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Early life of Neanderthals

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Author Contributions

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

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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 and one

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

Significance Statement

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 constraints, both of which are still poorly understood. Here we use chemical and isotopic patterns in tandem with enamel growth rates of three Neanderthal milk teeth from Northeastern Italy to explore the early life of these individuals. Our study shows that these late Neanderthals started to wean children at 5-6 months akin to modern humans, implying similar energy demands during early infancy. Dental growth rates confirm this and follow trajectories comparable with modern humans. Contrary to previous evidence, we suggest that differences in weaning age did not contribute to the demise of Neanderthals.

Main Text

Introduction

Maternal physiology, breastfeeding and the first introduction of supplementary foods are key determinants of human growth (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, 3). Indeed, supplementary food is needed when an infant's nutritional requirements exceeds what the mother can provide through breastmilk only (4) and this dietary development can introduce foods that are higher in protein, calories and key micro-nutrients than maternal milk (4, 5). Weaning onset occurs in contemporary non-industrial human societies at a modal age of 6 months (6).

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

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

comparative specimen from the Fumane site (Fumane 2, an upper right deciduous second incisor (21), Protoaurignacian, ~40 ka) (Fig. 1).

[Insert Figure 1 here qui entrano in didascalia le citazioni Rasmussen 2014 e Seguinot 2018 (22, 23)]

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

We quantified enamel incremental growth parameters such as postnatal crown formation time and daily enamel secretion rates (25), and we detected the presence of the neonatal line as birth marker (26) by optical light microscopy on thin sections of the deciduous dental crowns. Weaning onset was investigated via Sr/Ca profiles on the same histological sections along the enamel-dentine junction (EDJ) by laser-ablation inductively-coupled-plasma mass spectrometry (LA-ICPMS) (15). In order to detect mobility and/or potential non-local food sources in maternal diet, $^{87}Sr/^{86}Sr$ isotope ratio profiles were measured by LA-multi-collector-ICPMS (see Materials and Methods) (16, 17). Moreover, we evaluated elemental ratio profiles in teeth from children with known life history (*SI Appendix*, Text S3, (15)).

Results

The neonatal lines marking birth were visible in all four archaeological specimens, despite their worn crowns (*SI Appendix*, Fig. S1), allowing the precise estimation of postnatal crown formation times (Fig. 2a). The deciduous first molar Nadale 1 and the deciduous canine Riparo Broion 1 lie within the modern human variability (27-30), while the second deciduous molar Fumane 1 shows a shorter postnatal crown formation time compared with the known archaeological and modern human range (27). The UPMH Fumane 2 deciduous lateral incisor postnatal crown formation time falls instead in the lower limit of the modern human range (28, 30). Overall, the enamel growth rates and the

time to form postnatal enamel compares well with modern human data, regardless of differences in their relative tissue volumes and morphologies (7-9).

Daily enamel secretion rates (DSRs) of all specimens, collected in the 100 µm thickness along the enamel dentine junction where laser tracks were run, are reported in Figure 2b, compared with range of variation (min., mean, max.) of modern humans (27-30). Neanderthal DSRs in the first 100 µm of enamel thickness are slower than the corresponding modern human range of variability. However, when the entire dental crown is considered, the distributions of Neanderthal DSRs lie within the lower 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 Neanderthals couple with slower DSRs than in modern humans, as expected given the thinner enamel in Neanderthals' permanent and deciduous teeth (31, 32).

[Insert Figure 2 here] references in caption: (27-30)

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

breastfeeding, yet due to even stronger discrimination across biological membranes, Ba/Ca behavior is expected to be similar to Sr/Ca (34), as indeed unequivocally observed here (*SI Appendix*, Text S3 and Fig. S6-S8) and elsewhere (15, 40-42).

