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Sex and age-related social organization in the Neolithic: a promising survey from the Paris Basin

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Abstract:

Previous studies have identified differences based on sex in adult diet and age-based changes in the funerary treatment of children in the Neolithic of northern France. This preliminary study investigates sex-based differences and age-related changes in diet and mobility in childhood in comparison to adulthood.

Nineteen individuals from the Neolithic Yonne valley (Paris Basin) were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in dentine of the second permanent molar (M2) and 15 also for $\delta^{34}\text{S}$. Analyses were performed on different tooth parts depending on the individuals and targeted different age groups: bulk coronal (ca. 2.5-8.5 years; n=16 adults) and root dentine (ca. 8.5- 12 years; n=1 juvenile) and dentine microsections of the entire tooth (6 age groups between 2.5 and 16 years old; n=2 adults).

Higher $\delta^{15}\text{N}$ values in dentine than in adult bone reflect children's dietary specificity, possibly due to their nutritional needs during growth. There is no mean isotopic difference in coronal dentine between males and females under 9 years old. Dietary sex-based division occurs later. Two isotopic shifts are observed on the incremental profiles, indicative of social transitions, possibly sex-related, around 8-9 and 14 years old. The variability of $\delta^{34}\text{S}$ values highlights different mobility patterns between the sexes and argues for patrilocality. These results echo previous studies and offer a promising perspective which must be investigated further on a larger sample size.

Keywords: stable isotope ratios, incremental dentine, early farmers, diet, mobility

1 1. Introduction

2 Studies of eating behaviors and mobility patterns help address the issue of social
3 organization in human communities. Stable carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and more recently sulfur
4 ($\delta^{34}\text{S}$) isotope analyses are routinely used in archaeology to reconstruct the diet and mobility of past
5 populations (*e.g.*, Fernández-Crespo et al. 2020; Goude et al. 2020b; Moghaddam et al. 2016; Nehlich
6 et al. 2014; Siebke et al. 2020). While bulk collagen stable isotope analysis of bones and teeth is the
7 common approach (a brief methodological background is provided in Supporting Information 1),
8 sequential analysis of dentine recently demonstrated the possibility of tracking individual early life
9 history at a high resolution (*e.g.*, Beaumont et al. 2013a; Beaumont et al. 2015; Goude et al. 2020a;
10 King et al. 2017). Moreover, sampling in both bone and teeth enables identifying differences
11 registered at distinct moments of life.

12 This paper presents a preliminary investigation of dietary and mobility patterns in childhood,
13 including sex-based differences and age-related changes, in a population with known sexual
14 differences in adult food consumption (Rey et al. 2017; Rey et al. 2019). The samples come from
15 three Middle Neolithic sites located in the Yonne Valley (Paris Basin, France; Fig 1): Gurgy “Les
16 Noisats” (GLN), Monéteau “Macherin” (Mon) and Chichery “Sur les Pâturaux” (CHI-Pat).
17 Archaeological and anthropological information is available in Chambon et al. (2013) and
18 summarized in Rey et al. (2019). Previous isotopic analyses of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ of bone collagen
19 have pinpointed significant differences between adult males and females (Rey et al. 2019). On the
20 other hand, trace element analysis (Ba/Ca; Sr/Ca) on tooth enamel did not support those sex-based
21 differences in trophic levels during the early phases of life (Rey et al. under review). This
22 inconsistency throughout the life course raises the question of the emergence of sexual division and
23 social organization during a person’s lifespan. For the Neolithic period, when social differences
24 become more pronounced, sexual division is one of the most frequently addressed topics in human
25 bioarchaeology (*e.g.*, Cintas-Peña and García Sanjuán 2019; Goude et al. 2019; Knipper et al. 2017;
26 Robb and Harris 2018; Siebke et al. 2020), but it is somewhat less well-known in children (*e.g.*, Bickle
27 and Fibiger. 2014; Fernández-Crespo et al. 2018).

28 Furthermore, in northern France, the study of funerary practices has highlighted two age
29 related changes, around 8-9 and 14-15 years of age, probably linked to a change in social status (Le

30 Roy et al. 2018; Supporting Information 2). “Adolescence”¹ could be a transitional period towards
31 adulthood in the social organization of these farmers, including changes in diet and/or mobility.

32 2. To investigate this issue and track possible variations in lifetime, carbon, nitrogen and
33 sulfur stable isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) were analyzed in the dentine and
34 compared to previous results obtained in bone collagen. While $\delta^{13}\text{C}$ is rather linked to
35 environmental variations, $\delta^{15}\text{N}$ is a good indicator of trophic level, as high $\delta^{15}\text{N}$ values
36 often reflect high animal protein intake (as well as breastfeeding signal; e.g. Lee-Thorp
37 2008; Tsutaya and Yoneda 2015). In addition, S isotope ratios vary among resources
38 depending on location and can reflect both dietary specificities and/or mobility patterns
39 (for a review of methodologies, see Nehlich 2015). A previous study on bone collagen
40 (Rey et al. 2019) provides more details about specific isotopic variations linked to diet
41 and mobility in this population. Material and methods

42 2.1. Sample selection and age alignment

43 Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analysis was performed on dentine samples
44 of 19 human second molars (M2), 15 of which were also analyzed for sulfur ($\delta^{34}\text{S}$). Four samples did
45 not contain sufficient collagen to conduct S analysis after the C and N analyses (which required here
46 between 0.9 and 1.1 mg for CN and ca. 8 mg for S). Most of the analyses were performed on the bulk
47 coronal dentine of completely formed permanent teeth of adult individuals (Fig 2). The crown only
48 was sampled for two reasons: to match, in terms of age signal, other analyses performed in the
49 enamel and to avoid averaging samples of widely different age categories. According to the London
50 Atlas of Queen Mary University of London (AlQahtani et al. 2010), the crown of the second molars
51 form approximately between 2.5 ± 0.5 and 8.5 ± 0.5 years of age (stages Ci to Crc). For these
52 samples, the comparison with bone values thus reflects the difference between childhood and
53 adulthood. For one child sample (GLN 215B), whose age-at-death was estimated to be between 8
54 and 14 years old (according to the stage of dental development of several teeth and considering the
55 broadest range of variation given by AlQahtani et al. 2010, namely including minimum and maximum
56 development stages), the root dentine was analyzed to directly compare it to the bone results. This
57 root (of a lower second molar) was incompletely formed (stage R1/2). Its growth period (stages Ri to

¹The word “adolescence” is used here to refer to the transitional period between the end of childhood and the beginning of adulthood, in this study defined between 8-9 years and 14-15 years of age according to the results published in Le Roy et al. (2018) and here. This definition is not meant to necessarily apply elsewhere and the cultural definition of this concept is not discussed here.

58 R1/2) starts in average at 8.5 ± 0.5 and stops in average at 12 ± 0.5 years old according to AlQahtani
59 et al. (2010).

60 Moreover, two complete permanent molars belonging to two adults were cut into six
61 horizontal sections of ca. 2 mm each for incremental dentine analysis (Fig 2). This sectioning strategy
62 (thicker than in other studies) was chosen to ensure sufficient data was available, considering the
63 already known heterogeneity of the collagen preservation in the samples. This strategy only allows
64 for preliminary insights into the potential of such an approach. The approximate age ranges
65 represented by each section were estimated according to Czermak et al. (2020). Since the actual
66 growth rate within past populations is not known, it is generally assumed that dentine develops at a
67 regular rate of approximately 4-6 μm per day (Dean and Scandrett 1995). The duration (years) in the
68 development of dentine was usually divided by the number of sections according to the tooth length
69 so as to obtain the midpoint age for each section (Beaumont and Montgomery 2015). However,
70 some studies have shown that the growth rate is variable throughout the formation of a tooth (Dean
71 and Cole 2013; Scharlotta et al. 2018). Recently, Czermak et al. (2020) reassessed the age-alignment
72 from a “more detailed reading of AlQahtani et al. (2010)” and proposed a new approach by using
73 various growth rates according to anatomical tooth areas. The present study relies on this calibration
74 method to assign an approximate age range to each section (a calibration scheme is provided in
75 Supporting Information 3) and from this the midpoint age estimation. The latter will be used in order
76 to simplify the discussion, but the error range is displayed on the graphs. According to AlQahtani et
77 al. (2010), the dentine of M2 starts to develop at 2.5 ± 0.5 years and ends at 16 ± 0.5 years (from
78 stage Ci to Ac). The development of both teeth sampled was completed. Figure 2 shows the
79 estimated approximate ages delimiting each section. Considering the large thickness of the sections,
80 the variability in growth rate is averaged, like the isotopic ratios registered, making the possible error
81 in age determination less significant. The sections d1 and d2 were averaged to obtain a coronal
82 dentine mean (Fig 2) in order to compare them to the other bulk coronal dentine samples.

