Old Crow, Yukon, Canada: implications
481		for Pleistocene extinctions. <i>Palaeogeography</i> , <i>Palaeoclimatology</i> , <i>Palaeoecology</i>
482		298(3-4):257-270.
483	35.	Tacail T, Kovačiková L, Brůžek J, & Balter V (2017) Spatial distribution of trace
484		element Ca-normalized ratios in primary and permanent human tooth enamel.
485		Science of the Total Environment 603:308-318.
486	36.	Mahoney P (2011) Human deciduous mandibular molar incremental enamel
487		development. American Journal of Physical Anthropology 144(2):204-214.
488	37.	Mahoney P (2012) Incremental enamel development in modern human deciduous
489		anterior teeth. American Journal of Physical Anthropology 147(4):637-651.
490	38.	Dean MC, Humphrey L, Groom A, & Hassett B (2020) Variation in the timing of
491		enamel formation in modern human deciduous canines. Archives of Oral
492		Biology:104719.
493	39.	Birch W & Dean MC (2014) A method of calculating human deciduous crown
494		formation times and of estimating the chronological ages of stressful events
495		occurring during deciduous enamel formation. Journal of Forensic and Legal
496		Medicine 22:127-144.
497	40.	Fornai C, et al. (2014) Enamel thickness variation of deciduous first and second
498		upper molars in modern humans and Neanderthals. Journal of human evolution
499		76:83-91.
500	41.	Olejniczak AJ, et al. (2008) Dental tissue proportions and enamel thickness in
501		Neandertal and modern human molars. Journal of Human Evolution 55(1):12-23.
502	42.	López-García JM, Berto C, & Peresani M (2019) Environmental and climatic
503		context of the hominin occurrence in northeastern Italy from the late Middle to
504		Late Pleistocene inferred from small-mammal assemblages. <i>Quaternary Science</i>
505		<i>Reviews</i> 216:18-33.
506	43.	López-García JM, dalla Valle C, Cremaschi M, & Peresani M (2015)
507		Reconstruction of the Neanderthal and Modern Human landscape and climate
508		from the Fumane cave sequence (Verona, Italy) using small-mammal
509		assemblages. Quaternary Science Reviews 128:1-13.
510	44.	Miller EM (2018) Beyond passive immunity: Breastfeeding, milk and
511		collaborative mother-infant immune systems. Breastfeeding: New
512		Anthropological Approaches, (Routledge, New York), pp 26-39.
513	45.	Campbell KL & Wood JW (1988) Fertility in traditional societies. Natural human
514		fertility, (Springer), pp 39-69.
515	46.	Lee PC (1996) The meanings of weaning: growth, lactation, and life history.
516		Evolutionary Anthropology: Issues, News, and Reviews: Issues, News, and
517		<i>Reviews</i> 5(3):87-98.
518	47.	World Health Organization (2009) Infant and young child feeding: model chapter
519		for textbooks for medical students and allied health professionals.
520	48.	Prentice P, et al. (2016) Breast milk nutrient content and infancy growth. Acta
521		Paediatrica 105(6):641-647.

522	49.	Taylor HW, Vázquez-Geffroy M, Samuels SJ, & Taylor DM (1999) Continuously
523		recorded suckling behaviour and its effect on lactational amenorrhoea. Journal of
524		biosocial science 31(3):289-310.
525	50.	Prüfer K, et al. (2014) The complete genome sequence of a Neanderthal from the
526		Altai Mountains. Nature 505(7481):43-49.
527	51.	Garber CM (1947) Eskimo infanticide. The Scientific Monthly 64(2):98-102.
528	52.	Trinkaus E (1995) Neanderthal mortality patterns. J. Archaeol. Sci. 22(1):121-
529		142.
530	53.	Nava A (2018) Hominin dental enamel: an integrated approach to the study of
531		formation, maturation, and morphology (Unpublished doctoral dissertation). PhD
532		(Sapienza University of Rome, Rome).
533	54.	Caropreso S, et al. (2000) Thin sections for hard tissue histology: a new
534		procedure. Journal of Microscopy 199(3):244-247.
535	55.	Weber M, Lugli F, Jochum KP, Cipriani A, & Scholz D (2018) Calcium
536		carbonate and phosphate reference materials for monitoring bulk and
537		microanalytical determination of Sr isotopes. Geostandards and Geoanalytical
538		<i>Research</i> 42(1):77-89.
539	56.	Steiger RH & Jäger E (1977) Subcommission on geochronology: convention on
540		the use of decay constants in geo-and cosmochronology. Earth and planetary
541		science letters 36(3):359-362.
542	57.	McArthur JM, Howarth R, & Bailey T (2001) Strontium isotope stratigraphy:
543		LOWESS version 3: best fit to the marine Sr-isotope curve for 0–509 Ma and
544		accompanying look-up table for deriving numerical age. The Journal of Geology
545		109(2):155-170.
546	58.	Müller W, Shelley M, Miller P, & Broude S (2009) Initial performance metrics of
547		a new custom-designed ArF excimer LA-ICPMS system coupled to a two-volume
548		laser-ablation cell. Journal of Analytical Atomic Spectrometry 24:209-214.
549	59.	Klemme S, et al. (2008) Synthesis and preliminary characterisation of new
550		silicate, phosphate and titanite reference glasses. Geostandards and Geoanalytical
551		<i>Research</i> 32(1):39-54.
552	60.	Evans D & Müller W (2018) Automated extraction of a five-year LA-ICP-MS
553		trace element data set of ten common glass and carbonate reference materials:
554		Long-term data quality, optimisation and laser cell homogeneity. Geostandards
555		and Geoanalytical Research 42(2):159-188.
556	61.	Cleveland WS, Grosse E, & Shyu WM (1992) Local regression models.
557		Statistical Models in S 2:309-376.
558	62.	R-Core-Team (2020) R: A language and environment for statistical computing.
559		(R Foundation for Statistical Computing, Vienna, Austria).
560	63.	Antoine D, Hillson S, & Dean MC (2009) The developmental clock of dental
561		enamel: a test for the periodicity of prism cross-striations in modern humans and
562		an evaluation of the most likely sources of error in histological studies of this
563		kind. Journal of Anatomy 214:45-55.
564	64.	Dean MC (2006) Tooth microstructure tracks the pace of human life-history
565		evolution. Proceedings of the Royal Society of London B: Biological Sciences
566		273(1603):2799-2808.