[Insert Figure 3 here] references in caption: (15)

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Nadale 1 (Fig. 3b), Fumane 1 (Fig. 3c) and Fumane 2 (Fi. 3e) are sufficiently well-preserved from a geochemical point of view. Riparo Broion 1 (Fig. 3d) instead shows some diagenetic overprint (overall Ba is far more affected than Sr; see *SI Appendix*, Text S4 for our diagenesis assessment strategy and detailed description of the diagenetic imprints), but the overall biogenic elemental pattern can still be discerned (Fig. 3f, where only the portions with [U]<0.05 ppm are included in the interpolated profiles). Overall, Ba is more diagenetically affected than Sr; see *SI Appendix*, Text S4 for our diagenesis assessment strategy and detailed description of the diagenetic overprints. Two out of the three Neanderthals, Fumane 1 and Riparo Broion 1, clearly show a decreasing trend in Sr/Ca ratio immediately post-birth, followed by slope changes with the first introduction of non-breastmilk food at 115 days (3.8 months) and 160 days (5.3 months; Fig. 3c,d), respectively. An even stronger signal of transitional food intake is visible in Fumane 1 at 200 days (6.6 months) in the form of a steep increase in Sr/Ca ratio. For the oldest Neanderthal specimen Nadale 1, following a marked variability before birth, the Sr/Ca profile slightly decreases until 140 days (4.7 months, Fig. 3b). We cannot determine the weaning onset for this individual, who was still being exclusively breastfed by ~5 months of life. The UPMH Fumane 2 has a substantial portion of the prenatal enamel preserved and only a short postnatal enamel growth record (~85 days vs ~55 days respectively, Fig. 3e). This precludes the chemical detection of the onset of weaning, although the Sr/Ca drop at birth clearly indicates breast-feeding. The prenatal Sr/Ca increase in Fumane 2 could be related to changing dietary habits of the mother during pregnancy. A similar trend in prenatal enamel is observable in MCS2 (Figure 3a), whose mother followed a diet poor in meat during pregnancy.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-members rather than diverse geographical provenance of food sources (Fig. 4). These data also give insights in Neanderthal mobility and resource gathering. The $^{87}Sr/^{86}Sr$ ratios of all Neanderthal teeth overlap with the respective local baselines, defined through archaeological micromammals (43). This suggests that the mothers mostly exploited local food resources. Fumane 1 and Fumane 2, both from the same archaeological site, are characterized by contrasting $^{87}Sr/^{86}Sr$ ratios (0.7094 vs 0.7087), indicative of a different use of resources between Neanderthal (local resources) and early UPMH (non-local resources). Such behavior might have been driven by climatic fluctuations, suggesting colder conditions at ~40 ka, dominated by steppe and Alpine meadows (44).

[Insert Figure 4 here]

Discussion

Nursing strategies are strictly linked to fertility rates, maternal energetic investment, immune development and infant mortality (45). All of these ultimately contribute to demographic changes of a specific population, with key relevance to the study of human evolution. Prolonged exclusive breastfeeding has a positive impact on an infant's immune system; however, longer breastfeeding negatively influences women's fertility via lactational amenorrhea and thus inter-birth intervals (46). It has been shown that the age peak for weaning onset is reached at around 2.1 times birth weight (47), implying that infants who grow more rapidly need to be weaned earlier than those with a slower pace of growth. Based on modern models, a sustainable timing for infant weaning onset would thus range between 3 and 5 months of age (4). However, contemporary non-industrial societies start weaning their children at a modal age of 6 months (6). Similarly, the World Health Organization recommends exclusive breastfeeding for the first six months of an infant's life (48). This time frame broadly corresponds to the age at which the masticatory apparatus develops, favoring the chewing of first solid foods (4). Such evidence suggests that both skeletal development and infant energy demand contribute to the beginning of the weaning transition. Introduction of non-breastmilk foods is also crucial in reducing

the energetic burden of lactation for the mother (6). Breastfeeding represents a substantial investment of energy resources (total caloric content of modern human breastmilk =~60 kcal/100 mL) (49), entailing an optimal energy allocation between baby feeding and other subsistence-related activities.