83 The main archaeological and biological data of the samples are provided in Supporting
84 Information 4. Since isotope analyses are destructive, as much information as possible was recorded
85 for future research purposes. All observations, external metrics and pictures were recorded. For
86 several teeth, calculus samples were collected for potential later investigations and an impression of
87 the crown was taken for dental microwear texture analysis (DMTA; see Rey et al. 2016 for
88 preliminary work). Some teeth had also been previously scanned (μCT) for an internal tooth structure
89 study (Le Luyer et al. 2016). All the individuals had already been analyzed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ in
90 bone collagen (Rey et al. 2017; Rey et al. 2019) and also for radiogenic strontium ($^{87/86}\text{Sr}$) and trace
91 element (Ba/Ca, Sr/Ca, Mg/Ca) ratios in dental enamel of the same teeth by means of laser ablation

92 (Rey et al. under review). The teeth had thus already been cut and embedded in resin for these
93 previous studies, and the remaining parts were used here. The samples were selected for their
94 archaeological interest, on the basis of the previous geochemical results and for their less damaged
95 condition. However, some caries and strong dental wear were also recorded for a few other teeth
96 when no other unworn M2 was available (Supporting Information 4). These teeth will be considered
97 with caution because they can include part of tertiary dentine (Nanci 2003; van Rensburg 1987). In
98 any case, the loss of dentine (even for the most worn teeth) remains negligible and should not have
99 had an influence on the results (interpretation in terms of age, especially, must be the same given
100 the already large average considered and the actual interindividual variation).

101 2.2. Sample preparation and analyses

102 The teeth had been previously cut longitudinally from the tip of the cusps to the tip of the
103 roots along the bucco-lingual axis. Mesial parts had been used in a previous study (Rey et al. under
104 review). The remaining distal half of each tooth were either entirely used for dentine micro-sampling
105 analysis (two individuals) or re-cut in half and then perpendicularly at the crown root junction (crj),
106 for the bulk coronal or root dentine analysis (Fig 2). The remaining fragments will be used in future
107 analyses. The teeth were then processed for collagen extraction according to the local laboratory
108 standard protocol (Goude et al. 2019) and, for the incremental stable isotope analysis, the protocol
109 described in Beaumont et al. (2013b) (more details in Supporting Information 1). Freeze-dried
110 collagen was then analyzed by EA-IRMS (Europa Scientific EA analyzer coupled to Europa Scientific
111 20-20 IRMS; IsoAnalytical Ltd, Crewe, UK). Isotopic values were corrected using at each run in-house
112 standards calibrated with IAEA standards. Internal error (calculated on within-run replicate
113 standards) is 0.1‰ for CN and 0.2‰ for S. The quality of collagen and the reliability of isotopic data
114 were controlled according to international recommendations: C>30 %, N>10 % (Ambrose 1993), C/N
115 between 2.9 and 3.6 (DeNiro 1985), S between 0.15 and 0.35%, C/S between 300–900 and N/S
116 between 100–300 (Nehlich and Richards 2009).

117 Statistical analyses were performed using R 4.0.2 (R Core Team 2020) within Org mode
118 (Schulte et al. 2012). Percentile bootstrap with 9999 replicates was used to compute confidence
119 intervals for differences in means or variances among groups (Zieffler et al. 2011). These confidence
120 intervals were computed at both the classical nominal level (95%) and an adjusted level provided by
121 Hesterberg (2014) as a correction for small samples. Permutation tests were also used to derive
122 empirical p-values in group comparisons. More theoretical details, along with additional results and
123 all the R code written for this study is available as Supporting Information 5, and the R packages used
124 are detailed in the Appendix. The present study's data are available online on Zenodo (Rey et al.
125 2020).

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3. Results and discussion

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All samples provided reliable results according to the quality control criteria (Table 1). The isotopic results are plotted in Figure 3 and summarized in Table 2. There is no difference in isotopic values for either teeth with caries or with strong wear (Supporting Information 6). For the bulk coronal dentine samples, $\delta^{13}\text{C}$ values range from -21.3 to -20.3‰ ($\Delta=1$, $n=18$), $\delta^{15}\text{N}$ from 10.8 to 12.8‰ ($\Delta=2$, $n=18$) and $\delta^{34}\text{S}$ from -2.6 to 5‰ ($\Delta=7.6$, $n=15$). For the root dentine sample (child GLN 215B), the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values fall within these ranges, although they are among the lowest values (Fig 3, Table 1). It is noteworthy that these values are similar to those from the bone of the same individual (Table 1), both tissues (tooth and bone) corresponding to a signal registered during two overlapping time periods (Beaumont et al. 2018). Furthermore, according to the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios (Fig3), the samples from CHI-Pat and Mon seem to cluster “by site”, supporting a previous tendency observed between these sites (Rey et al. 2019). However, considering the overall uniform dispersion of values (Fig3, Fig1 in Supporting Information 5), the sites will be considered as a whole in the rest of the paper.

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3.1. General comparison between dentine and bone

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Globally, the $\delta^{15}\text{N}$ data from coronal dentine samples are higher than in adult bone collagen (mean = 12.1‰ in dentine vs. 11.6‰ in bone, with an adjusted 97% percentile bootstrap interval of [0.0111, 0.9333] for the difference between coronal dentine and adult bone means; Table 2, Supporting Information 5). For the sake of consistency regarding the signal period, the child GLN 215B (bone and root dentine samples) is excluded from the comparison which only concerns adult bone and coronal dentine samples. Conversely, the observed $\delta^{13}\text{C}$ values for dentine and bone are closer (Fig3) and the percentile bootstrap confidence intervals do not support a difference in mean values between adult bone and coronal dentine (Table 2, detailed results available in Supporting Information 5). For $\delta^{34}\text{S}$, the dentine values are on average slightly lower than the bone values (Table2). Considering the great variability, this difference is small and the bootstrap intervals give only a weak or even no real difference between dentine and bone means (Supporting Information 5). This suggests that part of the resources consumed in childhood (especially by the individuals with the lowest values) may come from another environment. This will be discussed later. For $\delta^{15}\text{N}$, most of the dentine samples are higher than 12‰, while four samples are clearly lower, near 11‰, among which three from GLN have also lower $\delta^{13}\text{C}$ values (one of them is the “older” root sample GLN 215B; Fig 3). This pattern supports the trend previously observed in bones: most of the children (<15 years old) presented extreme $\delta^{15}\text{N}$ values (> 12 and \approx or < 11 ‰) compared to the adult group (mainly comprised between 11 and 12.5 ‰; Fig6a in Rey et al. 2019). In both cases (high and low $\delta^{15}\text{N}$ values relative to adult bones), this may express the physiological metabolism linked to growth, reflecting

160 particular nutritional needs (de Luca et al. 2012) as observed in previous archaeological studies
161 (Beaumont et al. 2018). The influence of metabolic processes involved during growth on the $\delta^{15}\text{N}$
162 values of bone collagen is poorly understood, and while some studies record an increase in $\delta^{15}\text{N}$ in
163 younger individuals (e.g. Ambrose 2002), others conversely record a decrease (e.g. Fuller et al. 2004)
164 or no significant impact (e.g. Waters-Rist and Katzenberg 2010). Moreover, some physiological stress
165 (more prevalent in children) can also impact the $\delta^{15}\text{N}$ values: if nutritional stress (malnutrition) or
166 some pathologies can cause an increase in $\delta^{15}\text{N}$ due to the recycling of body proteins (e.g. Fuller et al.
167 2005; Mora et al. 2021), several diseases can inversely lead to a decrease of $\delta^{15}\text{N}$ (e.g. Goude et al.
168 2020a; Hatch et al. 2006; Petzke et al. 2006). It is also possible that children consumed another diet
169 than that of adults due to their social age, or that some individuals, those with the most different
170 dentine values relative to adult bone, come from another environment (see later). Furthermore, the
171 inclusion of a breastfeeding signal could lead to higher $\delta^{15}\text{N}$ ratios (Beaumont et al. 2015; Tsutaya and
172 Yoneda 2015). However, here the ratios are averaged over the duration of the signal from 2.5 to 8.5
173 years old \pm 0.5 and it is unlikely that breastfeeding was still significant during this period. For the
174 Neolithic period, children are expected to be weaned around 2 - 3 years of age, although this may be
175 delayed in many circumstances (Ash et al. 2016; Goude et al. 2020a; Stuart-Macadam and Dettwyler
176 1995). Further information regarding the potential length of weaning can be obtained by analyzing
177 first molars from the same individuals.