567	65.	Hillson S (2014) Tooth development in human evolution and bioarchaeology
568		(Cambridge University Press, Cambridge).
569	66.	Lacruz RS, et al. (2012) The circadian clock modulates enamel development.
570		Journal of Biological Rhythms 27(3):237-245.
571	67.	Zheng L, et al. (2013) Circadian rhythms regulate amelogenesis. Bone 55(1):158-
572		165.
573	68.	Dean MC (1987) Growth layers and incremental markings in hard tissues; a
574		review of the literature and some preliminary observations about enamel structure
575		in Paranthropus boisei. Journal of Human Evolution 16(2):157-172.
576	69.	Nava A, Frayer DW, & Bondioli L (2019) Longitudinal analysis of the
577		microscopic dental enamel defects of children in the Imperial Roman community
578		of Portus Romae (necropolis of Isola Sacra, 2nd to 4th century CE, Italy). Journal
579		of Archaeological Science: Reports 23:406-415.
580	70.	Witzel C, et al. (2006) Reconstructing impairment of secretory ameloblast
581		function in porcine teeth by analysis of morphological alterations in dental
582		enamel. Journal of Anatomy 209(1):93-110.
583	71.	Sabel N, et al. (2008) Neonatal lines in the enamel of primary teeth—a
584		morphological and scanning electron microscopic investigation. Archives of Oral
585		<i>Biology</i> 53(10):954-963.
586	72.	Zanolli C, Bondioli L, Manni F, Rossi P, & Macchiarelli R (2011) Gestation
587		length, mode of delivery, and neonatal line-thickness variation. <i>Human Biology</i>
588		83(6):695-713.
589	73.	Guatelli-Steinberg D, Floyd BA, Dean MC, & Reid DJ (2012) Enamel extension
590		rate patterns in modern human teeth: two approaches designed to establish an
591		integrated comparative context for fossil primates. Journal of Human Evolution
592		63(3):475-486.
593		

594 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). 



Fig. 2. Dental crown growth parameters. (a) Postnatal crown formation time in days 612 from birth for the different deciduous teeth. The range of variability reported in 613 literature for modern and archaeological individuals is represented by red, blue, 614 615 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, 616 max) and range of variation (min, mean, max) of modern humans (MH), re-617 assessed from (36-39). (c) Boxplot of the daily secretion rate variation across the 618 whole crown (mean, second quartile, median, third quartile, max) and range of 619 variation (min, mean, max) of modern humans (MH), re-assessed from (36-39). 620 Ldm1 = lower deciduous first molar; Ldm2 = lower deciduous second molar; Udc621 622 = upper deciduous canine; Ldi2 = lower deciduous later incisor.



624 Fig. 3. Nursing histories from time-resolved Sr/Ca variation in Middle-Upper **Paleolithic deciduous teeth.** UPMH = Upper Paleolithic modern human; NEA = 625 Neanderthal. The elemental profiles were analyzed within enamel close to the enamel-626 dentine-junction (EDJ); [U] is reported as the most sensitive proxy for diagenetic 627 alteration (14) (see SI Appendix, Text S4). Grey portions of the profiles represent 628 diagenetically overprinted enamel domains, based on elevated U concentrations. The 629 630 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) 631 Fumane 1: Sr/Ca variation indicates breastfeeding until 4 months of age (fully 632 633 comparable with MCS1 sample, see Supplementary Figure S6); (c) Riparo Broion 1: 634 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 635 636 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 637 638 strong smoothing parameters to enhance the biogenic signal. See Material and Methods section for details. 639 640



Fig. 4. Mobility of the Middle-Upper Paleolithic infants via time-resolved <sup>87</sup>Sr/<sup>86</sup>Sr 642 profiles of their deciduous teeth. Grey horizontal bands represent the local Sr isotopic 643 baselines defined via the Sr isotopic composition of archaeological rodent enamel (SI 644 Appendix, Table S1). The birth event is indicated by a vertical line. (a,b) Nadale 1 / 645 Fumane 1: exploitation of local food resources through the entire period; (c) Riparo 646 Broion 1: possible limited seasonal mobility (non-local values between c. 25 and 75 days 647 648 = 4 months); (d) Fumane 2: exploitation of non-local food resources through the entire 649 period. 650

651	
652	
653	
654	
655	
656	
657	Supplementary Information for Early life of Neanderthals
658	
659 660 661 662 663 664	Alessia Nava, Federico Lugli, Matteo Romandini, Federica Badino, David Evans, Angela H. Helbling, Gregorio Oxilia, Simona Arrighi, Eugenio Bortolini, Davide Delpiano, Rossella Duches, Carla Figus, Alessandra Livraghi, Giulia Marciani, Sara Silvestrini, Anna Cipriani, Tommaso Giovanardi, Roberta Pini, Claudio Tuniz, Federico Bernardini, Irene Dori, Alfredo Coppa, Emanuela Cristiani, Christopher Dean, Luca Bondioli, Marco Peresani, Wolfgang Müller, Stefano Benazzi
665 666 667 668 669 670 671	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
672	This PDF file includes:
673	
674	Supplementary text S1 to S4
6/5	Figures S1 to S13
6/6 (77	Lagenda for Datagata S1 to S2
0// ۲۹	Legenus for Datasets 51 to 55 SI Deferences
0/ð	

- 680 Other supplementary materials for this manuscript include the following:
- 681682 Datasets S1 to S3

### 685 SUPPLEMENTARY INFORMATION TEXT S1: DENTAL MORPHOLOGY

686

The deciduous dental sample here investigated consists of three Neanderthals and oneUpper 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). Highresolution micro-CT images of Fumane 1 and 2 were obtained with a Skyscan 1172 microtomographic system using isometric voxels of 11.98  $\mu$ m (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  $\mu$ m (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 specimens (Fumane 1 and Riparo Broion 1) and one of the earliest modern humans in
Europe (Fumane 2), thus providing a unique opportunity to compare subsistence
strategies between the two human groups around the time of Neanderthal demise.

# 719 SUPPLEMENTARY INFORMATION TEXT S2: ARCHAEOLOGICAL AND 720 PALEOENVIRONMENTAL CONTEXTS

721

722 <u>Nadale 1</u>

De Nadale Cave is a small cavity located 130m a.s.l. in the middle of the Berici Hills. 723 724 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 campaigns were carried out between 2014 and 2017 in order to investigate the deposits 726 preserved in the cave entrance and the back (5). The excavations exposed a stratigraphic 727 728 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, lithic implements, and the Neanderthal deciduous tooth (2). A molar of a large-sized 732 733 ungulate was U/Th dated to 70,200±1,000/900 years as a minimum age (5) placing the human occupation to an initial phase of the MIS 4. The zooarchaeological assemblage is 734 735 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 stages of the butchery process and to the fragmentation of the bones for marrow 742 743 extraction. Burnt bone fragments and charcoal accumulations have been likely related to residual fire-places (6). Lithic industry from of De Nadale differentiates technologically 744 and typologically from the Mousterian elsewhere in the region, especially with regard to 745 the core reduction methods and the types of flakes and retouched tools. These are 746 represented from several scrapers with stepped-scaled invasive retouches and make the 747

De Nadale industry comparable to Quina assemblages in Italy and Western Europe (5).De Nadale peculiarity is also enhanced by the high number of bone retouchers (9).