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 (11, 12) and fully within the modern human pre-industrial figures (6). Neanderthals, therefore, were capable of being weaned at least from the fifth post-natal month in terms of supplementing the nutritional requirements of an infant that is growing a large brain with high energy requirements. This evidence, combined with deciduous dental growth akin to modern humans, indicates similar metabolic constraints during early life for the two taxa. The differential food exploitation of Fumane 1 and Fumane 2 mothers suggests a different human-environment interaction between Neanderthals and early UPMHs, as seen in Sr isotope profiles. The UPMH Fumane 2 mother was consuming low-biopurified non-local foodstuff with elevated Sr/Ca and possibly spent the end of her pregnancy and the first 55 days after delivery away from the Fumane site. The most parsimonious interpretation is that mother and child of Fumane 2 likely lived away from Fumane Cave and that, many years after, the UPMH child lost his tooth at Fumane Cave, away from his original birthplace. Conversely, all Neanderthal mothers 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). Such evidence of a seeming limited mobility for these Neanderthals women counters previous hypotheses of a large home-range of Neanderthals (50, 51).

The introduction of non-breastmilk food at ~5-6 months implies relatively short inter-birth intervals for Neanderthals due to an earlier resumption of post-partum ovulation (52). Moreover, considering the birth weight model (47), we hypothesize that 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 suggest that nursing mode and time among Late Pleistocene humans in Europe were 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 demise of Neanderthals (13). On the other hand, genetic evidence indicates that Neanderthal groups were limited in size (53), 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 (54, 55).

Materials and Methods

Thin slices of teeth preparation

Prior to sectioning, a photographic record of the samples was collected. Thin sections of the dental crowns were obtained using the standard method in dental histology described in (56, 57) and prepared at the Service of Bioarchaeology of the Museo delle Civiltà in Rome. The sectioning protocol consists of a detailed embedding-cutting-mounting procedure that makes use of dental adhesives, composite resins, and embedding resins. In order to be able to remove the crown from the resin block after sectioning and to restore the dental crowns, the teeth were initially embedded with a reversible resin (Crystalbond 509, SPI Supplies) that does not contaminate chemically the dental tissues and is soluble in Crystalbond cleaning agent (Aramco Products, Inc.). A second embedding in epoxy resin (EpoThin 2, Buehler Ltd) guarantees the stability of the sample during the cutting procedure. The sample was cured for 24 hours at room temperature. Teeth were sectioned using an IsoMet low speed diamond blade microtome (Buehler Ltd). After the first cut, a microscope slide previously treated with liquid silane (3 M RelyX Ceramic Primer) was attached on the exposed surface using a light curing adhesive (3M Scotchbond Multi-Purpose Adhesive) to prevent cracks and any damage during the cutting procedure. A single longitudinal bucco-lingual thin section, averaging 250 µm thick, was cut from each specimen. Each ground section was reduced to a thickness of ~150 µm using water resistant abrasive paper of different grits (Carbimet, Buehler Ltd). Finally, the sections were polished with a micro-tissue (Buehler Ltd) and diamond paste with 1 µm size (DB-Suspension, M, Struers).

Each thin section was digitally recorded through a camera (Nikon DSFI3) paired with a transmitted light microscope (Olympus BX 60) under polarized light, with different magnifications (40x, 100x, 400x, including the ocular magnifications). 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).

Sr isotopic analysis by solution MC-ICPMS

To determine local Sr isotope baselines we analyzed archaeological rodent teeth from the same sites where the human teeth were found (*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 (16, 58) 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 dissolution with 3M HNO_3, samples (1-5 mg in mass) were washed with MilliQ (ultrasonic bath) and leached with ~0.5 M HNO_3. Sr of the dissolved specimens was separated from the matrix using 30 µl columns and Triskem Sr-Spec resin.