178 A comparison with previous isotope data obtained in children's bones is proposed in Fig 4. A
179 decrease in $\delta^{15}\text{N}$ values with age is observed in the bones of children under 14 years old, especially
180 for males (b). However, the bone samples of children recorded the signal of those who did not
181 survive into adulthood. They may have had specific nutrition related to their health condition (e.g.,
182 Beaumont et al. 2018; Goude et al. 2020a). The lowest values, in particular, may reflect a specific diet
183 or some (patho-)physiological stress associated with a positive nitrogen balance (Petzke and Fuller.
184 2012). On the contrary, the highest $\delta^{15}\text{N}$ values probably include a breastfeeding signal, as bone
185 records a signal averaged over several years before death. The samples with the highest values also
186 seem to correspond to the youngest children. When excluding the extreme values, the difference
187 between males and females is statistically significant ($p < 0.05$ according to Mann-Whitney),
188 conversely to what is observed in the teeth (see later). Although the small sample size may have
189 biased this observation, it is possible that deceased (possibly as a result of illness) boys and girls did
190 not receive the same treatments/consideration according to their sex. Boys who died young may
191 have had a higher protein-rich diet than little girls. Because bone considers a much longer period
192 than the teeth (especially when, as in the present study, only the crown is considered) and because
193 this signal may be further disturbed by the specific physiological or pathological status of these

194 children who died young (and also because of the broad estimated range of age at death of some of
195 these children), it is difficult /not relevant to link any isotopic variation observed in the bone with a
196 specific age, due to different incorporation times of the elements and mineralization rates
197 (Beaumont et al. 2018).

198 3.2. Sex-related variations in dentine

199 While higher $\delta^{15}\text{N}$ values are observed in dentine compared to the bones of all adults, it is
200 noteworthy that the bone-dentine difference in $\delta^{15}\text{N}$ is emphasized by the low $\delta^{15}\text{N}$ values of female
201 bones (Fig 3). Previous results have shown that in adult bone, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios were
202 statistically different between males and females (Rey et al. 2019). In dentine, there is no evidence of
203 differences in means between males and females for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ (Table 2, Supporting
204 Information 7, detailed results available in Supporting Information 5). This supports previous trace
205 element results in dental enamel of the same teeth: the Ba/Ca and Sr/Ca ratios were not different
206 according to sex (Rey et al. under review). This may indicate that dietary sexual division occurs after
207 the M2 crown formation is completed, so after 9 years. However, the signal is averaged over the
208 duration of tooth development and the change may occur before the crown is completely formed
209 without it being visible here. This will be further discussed with the incremental dentine profiles.
210 Notwithstanding, the study of funerary practices in Middle Neolithic northern France has highlighted
211 substantial differences in grave goods and the position of the bodies both around 8-9 and 14-15
212 years of age (Supporting Information 2; Le Roy et al. 2018). Such changes of treatment in death
213 reflect a change in the role or place of the individuals within society, namely a change in social status.
214 These observations allow identifying different social age ranks: children under 8-9 years, adolescents
215 between 8-9 and 14-15 years, and adults over 14-15 years of age. A concomitant change in dietary
216 habits, revealed by isotopic data, would not be inconsistent. Over 14-15 years old, males and females
217 were probably feeding differently, maybe according to specific sex-related activities, as shown for
218 example in ethnological studies (*e.g.*, Berbesque et al. 2011; Kaplan et al. 2009; Umezaki et al. 2016).
219 Males could have had a more animal protein rich-diet for example (sex-related dietary differences
220 are further discussed by Rey et al. 2019). Sexual division of labor has been proposed for Prehistoric
221 Europe (*e.g.*, Villotte and Knüsel 2014; Wünsch and Gibaja Bao 2003). On the contrary, children
222 under 8-9 years old (maybe up to 14) were in all likelihood consuming the same resources regardless
223 of their sex. Figure 5 shows for each individual the isotopic difference registered between dentine
224 and bone. Except the juvenile GLN 215B, all individuals are adults so the comparison mainly reflects
225 childhood vs. adulthood. It is particularly evident that the $\delta^{15}\text{N}$ values of females almost
226 systematically decrease from dentine to adult bone, contrarily to the ones of males for whom they
227 vary indifferently and to a lesser extent. This confirms that the dentine-adult bone difference is

228 mainly sex-related and the transition from childhood to adulthood involves primarily dietary change
229 for females and/or mobility from different isotopic environments.

230 3.3. Sulphur isotope ratios and mobility patterns

231 Furthermore, the observed variance of $\delta^{34}\text{S}$ in dentine is slightly wider for females, who have
232 the lowest and the highest values (Fig 3b, Fig 5 in Supporting Information 5). The variance is also
233 wider in dentine (with values ranging from -2.6 to 5 ‰) than in bone (1.4 to 6.3 ‰), with a lower
234 mean in dentine (2.4 vs. 3.7 in bone; Table 2). Although the permutation test could not provide any
235 strong evidence in favor of this difference ($p = 0.117$), this might indicate a more varied environment
236 for females between 2 and 9 years of age, potentially associated with different origins (genetic work
237 is in progress within the ANR program Interact). Figure 5 highlights a great difference in $\delta^{34}\text{S}$ values
238 between childhood and adulthood in some females, without correlation with C and N isotopes. This
239 can reflect different origins. On the other hand, the individual $\delta^{34}\text{S}$ dentine-bone differences are also
240 relatively high for males, possibly indicating a certain degree of mobility too. This supports an
241 ongoing study of strontium (Sr) isotope ratios that suggested a sex-related mobility pattern with
242 possible foreign origins for females (Rivollat et al, forthcoming). The question of patrilocality has
243 been raised several times in similar contexts (Bentley et al. 2012; Goude et al. 2020b; Goude et al.
244 2019; Knipper et al. 2017; Rasteiro and Chikhi 2013; Rivollat et al. 2017) and a genetic study
245 combined to new Sr isotopic data is currently in progress to specifically address this issue.

246 3.4. Refining the age-alignment with incremental dentine analysis

247 The incremental section values along with the bulk dentine samples are represented against
248 age in Fig 6. Isotopic results of each dentine section are given in Table 3. The S analysis was
249 conducted on the remaining collagen after C and N analysis, which was only sufficient to provide
250 results for 5 sections of GLN 221B and 1 section of GLN 284. Globally, the male GLN 221B presents
251 higher $\delta^{15}\text{N}$ values and lower $\delta^{13}\text{C}$ values than the female GLN 284. For $\delta^{13}\text{C}$, both patterns are rather
252 similar although that of the female is more variable. The difference between them decreases with
253 age, though it is never very significant, even before 8 years of age where it remains lower than 0.6‰
254 (Fig 6). Between the midpoint ages of 8 and 9, the $\delta^{13}\text{C}$ ratios of both suddenly decrease and become
255 less variable, slightly decreasing, until 14 years of age. Then they are slightly higher in adulthood
256 (bone signal). On the contrary, for $\delta^{15}\text{N}$ the patterns are rather the opposite and the difference
257 between both individuals increases with age (Fig 6). Until 9 years old, both individuals have among
258 the highest $\delta^{15}\text{N}$ values of all the samples. Then, those of the male GLN 221B increase again until age
259 14, reaching the highest value of all the samples, and then slightly decreasing between 14 and
260 adulthood, where it still remains the highest value of all the samples. Inversely, those of female GLN

261 284 strongly decrease between 8 years old and adulthood, stabilizing around 10-12 years old, to
262 switch from the highest to the lowest value of all the samples. This indicates that the diet of these
263 children may be enriched in ^{15}N regardless of sex until 8-9 years of age and that differentiation
264 (possibly sexual) occurs later. This supports and specifies the previous results observed on bulk
265 dentine samples. A first shift could be identified around age 8-9, maybe mainly for female(s), and a
266 second shift, although less evident, could be observed around 14 years old, possibly mostly in
267 male(s). Although this must be observed on more samples, this already echoes the changes identified
268 in the funerary practices around 8-9 and again at 14-15 years old (Fig 6; Le Roy et al. 2018).
269 Generally, it is not possible to distinguish male from female treatments when studying the remains of
270 children since their sex cannot be determined without a genetic assessment (which was the case for
271 Le Roy et al. 2018). In such a context, this study shows that the present approach can highlight sex-
272 specificities. However, both GLN 221B and GLN 284 were chosen precisely because they presented
273 extreme values in bone, which may reflect a specific access to resources, maybe due to a particular
274 social status or a physiological effect possibly associated to a health condition, although no
275 pathologies were identified on the skeleton (Rey et al. 2019). It is therefore not excluded that factors
276 other than dietary and environmental change, such as growth or physiological stress, might have
277 been involved and would explain the variations observed between the curves. In addition, the
278 difference between only two individuals of either sex may not reflect a real social distinction based
279 on sex. More incremental dentine analyses are required to further define the age of dietary
280 change(s) in this population and the possible sex-related specificities.