Research at the De Nadale Cave is coordinated by the University of Ferrara (M.P.) in the
framework of a project supported by the Ministry of Culture – "SABAP per le province
di Verona, Rovigo e Vicenza" and the Zovencedo Municipality, financed by the H.
Obermaier Society (2015), local private companies (R.A.A.S.M., Saf and Lattebusche),
and local promoters.

755

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

Grotta di Fumane (Fumane Cave) is a cave positioned at the western fringe of the Lessini
plateau in the Venetian Pre-Alps. The site preserves a finely layered late Middle and early
Upper Paleolithic sequence with evidence of cultural change related to the demise of
Neanderthals and the arrival of the first Anatomically Modern Humans (3, 10-12). Teeth
Fumane 1 and Fumane 2 were found in Middle Paleolithic unit A11 and Upper
Paleolithic unit A2 associated to Mousterian and Aurignacian cultures respectively.

Of the late Mousterian layers, unit A11 is a stratigraphic complex composed of an 763 764 ensemble of thin levels with hearths that was surveyed in different years at the eastern entrance of the cave over a total area of 10 sqm. The chronometric position of A11 is 765 provided by only one U/Th date to 49,000±7,000 years for level A11a, given unreliability 766 to the radiocarbon dataset currently available (13) but see (14). New radiocarbon 767 measurements are in progress. Paleoecological indexes calculated on the composition of 768 769 the micromammal assemblage point for a temperate and relatively moist period related to an interstadial before HE5 (15), in a landscape dominated from open-woodland 770 771 formations in accordance with the previous indications based on the zooarchaeological assemblage. Cervids (red deer, giant deer and roe deer) largely prevail on bovids and 772 773 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

retouched tools like single and double scrapers, also transverse or convergent and fewpoints and denticulates (11).

Aurignacian layer A2 records an abrupt change in material culture represented from lithic 779 780 and bone industry (10, 17, 18), beads made of marine shells and bone (10, 19), use of red mineral pigment (20). Bone and cultural remains have been found scattered on a 781 paleoliving floor with fire-places, toss zones and intentionally disposed stones (21). A 782 783 revised chronology of the Mid-Upper Paleolithic sequence (14) has shown that the start and the end of level A2 date respectively to 41,900-40,200 cal BP and 40,300-39,400 cal 784 BP at the largest confidence interval. Macro- and micro-faunal remains show an 785 786 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 was mostly targeted adult individuals of ibex, chamois and bison and occurred 788 789 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 research and innovation programme (grant agreement No 724046 - SUCCESS, 796 797 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 the Venetian Pre-Alps in the Alpine foreland. This is a large alluvial plain that was 800 formed initially during the Middle and Late Pleistocene by a number of major rivers, 801 including the Po, the Adige and those of the Friulian-Venetian plain. The western zone of 802 the Berici is a gentle landscape which conjoins to the alluvial plain. Conversely, along its 803 eastern slope the plateau connects abruptly to the alluvial plain. Here, caves and 804 805 rockshelters have been archaeologically investigated since the XIX century up to present days by teams from the University of Ferrara. Of these cavities, Riparo del Broion is a 806

807 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 808 slope for cultivation during recent historical times. The shelter is 10m long, 6m deep and 809 810 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 811 cavities in the area (25, 26). Two additional Paleolithic cavities were investigated on the 812 813 western side of the same cliff, Grotta del Buso Doppio del Broion and Grotta del Broion 814 (27, 28).

The sedimentary deposits of Riparo Broion were partially dismantled in historical times 815 816 by shepherds with use to store hay and wood. Further damage occurred in 1984 when 817 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 818 819 Alberto Broglio (1998 -2008) and by two of us (M.P. and M.R.) in 2015 on a 20sqm area 820 bounded to north and west from the rock walls. Faunal remains and Middle and Upper 821 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 822 823 with large prevalence on loams: 16 stratigraphic units planarly bedded have been 824 identified. The lowermost (11, 9, 7 and 4) contain Mousterian artefacts, faunal remains 825 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 826 48,100±3100 years BP with range from 50.000 to 45.700 years cal BP as the most likely 827 age (31). Stone tools are too low in number to propose an attribution to one or another 828 Mousterian cultural complex. Preliminary zooarchaeological data report a variety of 829 830 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 831 832 shells. This association reflects the presence of a patchy environmental context, with closed to open-spaced forests, Alpine grasslands and pioneer vegetation complemented 833 834 by humid-marshy environments and low-energy water courses, wet meadows and shallow 835 lacustrine basins.

Research at Riparo Broion is coordinated by the Bologna (M.R.) and Ferrara (M.P.) 836 Universities in the framework of a project supported by the Ministry of Culture -837 "SABAP per le province di Verona, Rovigo e Vicenza", public institutions (Longare 838 Municipality), institutions (Leakey Foundation, Spring 2015 Grant; Istituto Italiano di 839 Preistoria e Protostoria). Research campaigns 2017-2019 have received funding from the 840 European Research Council (ERC) under the European Union's Horizon 2020 research 841 and innovation programme (grant agreement No 724046 - SUCCESS, http://www.erc-842 843 success.eu).

844

### 845 <u>Paleoenvironmental contexts</u>

846 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 847 848 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 849 850 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 851 852 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 853 854 forest (TF): sum of deciduous Quercus, Alnus glutinosa type, Fagus, Acer, Corylus, 855 Carpinus, Fraxinus, Ulmus, Tilia and Salix; Xerophytic steppe (XS): sum of Artemisia and Chenopodiaceae. Other herbs: sum of terrestrial herbs, Chenopodiaceae excluded. 856 Original pollen data used for % calculation for the Palughetto basin are from (32). 857

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

- subordinated temperate taxa (10%). Open environments are identified by pollen of
  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
- of eurythermic conifers sum up to 37-38%. Temperate trees, notably Tilia, persisted in
- 870 very low percentages (4%) up to  $\sim 40$  ka (34).
- 871

# 872 SUPPLEMENTARY INFORMATION TEXT S3: TOWARDS A CONCEPTUAL 873 MODEL FOR Sr/Ca AND Ba/Ca BEHAVIOR IN HUMAN INFANTS: 874 THEORETICAL FRAMEWORK AND EMPIRICAL EVIDENCE FROM 875 CONTEMPORANEOUS INFANTS WITH KNOWN FOOD INTAKE

Strontium and barium are non-bioessential trace elements with no major metabolic 876 functions in the human body. Strontium and Ba mimic Ca, given their coherent behavior 877 878 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<sup>-10</sup> m); (35). Overall, they both follow 879 the Ca metabolism but due to their larger ionic size are discriminated against in the 880 881 gastrointestinal tract (GIT) (36, 37). Given the larger size, Ba is even more strongly 882 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 883 884 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 885 886 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 887 888 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 889 890 might be used as tools for paleodiet and trophic chain reconstruction (36).