Sr isotope ratios were measured using a Neptune (ThermoFisher) multi-collector inductively-coupled-plasma mass spectrometer (MC–ICPMS) housed at the Centro Interdipartimentale Grandi Strumenti (UNIMORE) during different analytical sessions. Seven Faraday detectors were used to collect signals of the following masses: ^{82}Kr, ^{83}Kr, ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr, ^{88}Sr. Sr solutions were diluted to ~50 ppb and introduced into the Neptune through an APEX desolvating system. Corrections for Kr and Rb interferences follow previous works (16). Mass bias corrections used an exponential law and a $^{88}Sr/^{86}Sr$ ratio of 8.375209 (59). The Sr ratios of samples were reported to a SRM987 value of

0.710248 (60). During one session, SRM987 yielded an average $^{87}Sr/^{86}Sr$ ratio of 0.710243 ± 0.000018 (2 S.D., n = 8). Total laboratory Sr blanks did not exceed 100 pg.

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

LA-MC-ICPMS analyses were conducted at the Frankfurt Isotope and Element Research Center (FIERCE) at Goethe University, Frankfurt am Main (Germany) and closely follow analytical protocols described by Müller & Anczkiewicz (2016) (17); only a brief summary is provided here aiming at highlighting project-specific differences. A 193 nm ArF excimer laser (RESOlution S-155, formerly Resonetics, ASI, now Applied Spectra Inc.) equipped with a two-volume LA cell (Laurin Technic) was connected to a NeptunePlus (ThermoFisher) MC-ICPMS using nylon6-tubing and a 'squid' signal-smoothing device (61). Ablation took place in a He atmosphere (300 ml/min), with ~1000 ml/min Ar added at the funnel of the two-volume LA cell and 3.5 ml/min N_2 before the squid. Laser fluence on target was ~5 J/cm^2.

Spatially-resolved Sr isotopic analyses of dental enamel were performed on the thin sections (100-150 µm thick) used for enamel histology and trace element analysis (see below), in continuous profiling mode following the enamel-dentine-junction (EDJ) from apex to cervix (14), less than 100 µm away from the EDJ. Tuning of the LA-MC-ICPMS used NIST 616 glass for best sensitivity (^{88}Sr) while maintaining robust plasma conditions, i.e. $^{232}Th^{16}O/^{232}Th$ <0.5% and $^{232}Th/^{238}U$>0.95 with RF-power of ~1360 W. In view of the low Sr concentrations in these human enamel samples (~60-100 µg/g), we utilized 130 µm spots, a scan speed of 5 µm/s and a repetition rate of 20 Hz to maintain ^{88}Sr ion currents of ~2-3.5 x 10^{-11} A. Nine Faraday detectors were used to collect the ion currents of the following masses (m/z): ^{83}Kr, ~83.5, ^{84}Sr, ^{85}Rb, ^{86}Sr, ~86.5, ^{87}Sr, ^{88}Sr, ^{90}Zr. Baseline, interference and mass bias corrections follow (17). The isotopically-homogenous (Sr) enameloid of a modern shark was used to assess accuracy of the Sr-isotopic analysis and yielded $^{87}Sr/^{86}Sr$ = 0.70916 ± 2 and $^{84}Sr/^{86}Sr$ = 0.0565 ± 1 (2 S.D.). Raw data are reported in Dataset S1.

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

All LA-ICPMS analyses of archaeological samples were conducted at the Frankfurt Isotope and Element Research Center (FIERCE) at Goethe University, Frankfurt am Main (Germany), using the same LA system described above, but connected via a squid smoothing-device to an Element XR ICPMS. Analytical protocols follow those by Müller et al (2019) (15); and only a brief summary is provided here aimed at highlighting differences. LA-ICPMS trace element ratios/concentrations of the comparative contemporary teeth were obtained at Royal Holloway University of London (RHUL) using the RESOlution M-50 prototype LA system featuring a Laurin two-volume LA cell (58), coupled to an Agilent 8900 triple-quadrupole-ICPMS (ICP-QQQ or ICP-MS/MS).