281 Nevertheless, this result is consistent with the concept of "middle childhood" proposed by
282 different authors for past and present populations (e.g. Eccles et al. 1999, Bickle and Fibiger 2014).
283 Based on a review of the literature on the Linearbandkeramic Culture (LBK) of the Early European
284 Neolithic, Bickle and Fibiger (2014) suggest that from around 8 years of age and until puberty (the
285 period may vary from one individual to another, according to the child's capacity more than his
286 chronological age) children may have start to be increasingly gender recognized. "Middle childhood"
287 represents a period of biological and cognitive changes, where children develop different social
288 relationships, roles and desire for autonomy (Eccles et al. 1999). As proposed for the LBK culture, at
289 this age they could then learn to become adults and take part in gender-related tasks (Bickle and
290 Fibiger 2014). In our study, this could correspond to an increasing differentiation in diets and/or
291 mobility, probably associated with a gradual change in social status that could result in a division of
292 labor between sexes with specific sex-related diets. For $\delta^{34}\text{S}$, the female GLN 284 also has the highest
293 value in bone, but the dentine value falls within the range of all the bulk dentine samples. If this
294 female spent her childhood in another environment, the S signature of this environment was not

295 different to the local one. During adulthood, the high value can, on the contrary, reflect diet
296 specificity. Indeed, the S isotope ratio is strongly linked to both the C and N isotope ratios ($p < 0.05$
297 according to a Spearman correlation, Supporting Information 8), mainly reflecting both the
298 environmental and dietary aspects, respectively. On the other hand, the male $\delta^{34}\text{S}$ values are highly
299 variable until the midpoint age of 10 years old, although still within the range of the whole sample
300 (as there is only one data point for the female, a comparison of their $\delta^{34}\text{S}$ variability is not possible).
301 This is in line with a high degree of mobility in childhood, which has been previously proposed from
302 bulk dentine results. To the authors' knowledge, this is the first time that high mobility for male
303 children is proposed for the Neolithic period. Indeed, mobility may be characterized by several forms
304 of movement: different origin vs. residential mobility, training period in a neighboring group or
305 fosterage (Massard-Vincent 1996), or transhumance and herding outside the villages (Bentley and
306 Knipper 2005). Further incremental dentine analysis and new strontium isotope results by laser
307 ablation (in progress) could lead to a better understanding of the question of mobility by identifying
308 the recurrence and the diversity of residential environments.

309 4. Conclusion and perspectives

310 This preliminary work was conducted in addition to a set of various other analyses and aims
311 to help establish an initial overview of social organization in the Neolithic correlated to sex and age in
312 childhood. Along with the results from the study of funerary practices, the analyses presented here
313 offer new insights and a promising perspective into the change of social status during childhood as
314 revealed through diet. The combined results from a small set of bulk dentine samples and two
315 incremental dentine analyses of both a male and a female helped identify two possible transitional
316 steps during life. Children under 8-9 years of age in all likelihood consumed the same resources
317 regardless of their sex, while some of them, mainly females, probably originated from other places.
318 This raises once again the question of patrilocality in a Neolithic context. Furthermore, a new vision
319 of early farmers' organization emerges: under approximately 10 years of age, the high variability of S
320 values over time for GLN 221B may reflect certain mobility. This is not necessarily linked to a
321 different geographic origin but may reflect occasional travel(s). As this is only observed in one
322 individual, further analysis is needed to determine whether this type of mobility is a cultural trait,
323 and whether it can be extended to other individuals. Moreover, around the age of 8-9 years, children
324 presumably became adolescents, an intermediate step on the path to adulthood achieved at around
325 14-15 years of age when they became socially "gender-identified". Considering the relatively low
326 precision scale of this study and its preliminary approach, further analyses must be performed in
327 order to better assess dietary and mobility variation throughout the lifespan. The combined analysis

328 of first and third molars could allow, on the one hand, to identify the age of weaning according to sex
329 through the first molar (see for example Eerkens and Bartelink 2013) and, on the other hand, to
330 refine the transitional age from adolescence to adulthood through the third molar. Moreover, this
331 approach enables the assessment of sexual differences in children, while the study of funerary
332 practices fails in this regard as the sex of deceased children cannot be reliably determined without
333 genetic analysis.

334 Declaration of interest

335 None

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343 millennium BC, France: a bio-anthropological approach; Dir. G. Goude 2016-2017;
344 [http://institutdanone.org/nos-prix/femmes-alimentation-les-premieres-societes-agropastorales-ve-
345 iiiie-millennaires-av-j-c-france-approche-bio-anthropologique/](http://institutdanone.org/nos-prix/femmes-alimentation-les-premieres-societes-agropastorales-ve-iiiie-millennaires-av-j-c-france-approche-bio-anthropologique/)) and funded by a ministerial grant from
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348 Rottier; Université de Bordeaux, LaScArBx-ANR; 2012–14)

349 Data availability statement

350 The authors declare that the data supporting the findings of this study are all available within
351 the article, its supporting information (SI) files and online on Zenodo
352 (<http://doi.org/10.5281/zenodo.4047736>).

353 Appendix: R packages used

- 354 • boot 1.3-25 (Canty and Ripley 2020)
- 355 • lawstat 3.4 (Gastwirth et al. 2020)
- 356 • resample 0.4 (Hesterberg 2015)

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Figure Legends

Fig1: Location map of the sites mentioned in the text, modified after Chambon et al. (2013).

Fig2: Summary of sampling selection: quantity of samples and analyses performed, tooth part sampled and corresponding age. Edj = enamel dentine junction, crj = crown root junction.

Fig3: Distribution of isotopic results in dentine by site and by sex. The bone mean results by sex from a previous study (Rey et al. 2019) are given for comparison. (a) $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ and (b) $\delta^{34}\text{S}$ vs. $\delta^{15}\text{N}$.

Fig4: Distribution of carbon (a) and nitrogen (b) isotopic values of children's bones from GLN (those who did not survive into adulthood) according to their age-at-death and compared to the isotopic values of adults' bones from the same site. These results come from a previous study (Rey et al. 2019) and the sex of the children was recently determined by genetic analysis (publication forthcoming).

Fig5: Individual differences in isotopic ratios between dentine and bone for females and males. *No $\delta^{34}\text{S}$ value.