Interestingly, significant GIT discrimination of Sr and Ba over Ca ions progressively 891 892 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 893 894 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 895 896 ~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 897 have shown that Ca is actively transported (47), resulting in lower Sr/Ca ratios in both 898 umbilical cord sera and breastmilk than in mother sera due to the larger size of Sr ions 899 900 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 901

902 breastmilk Sr/Ca values lower than umbilical cord (fetal) values (48). Crucially, fetal blood chemistry is recorded in prenatal dental enamel and breastmilk consumption in 903 postnatal enamel and can be reconstructed via high-spatial resolution chemical analysis of 904 905 teeth (49, 50). Thus, higher Sr/Ca signals in prenatal domains followed by lower postnatal Sr/Ca indicate breastmilk consumption (see Fig. S4). This has been previously 906 907 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 cord sera than in breast-fed infant sera. On the other hand, due to the nominal lower 909 trophic level of herbivores, their milk has higher Sr/Ca than human milk. Hence, when a 910 911 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 retain higher Sr/Ca than breastmilk (see e.g. 53). In general, an increased Sr/Ca signal 923 924 from transitional foods is also expected for formula-fed babies. However, due to the compositional variability of some formulas (e.g. soy-based) and non-human milk, a 925 decrease of the Sr/Ca ratio may occur if a highly-biopurified food (e.g. close to human 926 927 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 932 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 933 compared to umbilical cord sera (Tab. S3). However, colostrum is not a good proxy for 934 935 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 936 Krachler et al. (55) are about 2 times higher than other human milk samples (Figure S5). 937 938 Moreover, other studies suggested that only a very limited portion of the absorbed Ba  $(\sim 3\%)$  is transferred to the breast-milk (48). 939

Studies of dental enamel indicate that Ba overall behaves akin to Sr (50, 53, 58, 59), 940 941 decreasing with breastmilk consumption and increasing along with the introduction of 942 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 943 944 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 945 946 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 947 948 with a non-milk diet. In the same publication, the authors reported that Ba absorption 949 increased two-fold in young starved rats, whereas Ca absorption decreased in the same 950 individuals, pointing towards an association of Ba/Ca with dietary stress rather than 951 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.

956

### 957 <u>The modern reference sample</u>

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.

973 MCS1 is a lower deciduous second molar from an individual exclusively breastfeed until 974 the fifth month of life (154 days; Fig. S6). No supplementary food was given to the infant 975 during this period. The Sr/Ca profile analyzed parallel to the enamel-dentine junction 976 (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 977 978 once a day (reported from day 155), the slope of the profile becomes gradually shallower, 979 particularly, this was coincident with the introduction of some formula milk (reported 980 from day 182). Fifteen days after cutting down breastfeeding during daytime (reported on 981 day 209) the profile begins to show a sharp increase of the Sr/Ca values. At 8.5 months of 982 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 983 984 the last part of the profile (after day ~340) likely reflects the effects of maturationoverprint due to the thin enamel closest to the crown neck (50). The striking 985 correspondence of the independently recorded dietary transitions in MCS1 with the Sr/Ca 986 trend fully supports the use of Sr/Ca as a proxy for making nursing events. In this sense, 987 based on modelled values reported in Tab. S2, the theoretical ratio between Sr/Ca in 988 prenatal enamel and breastfeeding signal is ~0.7. In MCS1, this ratio is ~0.8, indicating a 989 990 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 991

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

- MCS2 is a deciduous canine from an exclusively formula-fed individual (Fig. S7), whose 997 998 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 increase after birth until ~130 days (~4.3 months), and then it starts to decrease as a 1000 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 higher than  $5*10^{-4}$  and thus higher than those observed in the other contemporary 1003 reference individuals (Figure S4b). The model reported in Fig. S4 and Table S2 specifies 1004 a ratio between prenatal enamel and formula Sr/Ca signal equal to ~2.2. In MCS2, this 1005 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).
- MCS3 is an upper deciduous canine from a mixed breast- formula-fed individual (Fig. S8). This infant was exclusively breastfed for the first 30 days. After that, the mother complemented the infant diet with formula milk. Mixed feeding was carried on until 4 months of age, at which time the mother underwent surgery. During this period of illness, the mother used a breast pump to continue breastfeeding. After the surgery, the mother continued to breastfeed the infant with formula milk supplements, until the onset of weaning at six months.
- 1017 The X/Ca profiles were nominally analyzed close to daily-resolution ( $6 \mu m$  spots vs. 10.3 1018  $\mu m$ /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  $\mu$ m) 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>

In Nadale1, Sr/Ca profile slightly decreases until the end of the crown, depicting a breastfeeding signal until the end of the crown formation. Unusually, Ba/Ca shows the opposite trend to Sr/Ca (Fig. S9), and appears to follow the dietary model proposed by (54). Mg/Ca is largely invariant across the whole crown, and only very minor diagenetic alteration is apparent via U peaks at the very beginning and end of the crown that have 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 reported above. According to (54), this individual falls outside the bounds of their model, 1046 because a decrease in Ba/Ca after birth is never detected in their data. 1047

1048

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

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

1058

1059 <u>Fumane 2 - Aurignacian</u>

1060 The Ba/Ca profile of Fumane 2 follows that of Sr/Ca (Fig. S12), slightly decreasing in the 1061 first month of postnatal life and then increasing in the most cervical enamel. The short 1062 postnatal portion of available enamel (~55 days) precludes the chemical detection of the 1063 onset of weaning but a clear breast-feeding signal is detectable after birth since Sr/Ca 1064 decreases. Ba/Ca also decreases accordingly, and all is independent of diagenesis that is 1065 very low.

# 1067 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. During the post-depositional history, however, bioapatite may react with soils and 1072 underground waters, which can modify the initial biogenic chemical composition. 1073 1074 Depending on apatite crystal-size, organic content and porosity, the distinct dental tissues behave differently in a soil environment. Bone and dentine are most susceptible to 1075 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 environment and bioapatite tissue as well as the partition coefficient for the element(s) 1078 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.  $>1 - 10^3 \mu g/g$ ) 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).

In particular, uranium as water soluble (as uranyl  $(UO_2)^{2+}$ ) and highly mobile element is 1086 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  $\mu$ g/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). To monitor diagenetic alterations of our fossil dental specimens, we monitored <sup>25</sup>Mg, 1094 <sup>27</sup>Al, <sup>55</sup>Mn, <sup>89</sup>Y, <sup>140</sup>Ce, <sup>166</sup>Er, <sup>172</sup>Yb and <sup>238</sup>U signals during the LA-ICPMS analyses and 1095 found that U (and Al) were the most sensitive indicators of diagenetic alteration, while 1096

1097 commonly utilized REEs plus Y were rather insensitive in all cases as they remained at
1098 detection limit even in domains with clearly elevated U and Al. As a result, REE + Y are
1099 not shown here and we focus on U as main proxy for post-mortem diagenesis.