Compositional profiles were analyzed parallel and as close as possible to the EDJ, following the same tracks used for Sr isotope analyses. We employed 15 µm spot sizes (FIERCE) or 6 µm (MCS3, RHUL) and 34 µm (MCS1 and 2, RHUL), respectively, as well as a scan speed of 5 µm/s and a repetition rate of 15 Hz; prior to acquisition, samples were pre-cleaned using slightly larger spot sizes (22 - 57 µm), 20 Hz and faster scan speeds (25 - 50 µm/s); laser fluence was ~5 J/cm^2. The following isotopes (*m/z*) were analyzed: ^{25}Mg, ^{27}Al, ^{43}Ca, (^{44}Ca), ^{55}Mn, ^{66}Zn, ^{85}Rb, (^{86}Sr), ^{88}Sr, ^{89}Y, ^{138}Ba, ^{140}Ce, (^{166}Er, ^{172}Yb), ^{208}Pb, ^{238}U. The total sweep times for the Element XR and the 8900 ICP-MS/MS were ~0.8 and 0.4-0.5 s, respectively; however, because of the slow scan speeds, this small difference has no effect on the compositional profiles presented here. Primary standardization was achieved using NIST SRM612. Ca was employed as internal standard (^{43}Ca); [Ca] at 37 %m/m was used to calculate concentrations for unknown bioapatites, although not required for X/Ca ratios. Accuracy and reproducibility were assessed using repeated analyses of the STDP-X-glasses (62) as secondary reference materials; the respective values for Sr/Ca and Ba/Ca (the element/Ca ratios of principal interest) here are 1.8 ± 6.6% and -0.2 ± 6.0 % (%bias ± 2 S.D. (%)); this compares well with the long-term reproducibility for these analytes reported previously (63). Raw data are reported in Dataset S2 and S3.

The compositional/isotopic profiles were smoothed with a locally weighted polynomial regression fit (LOWESS), with its associated standard error range (±3 S.E.) for each

predicted value (64). The statistical package R (ver. 44.0.0) (65) was used for all statistical computations and generation of graphs.

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

Dental enamel is capable of recording, at microscopic level during its formation, regular physiological and rhythmic growth markers (66-68). These incremental markings are visible under transmitted light in longitudinal histological thin sections of dental crowns. Enamel forms in a rhythmic manner, reflecting the regular incremental secretion of the matrix by the ameloblasts (i.e. the enamel forming cells). The rhythmical growth of enamel is expressed in humans at two different levels: a circadian rhythm that produces the daily cross striations(69, 70) and a longer period rhythmic marking (near- weekly in humans) that give rise to the Retzius lines (71). Physiological stresses affecting the individual during tooth growth cause a disruption of the enamel matrix secretion and mark the corresponding position of the secretory ameloblast front, producing Accentuated (Retzius) Lines (ALs) (72, 73). The birth event is recorded in the forming enamel of individuals surviving the perinatal stage, and leaves - usually the first - Accentuated Line, namely the Neonatal Line (NL) (26, 74, 75).

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

A prism segment starting from the most apical available point on the enamel dentine junction (EDJ) and extending from this point to an isochronous incremental line (i.e. the NL, an AL or a Retzius line) was measured. The incremental line was followed back to the EDJ and a second prism segment was measured in the same way. The process was repeated until the most cervical enamel was reached. The crown formation time is equal to the sum of the single prism segments. To obtain time (in days) from the prism length measurements, local daily secretion rates (25) (DSR) were calculated around the prism segments and within 100 µm from the EDJ, by counting visible consecutive cross striations and dividing it by the corresponding prism length. The chronologies of accentuated lines (ALs) in the modern sample closely match the timing of known

disruptive life history events in the mother (illness, surgery) and infant, and so are well within the range or error (1.2-4.4%) observed for this histological ageing method (67).