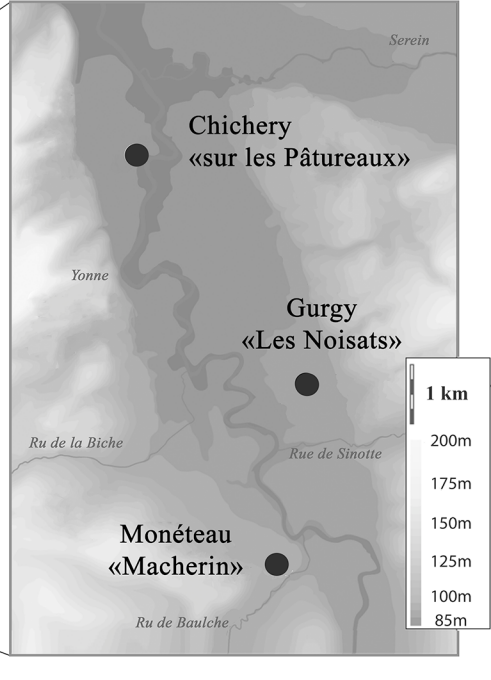
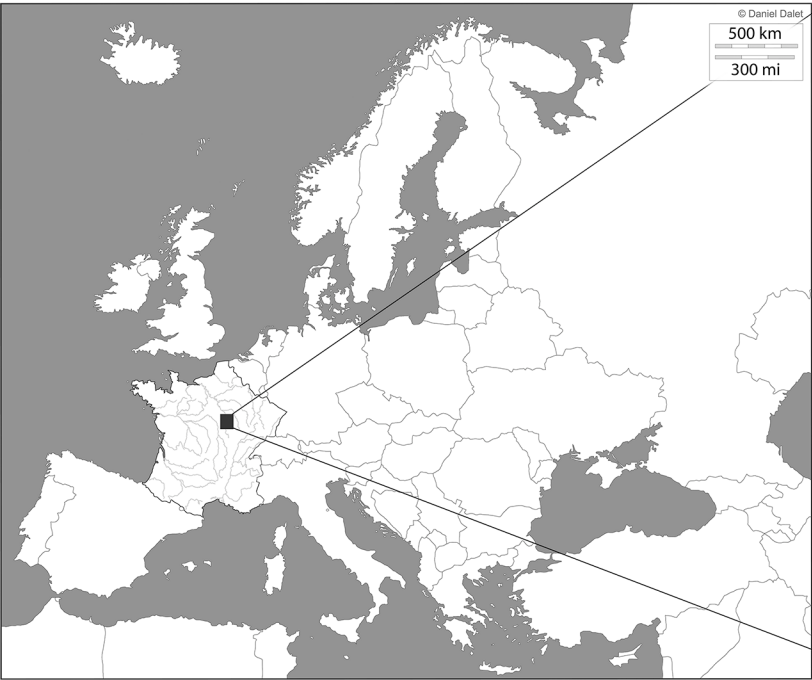
Fig6: On the left, isotopic ratios of bulk and incremental dentine samples by sex according to the corresponding estimated age. On the right, detail of the incremental dentine profiles of both a male and a female according to the corresponding estimated age and alignment with the transitional age identified through the study of funerary practices (Le Roy et al. 2018).