1100 Scatter plots between U and the residuals of Sr, Ba or Mg variation for the diagenetically 1101 most affected segments (Fig. S13) illustrate well the nature of element-specific diagenetic overprint of the four teeth. In samples with overall low [U] ( $<0.2 \mu g/g$ ), i.e. Nadale 1 and 1102 1103 Fumane 2, there are no significant positive or negative correlations discernible. In case of 1104 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. 1105 1106 It should be noted that spatially-resolved analysis by LA-ICPMS not only allows the 1107 retrieval of time-resolved chemical signals, but is equally ideally-suited for the delineation of well-preserved segments in partially diagenetically-overprinted samples. 1108 1109 We employ the following strategy to delineate well-preserved from diagenetically overprinted segments in our enamel profiles: 1110

1111 1) The visible co-variation between U and Sr/Ca (Fig. 3) as well as above mentioned 1112 correlations between Sr, Ba, Mg residuals with U (Fig. S13) show that especially Ba and 1113 less so Sr (only Riparo Broion 1) were added during diagenesis, while Mg was lost. 1114 Consequently, only data segments with lowest U ([U] <~0.05  $\mu$ g/g) were used for further 1115 considerations.

1116 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, 1117 Ba, Sr, Mg in Riparo Broion 1 (Fig. S11) characterize diagenetic signals, while variations 1118 1119 in low-U domains are far more gradual and occur over tens of days. The latter is more in 1120 line with biologically-mediated variations that are additionally modulated by the protracted nature of enamel mineralization (50), which precludes, for example, the up to 1121 fourfold variability in Ba/Ca occurring at the profile start of Fumane 1 to be of in-vivo 1122 1123 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

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.

1129 Overall, we note that diagenesis appears to affect the early formed enamel segments more 1130 than later mineralized areas. As the former are characterized by higher enamel extension 1131 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, 1132 the initial portions of Nadale 1, Fumane 1 and Riparo Broion 1 crowns show enrichments 1133 in U, Al and Mn, with a concurrent decrease of Mg (Figure 3 and S9-S12). While Sr 1134 seems only partly affected by this overprint, Ba tends to precisely resemble the small-1135 scale chemical fluctuations of the diagenetic proxies (clearly visible in Riparo Broion 1 1136 1137 and Fumane 1), suggesting a lack of post-burial stability for the latter element.

1138 Taken together, we observe that the areas of interest (i.e. weaning onset) of our 1139 specimens are sufficiently free from diagenetic alterations to reliably deduce time-1140 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 1143 1144 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 1145 left deciduous second molar, buccal aspect, he section pass through the hypoconid; (c) 1146 Riparo Broion 1, Neanderthal, upper left deciduous canine, buccal aspect; (d) Fumane 2, 1147 UPMH, upper right lateral deciduous incisor, buccal aspect. Red lines highlight the 1148 1149 position of the Neonatal line marking birth event; green lines highlight the laser ablation paths. 1150



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



1158

Figure S3. Pollen record summary of different vegetation types during selected 1159 time-frames. Pollen % are calculated based on the sum of terrestrial taxa and represent 1160 mean values over the selected time frame. Taxa are grouped according to their ecology 1161 and climatic preferences. Eurythermic conifers (EC): sum of Pinus and Juniperus; 1162 Temperate forest (TF): sum of deciduous Quercus, Alnus glutinosa type, Fagus, Acer, 1163 1164 Corylus, Carpinus, Fraxinus, Ulmus, Tilia and Salix; Xerophytic steppe (XS): sum of Artemisia and Chenopodiaceae; other herbs (OH): sum of terrestrial herbs; other woody 1165 taxa (OWT) are also reported. 1166





**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.





1180 Figure S5. Sr/Ca and Ba/Ca data of animal milks, human milks and formulas from

literature. Formulas are from Ikem et al. (72); cow and goat milks are from Bilandžić et
al. (73); human colostrum is from Krachler et al. (55); human milks are from Bilandžić et
al. (73), Björklund et al. (74), Li et al. (75) and Friel et al. (76). The geographical
provenance of the samples is also reported. Error bars are standard deviations.



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).



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).



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).



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-dentinejunction (EDJ).



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 postburial history, and the likely precipitation of low-Mg phases. Black arrows highlight the worst diagenetically-affected domains of the enamel.



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 postburial history, and the likely precipitation of low-Mg phases. Black arrows highlight the worst diagenetically-affected domains of the enamel.



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).





Site	Local geology	Rodent species	Sample type	<sup>87</sup> Sr/ <sup>87</sup> Sr	2 S.E.
		Microtinae	enamel	0.70847	0.00001
			enamel	0.70843	0.00001
Nadala	E P		enamel	0.70825	0.00003
Nauale	Eocerie inflestorie	indet.	enamel	0.70864	0.00001
			enamel	0.70857	0.00001
			mean (± 2 S.D.)	0.70847	0.00030
		<i>Microtinae</i> indet.	whole tooth	0.70826	0.00001
	Eocene Oligocene limestone		whole tooth	0.70820	0.00001
Riparo			whole tooth	0.70814	0.00001
Broion			whole tooth	0.70827	0.00001
			whole tooth	0.70838	0.00001
			mean (± 2 S.D.)	0.70825	0.00018
		<i>Microtinae</i> indet.	enamel	0.70948	0.00001
			enamel	0.70937	0.00001
	Jurassic-Cretaceous limestone and marl		enamel	0.70947	0.00001
Fumane Cave			enamel	0.70940	0.00001
			enamel	0.70962	0.00001
			enamel	0.70958	0.00001
			mean (± 2 S.D.)	0.70948	0.00020

<b>Table 51:</b> St isotopes of local rodent teeth by MC-ICP
--

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

1248

**Table S3.** Ba, Sr, Ca, Ba/Ca and Sr/Ca values of umbilical cord sera, breast-fed infant1251sera and formula-fed infant sera from (52, 55). Values are reported as mean  $\pm$  sd.

Elemental contents and ratios	Maternal seraª	Umbilical cord sera <sup>b</sup>	Umbilical cord sera <sup>a</sup>	Breast-fed infant (ca. 3 months) sera <sup>b</sup>	Formula-fed infant (ca. 3 months) sera <sup>b</sup>	Colostrum <sup>a</sup>
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*10 <sup>3</sup>	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*10 <sup>3</sup>	0.267 ± 0.135	0.228 ±0.121	0.204 ± 0.096	0.108 ±0.031	0.361 ±0.238	0.218 ±0.126

<sup>a</sup>Krachler et al. (1999, European Journal of Clinical Nutrition); <sup>b</sup>Krachler et al. (1999, Biological Trace Element Research)

### 1254 Legends for Datasets

1255

- Dataset S1. <sup>87</sup>Sr/<sup>86</sup>Sr, <sup>84</sup>Sr/<sup>86</sup>Sr and <sup>85</sup>Rb/<sup>86</sup>Sr data of Middle-Upper Paleolithic deciduous
   teeth (baseline, interference, mass-bias/elemental-fractionation-corrected (see text); very
   minor offset of <sup>84</sup>Sr/<sup>86</sup>Sr from 0.0565 is due to residual variability of <sup>84</sup>Kr-backgrounds
   for protracted profile analyses).
- 1260
- 1261 Dataset S2. Sr/Ca and Ba/Ca data of modern reference deciduous teeth.

