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Compound specific isotope evidence points to use of freshwater resources as weaning food in Middle Neolithic Paris Basin

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Abstract

Objectives: A clear understanding of past weaning practices can provide invaluable insights into social issues such as infant care, fertility rate, and demographic patterns in past societies. This study presents the first archeological research employing compound specific isotope analysis (CSIA) for the reconstruction of past weaning practices.

Methods: Weaning practices of two Middle Neolithic communities in the Paris Basin region: Balloy (BLR) and Vignely (VPB), are evaluated by combining previously published bone collagen stable carbon, nitrogen, and sulfur ($n = 66$) isotope analysis with new compound specific carbon and nitrogen isotope compositions of bone collagen ($n = 10$).

Results: Our results demonstrate that the diets of individuals from BLR and VPB likely incorporated freshwater resources. The signals of freshwater resources consumption are even stronger among subadults, suggesting that freshwater resources were used as weaning food at these sites.

Conclusions: The implications of our result are threefold. Currently many CSIA studies in archeology only involve either carbon or nitrogen. Our data shows that it is important to conduct CSIA on both carbon and nitrogen for a more integrated picture. Secondly, our data demonstrates that the use of a protein-based weaning food—instead of a starch-based weaning food (such as cereal gruel)—was likely more prevalent among the Middle Neolithic communities in the Paris Basin Region than previously thought. The finding thus prompts a rethinking of the role of protein-based weaning food in other archeological contexts. Lastly, the common assumption that weaning foods and adult diets share similar isotopic compositions can be problematic, as the use of protein-based, high trophic-level weaning foods can skew the δ^{15}N weaning curve and produce an erroneously late estimation for weaning ages.

KEYWORDS
bulk collagen stable isotope analysis, compound specific isotope analysis, freshwater resources, Middle Neolithic, weaning

1 | INTRODUCTION

Weaning describes the process where young mammals transition from exclusively suckling/breastfeeding to incorporating complementary foods into their diet. An individual is considered "weaned" when they no longer consume breastmilk for sustenance. In human infants, the duration of the weaning period can range from several months to several years, depending on a host of environmental, biological, socio-cultural, economical, or even personal factors (Humphrey, 2010). In this study, "breastmilk" specifically refers to secretion from the mammary glands from the same species. Here, "breastfeeding" also does not differentiate between breastfeeding from the birth mother or wet nurses. The process of weaning in past human groups has received wide scholarly attention, as weaning practices can provide unique insights into a wide range of social issues such as general health and sanitary conditions of the community (Redfern & Gowland, 2012), demographic and socio-economic structures (Herrscher et al., 2017; Lancaster & Lancaster, 1987; Lindenberg et al., 1990), differential treatment of male and female infants (Eerkens & Bartelink, 2013; Miller et al., 2020; Odebode & Odebode, 2005), and birth spacing (Fildes, 2017).

Two questions frequently asked by archeologists pertaining ancient weaning practices concern the timing and nature of food being fed during weaning. However, tracking, or even identifying weaning activities in past societies with no surviving written records is a difficult challenge. Fortunately, recent advances in biochemical and spectroscopy technologies have provided many new tools to help detect breastfeeding/weaning activities in past populations. Today, two of the most common approaches involve examining the stable nitrogen isotope ratios (δ^{15}N) in proteinaceous tissues (i.e., bone collagen or dentin), whether as (i) bone collagen data plotted against the estimated age of death (Bourbou et al., 2013; Brundke et al., 2017; Fuller, Molleson, et al., 2006; Keenleyside et al., 2009; Prowse et al., 2008; Stantis et al., 2020) or (ii) as intra-tooth serial data plotted against the formation time of micro-sampled dentin (Eerkens et al., 2011; Fernández-Crespo et al., 2018). Stable nitrogen isotope measurement is a well-established trophic level indicator, where organisms in a higher trophic position (TP) typically have more enriched δ^{15}N values than those below (Hedges & Reynard, 2007; O'Connell et al., 2012). This trophic enrichment effect is also noted among breastfeeding individuals, where a tissue-to-tissue enrichment of 2‰–3‰ is observed between modern mother-and-child pairings (Fuller, Fuller, et al., 2006; Herrscher et al., 2017). Based on these approaches, studies have shown that children in most archeological populations were weaned between 1 and 3 years of age (Chinique de Armas et al., 2017; Oelze et al., 2011; Pfeiffer et al., 2017; Stantis et al., 2020; Tsutaya & Yoneda, 2013), occasionally up to 4 years (Fernández-Crespo et al., 2020; Xia et al., 2018). These findings are in general agreement with historical and ethnographic data, which shows similar average weaning ages across a wide range of historical, cultural, and socio-economic backgrounds (Fildes, 2017; Fulminante, 2015; Sellen & Smay, 2001). Despite its wide applications, there are serious concerns and criticisms regarding the reconstruction of past weaning practices using stable nitrogen isotope analysis (see Reynard and Tuross (2015) for detailed discussion). Uncertainties in analytical measurements, tissue turnover rate, and enrichment factor aside, one key problem in this approach is that the estimation is built on the assumption that weaning food has the same isotopic composition as those of adult foods. Thus, to better understand ancient weaning practices, it is also important to ask the question: what was being used as weaning food?