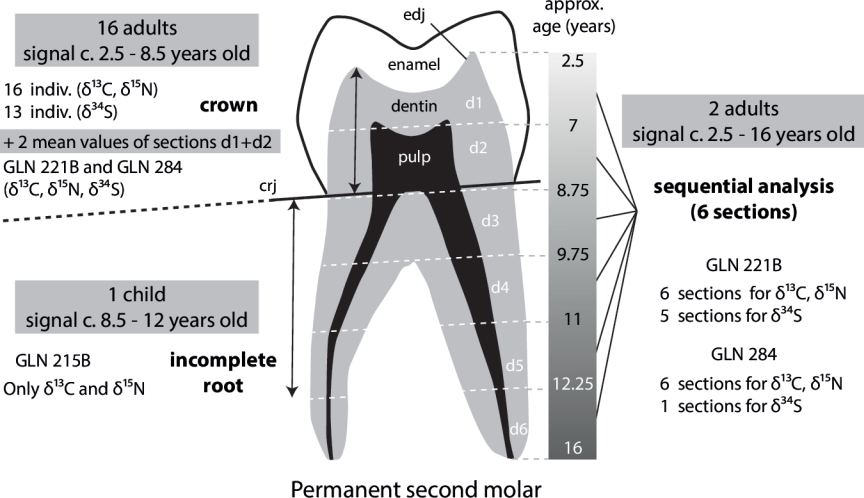
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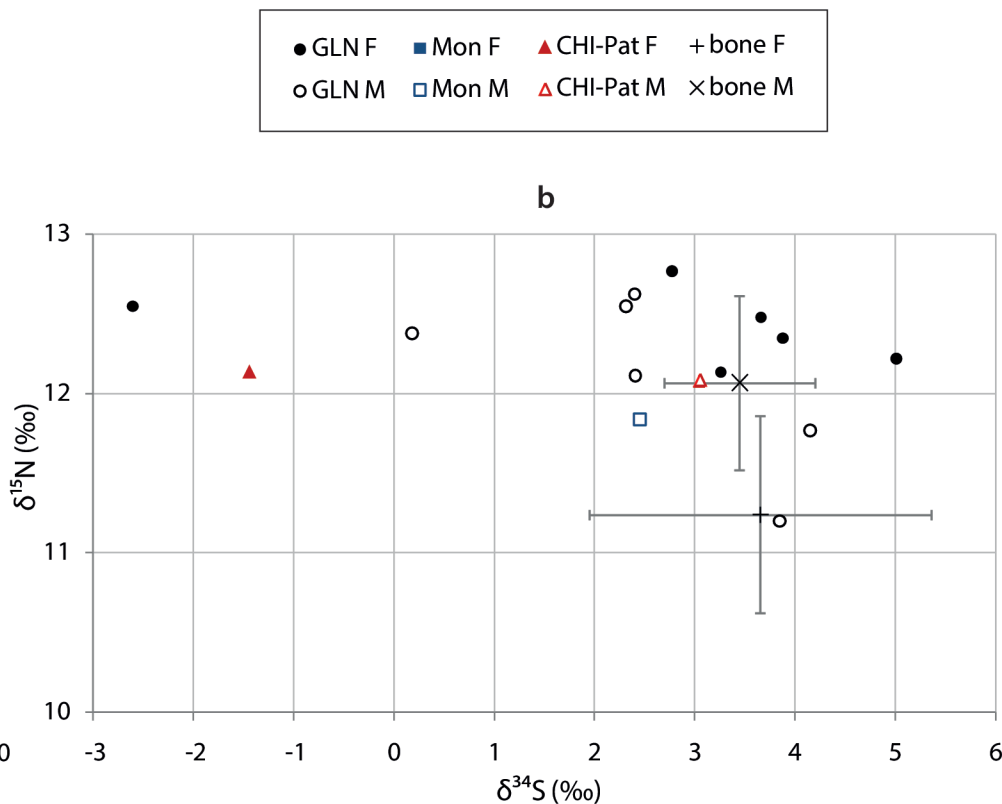
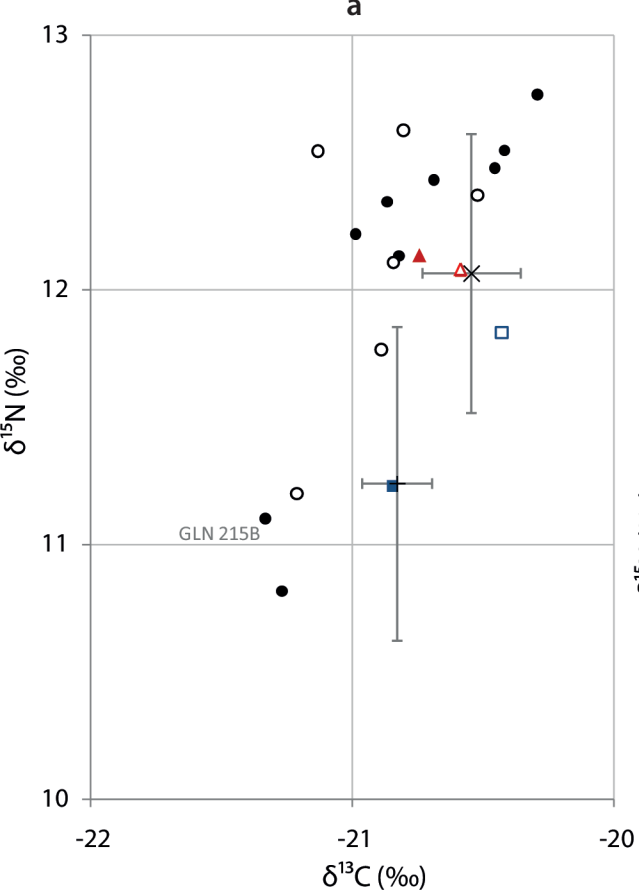
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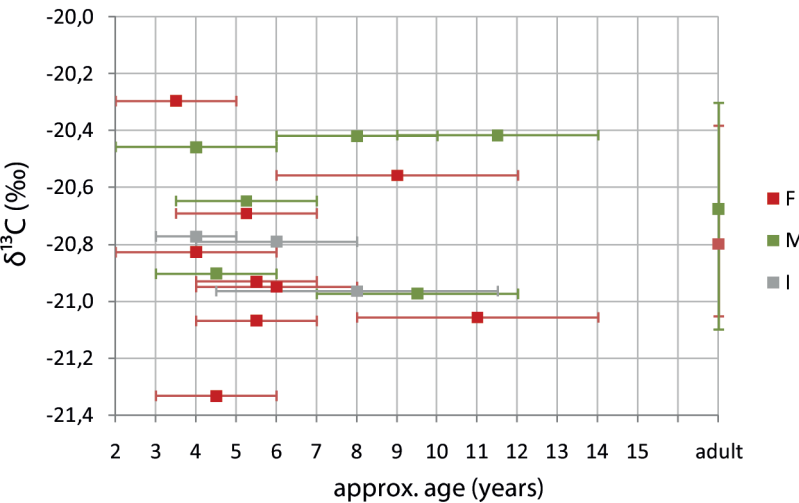
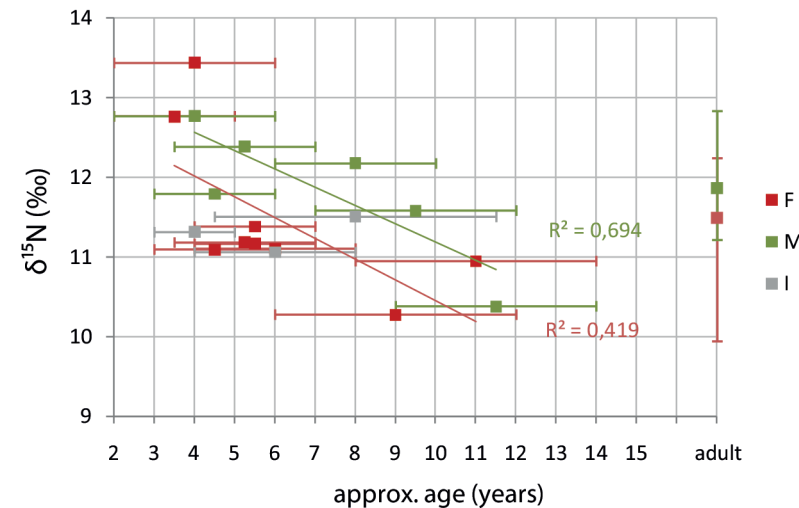
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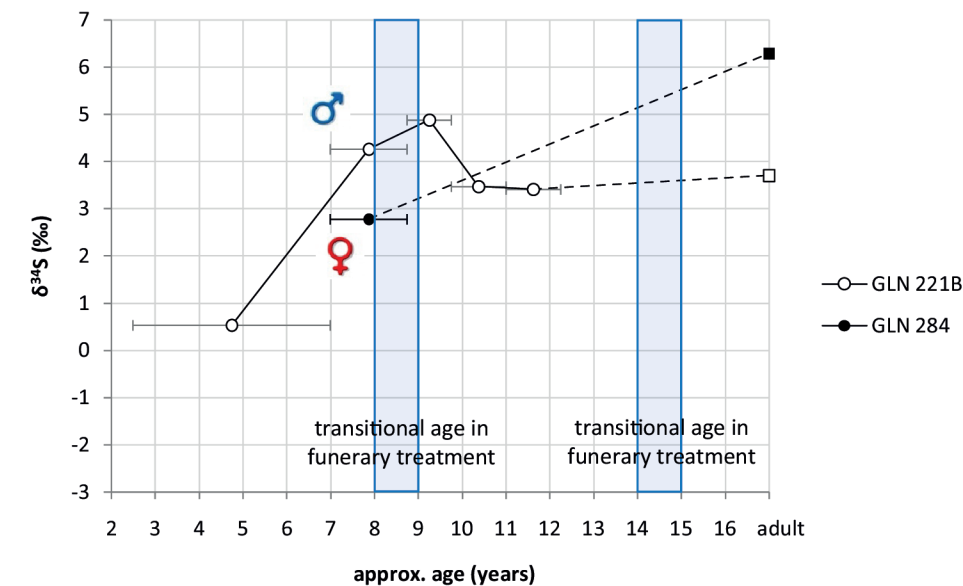
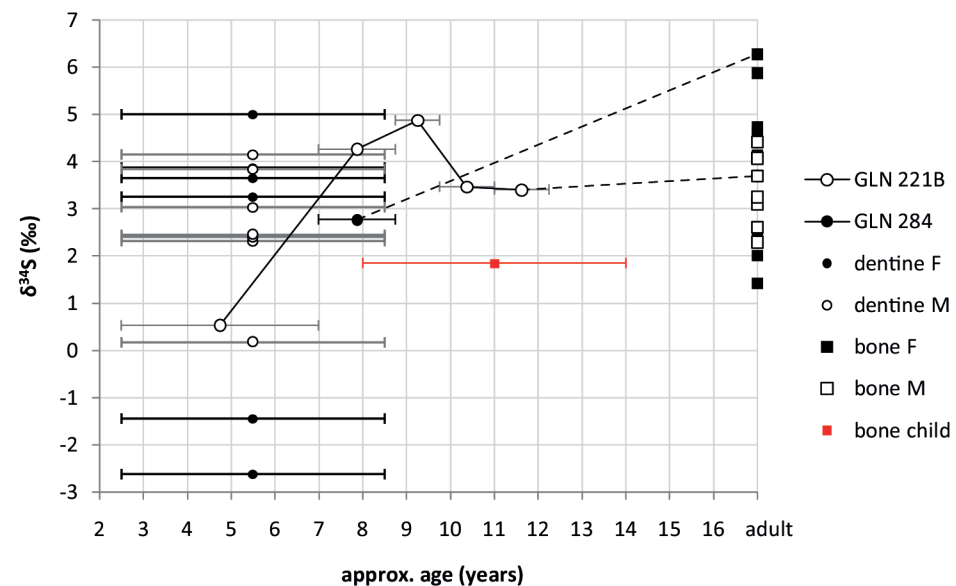
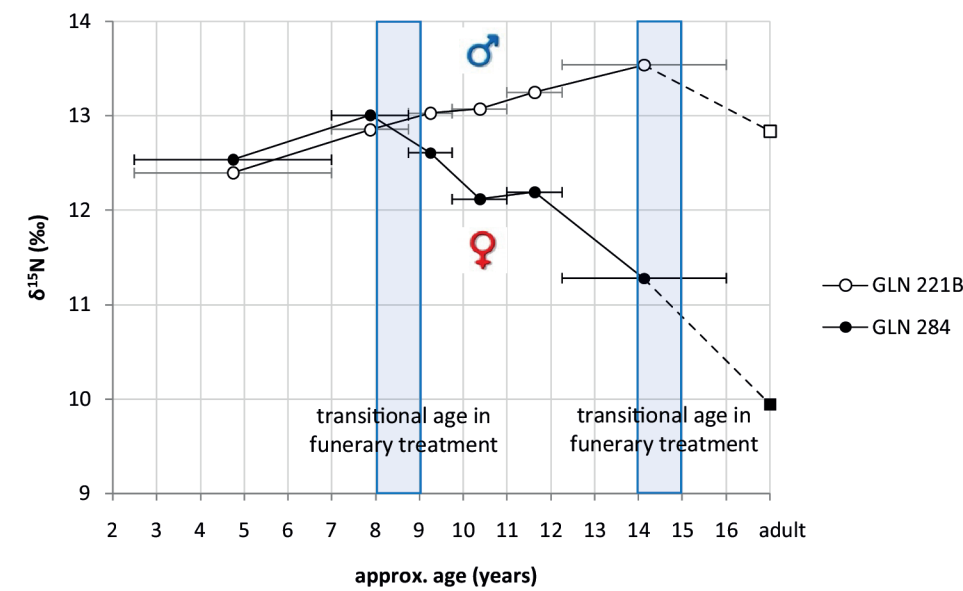
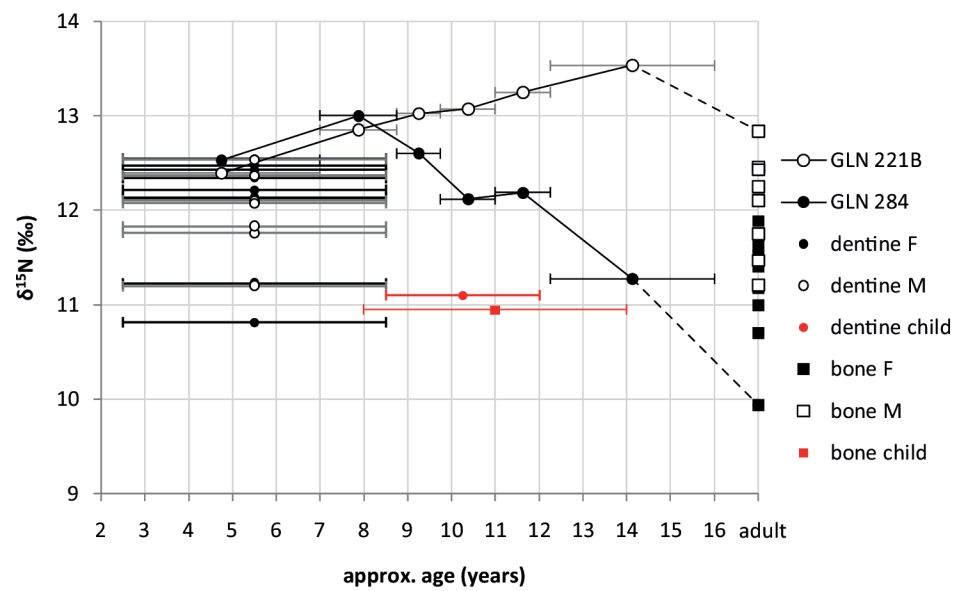
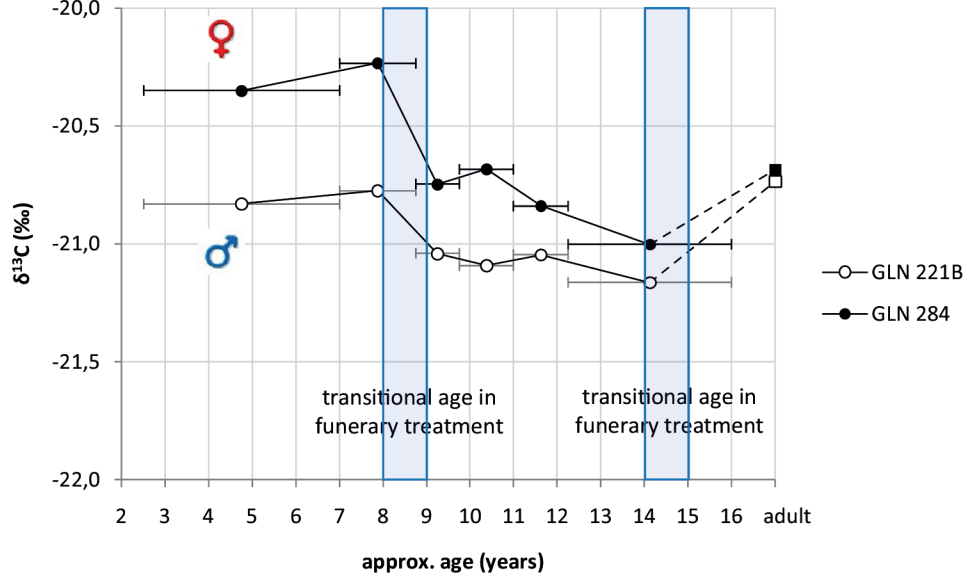
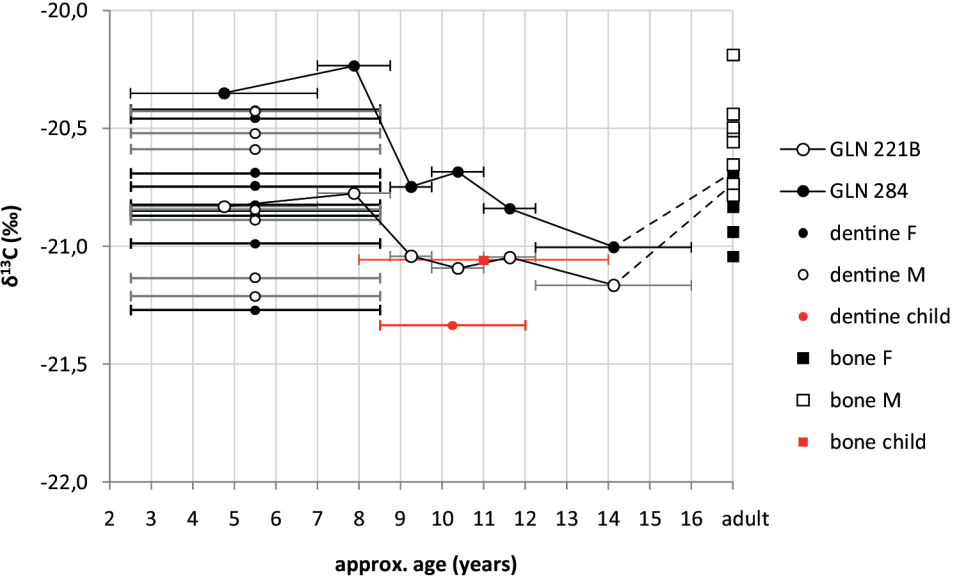