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

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

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596 **Figure legends**
597
598 **Figure 1. Geographical, paleoecological and chronological framework.** (a) Oxygen
599 isotope curve from NGRIP (22), with Greenland Stadials 5-21 highlighted. Chronologies
600 of the human specimens are also reported (see Supplementary Information for details);
601 Fumane 2 is UPMH (green), while Fumane 1, Riparo Broion 1 and Nadale 1 are
602 Neanderthals (yellow). (b,c,d) Modelled Alpine glacier extent during the time intervals of
603 the teeth recovered at the sites of Fumane Cave (b,c), Riparo Broion (c) and Nadale (d);
604 location within Italy is shown in the inset. Simulations show a high temporal variability
605 in the total modelled ice volume during Marine Isotope Stages 4 (70 ka snapshot) and 3
606 (50, 40 ka snapshots) with glaciers flowing into the major valleys and possibly even onto
607 the foreland (23).
608
609 **Fig. 2. Dental crown growth parameters.** (a) Postnatal crown formation time in days
610 from birth for the different deciduous teeth. The range of variability reported in literature
611 for modern and archaeological individuals is represented by red, blue, green lines. (b)
612 Boxplot of the daily secretion rate (DSR) variation in the first 100 µm from the enamel-
613 dentine-junction (min, second quartile, median, third quartile, max) and range of variation
614 (min, mean, max) of modern humans (MH), re-assessed from (27-30). (c) Boxplot of the
615 daily secretion rate variation across the whole crown (mean, second quartile, median,
616 third quartile, max) and range of variation (min, mean, max) of modern humans (MH),
617 re-assessed from (27-30). Ldm1 = lower deciduous first molar; Ldm2 = lower deciduous
618 second molar; Udc = upper deciduous canine; Ldi2 = lower deciduous later incisor.
619
620 **Fig. 3. Nursing histories from time-resolved Sr/Ca variation in Middle-Upper**
621 **Paleolithic deciduous teeth.** UPMH = Upper Paleolithic modern human; NEA =
622 Neanderthal. The elemental profiles were analyzed within enamel close to the enamel-
623 dentine-junction (EDJ); [U] and [Mg] are reported as sensitive proxies for diagenetic
624 alteration (15) (see *SI Appendix*, Text S4). The birth event is highlighted by a vertical
625 line. The compositional/isotopic profiles were smoothed with a locally weighted
626 polynomial regression fit (LOWESS), with its associated standard error range (±3 S.E.)
627 for each predicted value. (a) Comparison between two contemporary known feeding
628 history individuals, MCS1 (exclusively breastfed) and MCS2 (exclusively formula-fed);
629 t1=transitional period starts, first time solid food; t2=strongly reduced breastfeeding
630 during day; t3=transitional period ends, end of breastfeeding; (b) Nadale 1: the slight
631 decrease of Sr/Ca indicates exclusive breastfeeding until the end of crown formation (4.7
632 months); (c) Fumane 1: Sr/Ca variation indicates breastfeeding until 4 months of age
633 (fully comparable with MCS1 sample, see Supplementary Figure S6); (d) Riparo Broion
634 1: Sr/Ca profile indicates exclusive breastfeeding until 5 months of age; (e) Fumane 2: 55
635 days of available postnatal enamel shows exclusive breastfeeding. (f) Fossil specimens'
636 Sr/Ca profiles adjusted to the birth event; the interpolated modelled profiles were
637 calculated based on those portions unaffected by diagenesis ([U]<limit of detection, 0.05
638 ppm), with strong smoothing parameters to reveal the biogenic signal. Riparo Broion 1,

639 the specimen most affected by diagenesis, retains the expected breastfeeding signal (see
640 panel a). See Material and Methods section for details.

641

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

650