1262

- 1263 Dataset S3. Sr/Ca, Ba/Ca, Mg/Ca and [U] data of Middle-Upper Paleolithic deciduous
- teeth (LOD indicates that [U]<limit of detection).

1266	<b>SI References</b>
------	----------------------

1268	1.	Benazzi S, et al. (2014) Middle Paleolithic and Uluzzian human remains from
1269		Fumane Cave, Italy. Journal of Human Evolution 70:61-68.
1270	2.	Arnaud J, et al. (2017) A Neanderthal deciduous human molar with incipient
1271		carious infection from the Middle Palaeolithic De Nadale cave, Italy. American
1272		journal of physical anthropology 162(2):370-376.
1273	3.	Benazzi S, et al. (2015) The makers of the Protoaurignacian and implications for
1274		Neandertal extinction. Science 348(6236):793-796.
1275	4.	AlQahtani SJ, Hector M, & Liversidge H (2010) Brief communication: the
1276		London atlas of human tooth development and eruption. American Journal of
1277		Physical Anthropology 142(3):481-490.
1278	5.	Jequier CA, et al. (2015) The De Nadale Cave, a single layered Quina Mousterian
1279		site in the North of Italy.
1280	6.	Livraghi A, Fanfarillo G, Dal Colle M, Romandini M, & Peresani M (2019)
1281		Neanderthal ecology and the exploitation of cervids and bovids at the onset of
1282		MIS4: A study on De Nadale cave, Italy. Quaternary International.
1283	7.	Terlato G, Livraghi A, Romandini M, & Peresani M (2019) Large bovids on the
1284		Neanderthal menu: Exploitation of Bison priscus and Bos primigenius in
1285		northeastern Italy. Journal of Archaeological Science: Reports 25:129-143.
1286	8.	López-García JM, Livraghi A, Romandini M, & Peresani M (2018) The De
1287		Nadale Cave (Zovencedo, Berici Hills, northeastern Italy): A small-mammal
1288		fauna from near the onset of Marine Isotope Stage 4 and its palaeoclimatic
1289		implications. Palaeogeography, Palaeoclimatology, Palaeoecology 506:196-201.
1290	9.	Martellotta E, Livraghi A, & Peresani M (in press) Bone retouchers from the
1291		Mousterian Quina site of De Nadale Cave (Berici Hills, north-eastern Italy).
1292		Comptes Rendu Palevol.
1293	10.	Broglio A, Cilli C, Giacobini G, & Gurioli F (2006) Osso, palco, dente e
1294		conchiglia: i supporti in materia dura animale dei manufatti dei primi uomini
1295		moderni a Fumane (Verona). XXXIX Riunione Scientifica Istituto Italiano
1296		Preistoria e Protostoria" Materie prime e scambi nella preistoria italiana",
1297		(Istituto Italiano Preistoria e Protostoria), pp 815-827.
1298	11.	Peresani M (2012) Fifty thousand years of flint knapping and tool shaping across
1299		the Mousterian and Uluzzian sequence of Fumane cave. Quaternary International
1300		247:125-150.
1301	12.	Peresani M, Cristiani E, & Romandini M (2016) The Uluzzian technology of
1302		Grotta di Fumane and its implication for reconstructing cultural dynamics in the
1303		Middle–Upper Palaeolithic transition of Western Eurasia. Journal of human
1304		evolution 91:36-56.
1305	13.	Peresani M, et al. (2008) Age of the final Middle Palaeolithic and Uluzzian levels
1306		at Fumane Cave, Northern Italy, using 14C, ESR, 234U/230Th and
1307		thermoluminescence methods. J. Archaeol. Sci. 35(11):2986-2996.
1308	14.	Higham T, et al. (2009) Problems with radiocarbon dating the Middle to Upper
1309		Palaeolithic transition in Italy. <i>Quaternary Science Reviews</i> 28(13-14):1257-1267.
		C3

1310	15.	López-García JM, dalla Valle C, Cremaschi M, & Peresani M (2015)
1311		Reconstruction of the Neanderthal and Modern Human landscape and climate
1312		from the Fumane cave sequence (Verona, Italy) using small-mammal
1313		assemblages. Quaternary Science Reviews 128:1-13.
1314	16.	Fiore I, Gala M, & Tagliacozzo A (2004) Ecology and subsistence strategies in
1315		the Eastern Italian Alps during the Middle Palaeolithic. <i>International Journal of</i>
1316		Osteoarchaeology 14(3-4):273-286.
1317	17.	Falcucci A, Conard NJ, & Peresani M (2017) A critical assessment of the
1318		Protoaurignacian lithic technology at Fumane Cave and its implications for the
1319		definition of the earliest Aurignacian. <i>PloS one</i> 12(12).
1320	18.	Falcucci A. Peresani M. Roussel M. Normand C. & Soressi M (2018) What's the
1321		point? Retouched bladelet variability in the Protoaurignacian. Results from
1322		Fumane, Isturitz, and Les Cottés. Archaeological and Anthropological Sciences
1323		10(3):539-554
1324	19	Peresani M $et al$ (2019) Marine and freshwater shell exploitation in the Early
1325	17.	Upper Palaeolithic Re-examination of the assemblages from Fumane Cave (NE
1326		Italy)
1327	20	Cavallo G <i>et al.</i> (2018) Heat Treatment of Mineral Pigment During the Upper
1328	20.	Palaeolithic in North-East Italy Archaeometry 60(5):1045-1061
1320	21	Peretto C Biagi P Boschian G & Broglio A (2004) Living-floors and structures
1330	21.	from the Lower Paleolithic to the Bronze Age in Italy. Collegium antropologicum
1330		$28(1)\cdot63-88$
1332	22	Broglio A <i>et al.</i> (2003) I 'Aurignacien dans le territoire préalpin: la Grotte de
1332		Fumane XIV UISPP Congress (British Archaeological Reports) pp 93-104
133/	23	Cassoli P & Tagliacozzo $\Delta$ (1994) Considerazioni naleontologiche
1334	23.	paleoecologiche e archeozoologiche sui macromammiferi e gli uccelli dei livelli
1335		del Pleistocene superiore del Rinaro di Fumane (VR) scavi 1988–91 <i>Boll Mus</i>
1337		Civ Stor Nat Verona 18:349-445
1220	24	Broglio A Bertola S De Stefani M & Gurioli E (2009) The should red points of
1220	24.	the Early Enjargy ettian of the Berici Hills (Venetian Region, North Italy)
1240		Materiale Blanke Typology Exploitation
12/1	25	Sauro II (2002) The Monti Berici: a neguliar type of karst in the Southern Alps
1241	23.	Acta Carsologica 21(2):00 114
1342	26	Del Lago A & Miotto P (2002) Grotto dei Porioi Aspatti fisici a naturalistici
1245	20.	Dal Lago A & Micho F (2005) Ofotte del Benci. Aspetti fisici e naturalistici. Museo Naturalistico Arabeologico. Vicenza
1244	77	Museo Maturalistico Archeologico, Vicenza. Dereseni M & Derrez C (2004) Dé interprétation et mise en valour des niveeux
1245	27.	moustárians de la Grotte du Broion (Monti Borioi, Vánátia). Etudo techno
1240		áconomique des industries lithiques
1247	20	Romandini M. Rortala S. & Nannini N (2015) Nuovi dati sul Dalaalitica dai Calli
1348	20.	Romandini IVI, Bertola S, & Namini IV (2013) Nuovi dati sui Faleontico dei Com
1349		litiche delle Crette del Duse Donnie del Preier (Lurrignene Langer, Misser)
1350		nuche dena Groua dei Buso Doppio dei Broion (Lumignano, Longare, Vicenza).
1351		Nuovi aali sui Paleolitico ael Colli Berici: risultati preliminari aello studio
1352		arcneozoologico e aelle materie prime litiche della Grotta del Buso Doppio del
1353		Broion (Lumignano, Longare, Vicenza):53-59.