a**b**





ID	sex	status	tooth sample	tooth development stage	corresp. age group	%C dentine	%N dentine	%S dentine	C/N dentine	C/S dentine	N/S dentine	$\delta^{13}\text{C}$ (‰) dentine	$\delta^{15}\text{N}$ (‰) dentine	$\delta^{34}\text{S}$ (‰) dentine	$\delta^{13}\text{C}$ (‰) bone§	$\delta^{15}\text{N}$ (‰) bone§	$\delta^{34}\text{S}$ (‰) bone§
GLN 212	F	adult	LLM2 crown	complete	[2.5-8.5]	45.1	17.0	0.20	3.1	610	197	-20.8	12.1	3.3	-21.0	12.2	4.1
GLN 215B	F‡	child	LLM2 root	R 1/2	[8.5-12]	36.7	12.4		3.4			-21.3	11.1		-21.1	10.9	1.9
GLN 220	M	adult	LLM2 crown	complete	[2.5-8.5]	41.7	15.5	0.18	3.1	608	194	-21.1	12.5	2.3	-20.5	12.3	4.4
GLN 221B†	M	adult	ULM2 crown†	complete	[2.5-8.75]†	43.2†	16.1†	0.18†	3.1†	624†	199†	-20.8†	12.6†	2.4†	-20.7	12.8	3.7
GLN 225	F	adult	ULM2 crown	complete	[2.5-8.5]	39.9	14.9		3.1			-21.3	10.8		-20.8	11.4	4.5
GLN 231A	M	adult	LRM2 crown	complete	[2.5-8.5]	39.5	15.2	0.20	3.0	525	173	-21.2	11.2	3.9	-20.5	12.5	2.6
GLN 243A	M	adult	ULM2 crown	complete	[2.5-8.5]	39.6	14.8	0.19	3.1	560	179	-20.8	12.1	2.4	-20.6	11.2	4.1
GLN 243B	F	adult	URM2 crown	complete	[2.5-8.5]	38.1	14.1	0.19	3.1	535	170	-20.9	12.3	3.9	-20.9	11.5	2.0
GLN 257	M	adult	URM2 crown	complete	[2.5-8.5]	39.9	14.7	0.18	3.1	579	183	-20.5	12.4	0.2	-20.8	12.4	3.1
GLN 264	F	adult	ULM2 crown	complete	[2.5-8.5]	39.0	14.2	0.21	3.2	499	156	-21.0	12.2	5.0	-20.7	11.0	5.9
GLN 269	F‡	adult	URM2 crown	complete	[2.5-8.5]	41.9	15.8	0.21	3.1	539	174	-20.5	12.5	3.7	-20.7	11.6	2.4
GLN 284†	F	adult	URM2 crown†	complete	[2.5-8.75]†	41.5†	15.4†	0.20†	3.1†	558†	177†	-20.3†	12.8†	2.8†	-20.7	9.9	6.3
GLN 294	F‡	adult	LRM2 crown	complete	[2.5-8.5]	38.6	14.0		3.2			-20.7	12.4		-20.8	11.2	1.4
GLN 315	F	adult	URM2 crown	complete	[2.5-8.5]	39.6	14.6	0.19	3.1	560	178	-20.4	12.5	-2.6	-20.7	11.9	2.3
GLN 317	M	adult	LLM2 crown	complete	[2.5-8.5]	38.7	14.3	0.20	3.1	520	165	-20.9	11.8	4.2	-20.7	12.1	2.3
Mon 04-87	F	adult	LLM2 crown	complete	[2.5-8.5]	39.3	14.6		3.1			-20.8	11.2		-20.8	11.2	4.6
Mon 04-110	M	adult	ULM2 crown	complete	[2.5-8.5]	39.2	14.5	0.22	3.1	474	151	-20.4	11.8	2.5	-20.4	11.8	4.1
CHI-Pat 2	M	adult	LRM2 crown	complete	[2.5-8.5]	40.4	15.0	0.19	3.1	557	177	-20.6	12.1	3.0	-20.2	11.5	3.3
CHI-Pat 4	F	adult	ULM2 crown	complete	[2.5-8.5]	40.0	14.9	0.19	3.1	547	175	-20.7	12.1	-1.4	-20.8	10.7	4.7

Table 1: Summary of dentine isotopic values and previous data for bone collagen (C, N, S) results. †Mean values of micro-sections d1 + d2 (Table 3). ‡Sex determined by genetic analysis (Rivollat et al. 2020), §(Rey et al. 2019)

Rey L, Salazar-García DC, Chambon Ph, Santos F, Rottier S, and Goude G. 2019. A multi-isotope analysis of Neolithic human groups in the Yonne valley, Northern France: insights into dietary patterns and social structure. *Archaeol Anthropol Sci* 11(10):5591–5616.