Unfortunately, weaning food left little mark in the archeological record. Beyond occasional chance finds (Dunne et al., 2019; Stefanović et al., 2019), the current understanding of prehistoric weaning food is mainly informed by historical and ethnographic data. Based on these records, weaning food can be divided into two broad categories: starch-based and protein/lipid-based foods (Table 1).

Note that these categories are only roughly defined by the major component of the food, therefore the ingredients are not mutually exclusive (e.g., cereal gruel mixed with cow's milk would be considered "starch-based"). Of the two categories, starch-based foods, such as starchy vegetables, bread, or gruel, have been the dominant form of weaning food amongst most pre-industrial societies (Obladen, 2014; Sellen & Smay, 2001). Even though milk from other animals has been used across almost all pre-industrial agricultural groups, extensive consumption of milk is mostly limited to very young children. Other protein/lipid-based food such as meat, bone marrow, fish, or eggs are mentioned relatively sparingly. In fact, many cultures warn against feeding meat to weaning children too early (Levin, 1959; Rahman, 2017). Thus, the literature forms a very biased view toward the used of starch-based weaning food among past societies. However, has this always been the case?

To better understand the diversity of weaning foods used in past societies, we examine the weaning practices of two Middle Neolithic groups in the Paris Basin region: Balloy "Les Réaudins" (BLR) and Vignely "La Porte aux Bergers" (VPB). A recent study (Cheung et al., 2021) examining the bone collagen stable carbon, nitrogen, and sulfur isotope compositions of the two groups revealed unexpected patterns among the subadults, suggesting an unusual weaning food was consumed. Accordingly, compound specific isotope analysis (CSIA) of the bone collagen of 10 selected individuals was conducted, and all previously published bulk collagen C, N, and S isotope compositions from the two sites re-evaluated. Note that bone collagen isotopic compositions, whether bulk or compound specific, mostly provide dietary information from dietary protein (Ambrose & Norr, 1993; Fernandes et al., 2012; Hedges et al., 2007), therefore, in this study, the discussion of the "protein/lipid-based weaning food" will focus on the protein portion of this category.

2 | COMPOUND SPECIFIC ISOTOPE ANALYSIS

Bulk tissues stable carbon and nitrogen isotope analysis is arguably the most trusted and widely applied chemical approach to reconstruct past diets from human tissues. In recent years, improved

TABLE 1 Examples of weaning food used in pre-industrial societies

Type	Examples	Culture/period	References
Starch-based	Crumbs of bread softened by hydromel or milk; soup made from spelt; very moist porridge	1st–2nd century A.D. Roman world	(Temkin, 1991)
	Mashed banana; rice gruel; wild and cultivated taros and yams	Modern Southeast Asia	(Jelliffe, 1968)
	Pap: a mixture of flour or bread cooked in water (with or without milk)	Preindustrial Europe	(Fildes, 1986; Obladen, 2014)
	Mashed tubers such as cassava, sweet potatoes, and yams	Modern hunter-gatherer groups in Eastern and Central Africa	(Fouts et al., 2001; Pagezy & de Garine, 1990)
	Cereal gruels, sometimes fermented. Vegetables, legumes, and fruits are sometimes added to supplement the gruels.	Modern Zimbabwe and Nigeria	(Igbedioh et al., 1996; Simango, 1997)
Protein/lipid-based	An egg that can be sipped	1st–2nd century A.D. Roman world	(Temkin, 1991)
	Milk from cow, sheep, goat, donkey, camel, horse, or even pig	Throughout history in almost all agricultural groups	(Rebay-Salisbury, 2019; Stevens et al., 2009)
	Premasticated fish liver; a mixture of seal oil and moose or caribou tallow (agutuk); fish and meat broth	Preindustrial indigenous groups in Alaska	(Heller & Scott, 1967)
	Tender parts of game; smoked meat and fish	Modern hunter-gatherer groups in Central Africa	(Pagezy & de Garine, 1990)
	Boiled little fish; wild chicken broth, thickened with manioc bread	Modern Brazilian Warekena (indigenous Amerindians)	(Rahman, 2017)