1354 1355 1356	29.	De Stefani M, Gurioli F, & Ziggiotti S (2005) Il Paleolitico superiore del Riparo del Broion nei Colli Berici (Vicenza). <i>Il Paleolitico superiore del Riparo del</i> <i>Broion nei Colli Berici (Vicenza)</i> :93-108
1357	30	Peresani M. Bertola S. Delpiano D. Benazzi S. & Romandini M (2019) The
1358	201	Uluzzian in the north of Italy: insights around the new evidence at Riparo Broion
1359		Archaeological and Anthropological Sciences 11(7):3503-3536.
1360	31	Romandini M <i>et al.</i> (in review) A late Neanderthal tooth from northeastern Italy
1361	51.	<i>Journal of Human Evolution</i>
1362	32	Vescovi $\mathbf{F}_{etal}$ (2007) Interactions between climate and vegetation on the
1363	52.	southern side of the Alps and adjacent areas during the Late-glacial period as
1364		recorded by lake and mire sediment archives. <i>Quaternary Science Reviews</i>
1365		26.1650-1669
1266	33	Badino E <i>at al.</i> (2010) An overview of Alpine and Mediterranean
1267	55.	palaeogeography, terrestrial ecosystems and climate history during MIS 3 with
1260		focus on the Middle to Unner Palaeolithic transition. <i>Quaternary International</i>
1260	34	Dini P. Payazzi C. & Paimer D (2010) The vegetation and climate history of the
1270	54.	lest glasial cycle in a new pollon record from Lake Fimon (southern Alpine
1370		foreland N Italy) Quaternamy Science Paviaus 20(22-24):2115-2127
1371	25	Shannon D (1076) Devised effective ionic redii and systematic studies of
1372	<i>55</i> .	Shannon R (1976) Revised effective fonic radii and systematic studies of
13/3		interatomic distances in nandes and chalcogendes. Acta crystallographica section
1374		A: crystal physics, diffraction, theoretical and general crystallography 32(5): /51-
1375	26	$10^{-1}$
1376	30.	Burton JH, Price ID, & Middleton WD (1999) Correlation of bone Ba/Ca and
13//	07	Sr/Ca due to biological purification of calcium. J. Archaeol. Sci. 26(6):609-616.
1378	37.	Elias RW, Hirao Y, & Patterson CC (1982) The Circumvention of the Natural
1379		Biopurification of Calcium along Nutrient Pathways by Atmospheric Inputs of
1380	20	Industrial Lead. <i>Geochimica et Cosmochimica Acta</i> 46(12):2561-2580.
1381	38.	Balter V (2004) Allometric constraints on Sr/Ca and Ba/Ca partitioning in
1382	•	terrestrial mammalian trophic chains. <i>Oecologia</i> 139(1):83-88.
1383	39.	Dahl S, <i>et al.</i> (2001) Incorporation and distribution of strontium in bone. <i>Bone</i>
1384	10	28(4):446-453.
1385	40.	Kshirsagar S, Lloyd E, & Vaughan J (1966) Discrimination between strontium
1386		and calcium in bone and the transfer from blood to bone in the rabbit. <i>The British</i>
1387		Journal of Radiology 39(458):131-140.
1388	41.	Burton JH & Wright LE (1995) Nonlinearity in the relationship between bone
1389		Sr/Ca and diet: paleodietary implications. American journal of physical
1390		anthropology 96(3):273-282.
1391	42.	Price TD, Swick RW, & Chase EP (1986) Bone chemistry and prehistoric diet:
1392		strontium studies of laboratory rats. American Journal of Physical Anthropology
1393		70(3):365-375.
1394	43.	Gilbert C, Sealy J, & Sillen A (1994) An investigation of barium, calcium and
1395		strontium as palaeodietary indicators in the Southwestern Cape, South Africa. J.
1396		Archaeol. Sci. 21(2):173-184.
1397	44.	Rivera J & Harley JH (1965) The HASL Bone Program, 1961-1964. (Health and
1398		Safety Lab., New York Operations Office (AEC), NY).