Rey L, Tacail T, Santos F, Rottier S, Goude G, and Balter V. under review. Disentangling diagenetic and biogenic trace elements and Sr radiogenic isotopes in fossil dental enamel and bone using laser ablation analysis. *Geochimica et Cosmochimica Acta*.

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tissue	n	mean $\delta^{13}\text{C}$ (‰) \pm sd	mean $\delta^{15}\text{N}$ (‰) \pm sd	n	mean $\delta^{34}\text{S}$ (‰) \pm sd
adult bone†	18	-20.7 \pm 0.2	11.6 \pm 0.7	15	3.7 \pm 1.3
coronal dentine†	18	-20.8 \pm 0.3	12.1 \pm 0.5	15	2.4 \pm 2.1

sex (dentine)	n	mean $\delta^{13}\text{C}$ (‰) \pm sd	mean $\delta^{15}\text{N}$ (‰) \pm sd	n	mean $\delta^{34}\text{S}$ (‰) \pm sd
F	11	-20.8 \pm 0.3	12.0 \pm 0.7	7	2.1 \pm 2.9
M	8	-20.8 \pm 0.3	12.1 \pm 0.5	8	2.6 \pm 1.2

Table 2: Summary of isotopic mean ratios in adult bone vs. coronal dentine samples and of female vs. male dentine samples. †The bone and the root dentine samples of the child GLN 215B are excluded.

ID	sex	tooth	section	corresp. age group	corresp. approx. age	%C dentine	%N dentine	%S dentine	C/N dentine	C/S dentine	N/S dentine	$\delta^{13}\text{C}$ (‰) dentine	$\delta^{15}\text{N}$ (‰) dentine	$\delta^{34}\text{S}$ (‰) dentine
GLN 221B	M	ULM2	d1	[2.5-7]	5 ± 2.5	43.1	16.0	0.19	3.1	612	195	-20.8	12.4	0.5
			d2	[7-9]	8 ± 1	43.2	16.1	0.18	3.1	635	203	-20.8	12.9	4.3
			d3	[9-10]	9 ± 0.5	42.2	15.4	0.18	3.2	613	192	-21.0	13.0	4.9
			d4	[10-11]	10.5 ± 0.5	43.9	15.9	0.20	3.2	597	185	-21.1	13.1	3.5
			d5	[11-12]	11.5 ± 0.5	41.9	15.4	0.17	3.2	640	202	-21.0	13.2	3.4
			d6	[12-16]	14 ± 2	42.4	15.2	-	3.2	-	-	-21.2	13.5	-
GLN 284	F	URM2	d1	[2.5-7]	5 ± 2.5	40.7	15.1	-	3.1	-	-	-20.4	12.5	-
			d2	[7-9]	8 ± 1	42.4	15.7	0.20	3.1	558	177	-20.2	13.0	2.8
			d3	[9-10]	9 ± 0.5	42.1	15.6	0.20	3.1	555	176	-20.7	12.6	-
			d4	[10-11]	10.5 ± 0.5	43.1	15.9	-	3.1	-	-	-20.7	12.1	-
			d5	[11-12]	11.5 ± 0.5	41.3	14.9	-	3.2	-	-	-20.8	12.2	-
			d6	[12-16]	14 ± 2	39.6	13.6	-	3.4	-	-	-21.0	11.3	-

Table 3: Elemental and isotopic ratios of incremental dentine sections for two individuals. The corresponding (corresp.) age groups and approximate (approx.) ages are rounded for the sake of clarity and because of the uncertainty that exists in age estimation.

ID	sex	status	tooth sample	tooth development stage	corresp. age group	%C dentine	%N dentine	%S dentine	C/N dentine	C/S dentine	N/S dentine	$\delta^{13}\text{C}$ (‰) dentine	$\delta^{15}\text{N}$ (‰) dentine	$\delta^{34}\text{S}$ (‰) dentine	$\delta^{13}\text{C}$ (‰) bone§	$\delta^{15}\text{N}$ (‰) bone§	$\delta^{34}\text{S}$ (‰) bone§
GLN 212	F	adult	LLM2 crown	complete	[2.5-8.5]	45.1	17.0	0.20	3.1	610	197	-20.8	12.1	3.3	-21.0	12.2	4.1
GLN 215B	F‡	child	LLM2 root	R 1/2	[8.5-12]	36.7	12.4		3.4			-21.3	11.1		-21.1	10.9	1.9
GLN 220	M	adult	LLM2 crown	complete	[2.5-8.5]	41.7	15.5	0.18	3.1	608	194	-21.1	12.5	2.3	-20.5	12.3	4.4
GLN 221B†	M	adult	ULM2 crown†	complete	[2.5-8.75]†	43.2†	16.1†	0.18†	3.1†	624†	199†	-20.8†	12.6†	2.4†	-20.7	12.8	3.7
GLN 225	F	adult	ULM2 crown	complete	[2.5-8.5]	39.9	14.9		3.1			-21.3	10.8		-20.8	11.4	4.5
GLN 231A	M	adult	LRM2 crown	complete	[2.5-8.5]	39.5	15.2	0.20	3.0	525	173	-21.2	11.2	3.9	-20.5	12.5	2.6
GLN 243A	M	adult	ULM2 crown	complete	[2.5-8.5]	39.6	14.8	0.19	3.1	560	179	-20.8	12.1	2.4	-20.6	11.2	4.1
GLN 243B	F	adult	URM2 crown	complete	[2.5-8.5]	38.1	14.1	0.19	3.1	535	170	-20.9	12.3	3.9	-20.9	11.5	2.0
GLN 257	M	adult	URM2 crown	complete	[2.5-8.5]	39.9	14.7	0.18	3.1	579	183	-20.5	12.4	0.2	-20.8	12.4	3.1
GLN 264	F	adult	ULM2 crown	complete	[2.5-8.5]	39.0	14.2	0.21	3.2	499	156	-21.0	12.2	5.0	-20.7	11.0	5.9
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GLN 284†	F	adult	URM2 crown†	complete	[2.5-8.75]†	41.5†	15.4†	0.20†	3.1†	558†	177†	-20.3†	12.8†	2.8†	-20.7	9.9	6.3
GLN 294	F‡	adult	LRM2 crown	complete	[2.5-8.5]	38.6	14.0		3.2			-20.7	12.4		-20.8	11.2	1.4
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CHI-Pat 2	M	adult	LRM2 crown	complete	[2.5-8.5]	40.4	15.0	0.19	3.1	557	177	-20.6	12.1	3.0	-20.2	11.5	3.3
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M	8	-20.8 \pm 0.3	12.1 \pm 0.5	8	2.6 \pm 1.2

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			d4	[10-11]	10.5 \pm 0.5	43.9	15.9	0.20	3.2	597	185	-21.1	13.1	3.5
			d5	[11-12]	11.5 \pm 0.5	41.9	15.4	0.17	3.2	640	202	-21.0	13.2	3.4
			d6	[12-16]	14 \pm 2	42.4	15.2	-	3.2	-	-	-21.2	13.5	-
GLN 284	F	URM2	d1	[2.5-7]	5 \pm 2.5	40.7	15.1	-	3.1	-	-	-20.4	12.5	-
			d2	[7-9]	8 \pm 1	42.4	15.7	0.20	3.1	558	177	-20.2	13.0	2.8
			d3	[9-10]	9 \pm 0.5	42.1	15.6	0.20	3.1	555	176	-20.7	12.6	-
			d4	[10-11]	10.5 \pm 0.5	43.1	15.9	-	3.1	-	-	-20.7	12.1	-
			d5	[11-12]	11.5 \pm 0.5	41.3	14.9	-	3.2	-	-	-20.8	12.2	-
			d6	[12-16]	14 \pm 2	39.6	13.6	-	3.4	-	-	-21.0	11.3	-

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