methodologies to isolate and analyze the isotopic compositions of single amino acids (AAs) have allowed archeologists to examine the mechanisms by which isotopic signature is passed down the food chain with more specificity (O'Connell & Hedges, 2001; Whiteman et al., 2019).

The elemental compositions of each AAs vary according to their functions, chemical properties, as well as their synthetic and metabolic pathways. More specifically, some AAs, especially the essential ones (EAAs) are more tied to the sources (source AAs), while some fractionate strongly along the food chain and therefore reflect trophic levels (trophic AAs) (O'Connell, 2017; Takizawa et al., 2020). Generally speaking, δ^{13}C values of phenylalanine (Phe), valine (Val), and leucine (Leu) mostly reflect the δ^{13}C values of the basal resources. Higher $\delta^{13}C_{Gly}$ values have been observed in aquatic protein (marine and freshwater), as well as shown to be positively correlated with trophic level (Corr et al., 2005; Fantle et al., 1999; Honch et al., 2012; Webb et al., 2018). Thus, the difference between phenylalanine and glycine (Gly) ($\Delta^{13}C_{Gly-Phe}$) in consumer tissue has been used to help discriminate between diets based heavily on terrestrial versus aquatic resources (Corr et al., 2005; Honch et al., 2012; Webb et al., 2018). $\delta^{15}N_{AA}$ values are mostly used to understand the TP of the consumer, as the trophic discrimination factors in trophic AAs are considerably larger than those in source AAs (Chikaraishi et al., 2009; Chikaraishi et al., 2014; Takizawa et al., 2020). A commonly used AA pairing for this purpose is to compare the nitrogen isotope compositions of phenylalanine (Phe) and glutamic acid/glutamine (Glx) (Chikaraishi et al., 2014; Jaouen et al., 2019; Schwartz-Narbonne et al., 2015). Note that during the hydrolysis step in preparing the samples for analysis, asparagine (Asn) is converted to aspartic acid (Asp) and glutamine (Gln) to glutamic acid (Glu), the isotopic compositions of Asp and Asn are reported jointly as Asx, and Gln and Glu as Glx.

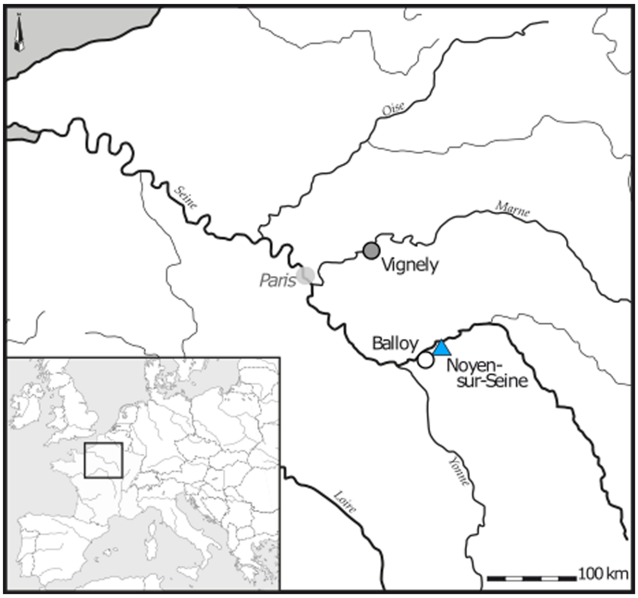

FIGURE 1 Map of Paris Basin showing the two middle Neolithic sites Balloy "les Réaudins" (BLR) and Vignely "La Porte aux Bergers" (VPB), as well as the nearby Mesolithic site Noyen-Sur-seine