1399	45.	Sillen A & Smith P (1984) Weaning patterns are reflected in strontium-calcium
1400		ratios of juvenile skeletons. J. Archaeol. Sci. 11(3):237-245.
1401	46.	Lough S, Rivera J, & Comar C (1963) Retention of strontium, calcium, and
1402		phosphorus in human infants. Proceedings of the Society for Experimental
1403		Biology and Medicine 112(3):631-636.
1404	47.	Rossipal E, Krachler M, Li F, & Micetic-Turk D (2000) Investigation of the
1405		transport of trace elements across barriers in humans: studies of placental and
1406		mammary transfer. Acta Paediatrica 89(10):1190-1195.
1407	48.	ICRP (2004) Doses to infants from ingestion of radionuclides in mother's milk.
1408		ICRP Publication 95. Ann. ICRP 34(3-4).
1409	49.	Humphrey LT, Dean MC, Jeffries TE, & Penn M (2008) Unlocking evidence of
1410		early diet from tooth enamel. Proceedings of the National Academy of Sciences of
1411		the United States of America 105(19):6834-6839.
1412	50.	Müller W, et al. (2019) Enamel mineralization and compositional time-resolution
1413		in human teeth evaluated via histologically-defined LA-ICPMS profiles.
1414		Geochimica et Cosmochimica Acta 255:105-126.
1415	51.	Humphrey LT, Dirks W, Dean MC, & Jeffries TE (2008) Tracking dietary
1416		transitions in weanling baboons (Papio hamadryas anubis) using
1417		strontium/calcium ratios in enamel. Folia Primatologica 79(4):197-212.
1418	52.	Krachler M, Rossipal E, & Micetic-Turk D (1999) Concentrations of trace
1419		elements in sera of newborns, young infants, and adults. Biological trace element
1420		research 68(2):121.
1421	53.	Peek S & Clementz MT (2012) Sr/Ca and Ba/Ca variations in environmental and
1422		biological sources: a survey of marine and terrestrial systems. Geochimica et
1423		Cosmochimica Acta 95:36-52.
1424	54.	Austin C, et al. (2013) Barium distributions in teeth reveal early-life dietary
1425		transitions in primates. Nature 498(7453):216-219.
1426	55.	Krachler M, Rossipal E, & Micetic-Turk D (1999) Trace element transfer from
1427		the mother to the newborn-investigations on triplets of colostrum, maternal and
1428		umbilical cord sera. European journal of clinical nutrition 53(6):486-494.
1429	56.	Dean MC, Spiers KM, Garrevoet J, & Le Cabec A (2019) Synchrotron X-ray
1430		fluorescence mapping of Ca, Sr and Zn at the neonatal line in human deciduous
1431		teeth reflects changing perinatal physiology. Archives of oral biology 104:90-102.
1432	57.	Matos C, Moutinho C, Almeida C, Guerra A, & Balcão V (2014) Trace element
1433		compositional changes in human milk during the first four months of lactation.
1434		International journal of food sciences and nutrition 65(5):547-551.
1435	58.	Metcalfe JZ, Longstaffe FJ, & Zazula GD (2010) Nursing, weaning, and tooth
1436		development in woolly mammoths from Old Crow, Yukon, Canada: implications
1437		for Pleistocene extinctions. Palaeogeography, Palaeoclimatology, Palaeoecology
1438		298(3-4):257-270.
1439	59.	Tacail T, Kovačiková L, Brůžek J, & Balter V (2017) Spatial distribution of trace
1440		element Ca-normalized ratios in primary and permanent human tooth enamel.
1441		Science of the Total Environment 603:308-318.

1442	60.	Taylor D, Bligh P, & Duggan MH (1962) The absorption of calcium, strontium,
1443		barium and radium from the gastrointestinal tract of the rat. <i>Biochemical journal</i>
1444		83(1):25.
1445	61.	Gillespie B, d'Arcy H, Schwartz K, Bobo JK, & Foxman B (2006) Recall of age
1446		of weaning and other breastfeeding variables. International Breastfeeding Journal
1447		1:4-4.
1448	62.	Hoppe KA, Koch PL, & Furutani TT (2003) Assessing the preservation of
1449		biogenic strontium in fossil bones and tooth enamel. International Journal of
1450		Osteoarchaeology 13(1-2):20-28.
1451	63.	Hinz EA & Kohn MJ (2010) The effect of tissue structure and soil chemistry on
1452		trace element uptake in fossils. Geochimica et Cosmochimica Acta 74(11):3213-
1453		3231.
1454	64.	Radosevich SC (1993) The Six Deadly Sins of Trace Element Analysis: A Case of
1455		Wishful Thinking in Science. Investigations of Ancient Human Tissue: Chemical
1456		Analyses in Anthropology, ed Sandford MK (Gordon and Breach), pp 269-332.
1457	65.	Kohn MJ & Moses RJ (2013) Trace element diffusivities in bone rule out simple
1458		diffusive uptake during fossilization but explain in vivo uptake and release.
1459		Proceedings of the National Academy of Sciences 110(2):419-424
1460	66	Revnard B & Balter V (2014) Trace elements and their isotopes in bones and
1461	00.	teeth diet environments diagenesis and dating of archeological and
1462		naleontological samples Palaeogeography Palaeoclimatology Palaeoecology
1463		416·4-16
1464	67	Millard AR & Hedges RFM (1996) A diffusion-adsorption model of uranium
1465	07.	uptake by archaeological hope <i>Geochim Cosmochim Acta</i> 60(12):2139-2152
1466	68	Krestou A Xenidis A & Panias D (2004) Mechanism of aqueous uranium (VI)
1/67	00.	uptake by hydroxyapatite <i>Minerals Engineering</i> 17(3):373-381
1468	69	Grün R Aubert M Joannes-Boyau R & Moncel M-H (2008) High resolution
1/60	07.	analysis of uranium and thorium concentration as well as U-series isotone
1405		distributions in a Neanderthal tooth from Payre (Ardèche, France) using laser
1470		ablation ICP-MS Geochim Cosmochim Acta 72(21):5278-5290
1471	70	Trueman CN & Tuross N (2002) Trace elements in recent and fossil hone anatite
1472	70.	Providence in minoralogy and acochemistry $48(1)$ :480 521
1475	71	Turner Welker G & Descock EE (2008) Preliminary results of hone diagenesis in
1474	/1.	Scondingvion bogs, Palacogeography, Palacoelimatology, Palacoecology 266(2)
1475		A).151 150
1470	72	4).131-139. Ikam A. Nuuankuuaala A. Oduauungha S. Nuauar K. & Egishar N. (2002) Lavala
1477	12.	of 26 elements in infant formula from USA UK and Nigeria by microwaya
1478		direction and ICD, OES. Each Chamistry 77(4):420,447
1479	72	Dilandžić N. et al. (2011) Trace element levels in row mills from northerm and
1480	15.	bilandzic in, et al. (2011) Trace element levels in raw milk from northern and
1481	74	Southern regions of Croatia. <i>Food chemistry</i> 12/(1):03-00.
1482	/4.	bjorkiunu KL, <i>et al.</i> (2012) Nietais and trace element concentrations in breast
1483		milk of first time nearing motners: a biological monitoring study. <i>Environmental</i>
1484		<b>Health</b> 11(1):92.

75. Li C, Solomons NW, Scott ME, & Koski KG (2016) Minerals and trace elements 1485 in human breast milk are associated with Guatemalan infant anthropometric 1486 1487 outcomes within the first 6 months. The Journal of nutrition 146(10):2067-2074. 76. Friel JK, et al. (1999) Elemental composition of human milk from mothers of 1488 premature and full-term infants during the first 3 months of lactation. *Biological* 1489 1490 trace element research 67(3):225-247. 77. Cleveland W, Grosse E, & Shyu W (1992) Local regression models. In 'Statistical Models 1491 in S'.(Eds JM Chambers, TJ Hastie) pp. 309–376. (Chapman & Hall: New York).1. 1492 1493 Sellen DW (2007) Evolution of infant and young child feeding: implications for contemporary public health. Annu. Rev. Nutr. 27:123-148. 1494 1495