One major advantage of CSIA is that by breaking down the analyzed substrate – protein in this case – to its single constituents, it is now possible to parse through the "noises" in bulk tissue isotopic compositions caused by issues such as particular dietary behaviors,

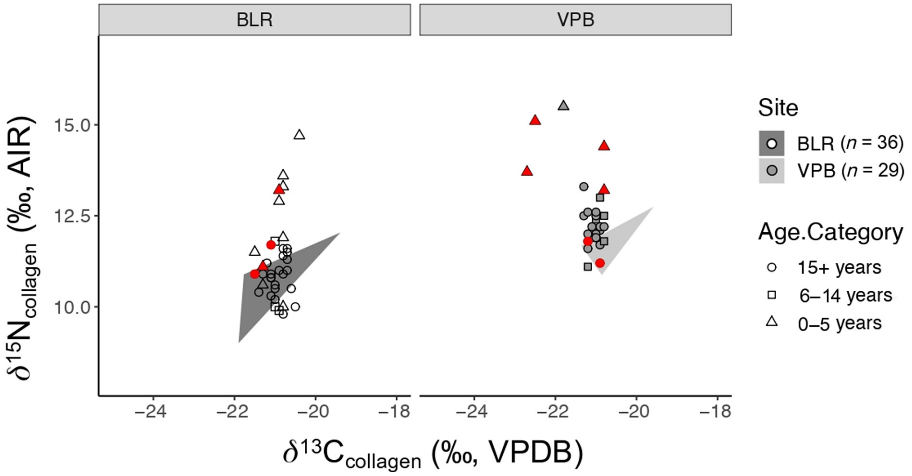

FIGURE 2 Previously published human bone collagen $\delta^{13}C$ and $\delta^{15}N$ values from the two sites (Cheung et al., 2021). The shaded areas in the graphs are mixing polygons consisted of the major terrestrial protein sources from each respective site. Points colored in red correspond to individuals also analyzed for compound specific isotope compositions

overlapping source values, climatic conditions, and gain a more direct understanding of the isotopic relationship between the consumer and its sources (Schwartz-Narbonne et al., 2015). For example, other than the trophic level effect, some confounding factors that could affect bulk collagen $\delta^{15}N$ values are the long-term consumption of intensely manured crops (Bogaard et al., 2013; Fraser et al., 2011; Szpak et al., 2012), nutritional stress (Fuller et al., 2005; Hobson et al., 1993), and aridity (Pate & Anson, 2008; Schwarcz et al., 1999). Recent studies have shown that CSIA can be used to help differentiate between these phenomena based on the fractionation patterns of different AAs. For example, an experimental study has shown that manuring tends to affect the $\delta^{15}N$ values of all AAs consistently, increasing the bulk $\delta^{15}N$ values of plants without changing the relative $\delta^{15}N_{AA}$ values (Styring et al., 2014). Therefore, we expect to see manured plants being in the same TP as unmanured plants, and consequently, humans subsisting intensively on manured plants would not be on a higher TP than those subsisting on unmanured plants. Another study that looked at southern elephant seals (*Mirounga leonina*) has shown that intense catabolism is registered differently in the $\delta^{15}N$ values of different AAs in whiskers (Lübcker et al., 2020). Specifically, while the $\delta^{15}N$ values of the bulk tissue and most glucogenic AAs (AAs that can be converted into glucose during gluconeogenesis) always increase during fasting periods, the $\delta^{15}N$ values of alanine (Ala) and threonine (Thr) always decrease. There are more examples of how CSIA can be used to help elucidate dietary behaviors in past populations, but only these two factors are described here as they are the two most pertinent factors to this particular study.

3 | BACKGROUND OF SITES

BLR and VPB are both situated within the Paris Basin and are associated with the Cerny culture (c.4700–4300 BC) (Figure 1). Based on archeological evidence, the Cerny people are generally considered as agrarian groups subsisted primarily on agricultural activities (Bakels, 1999; Bakels, 2009;Bostyn et al., 2016; Tresset, 1997), who occasionally supplemented their diet with wild games (Bostyn et al., 2016; Tresset, 1997) and possibly fish (Bostyn et al., 2018). For more detailed descriptions of these sites, please refer to the original reports (in French) (Chambon et al., 2018; Mordant, 1997), or a summary in English in Cheung et al. (2021).

Recently, bulk bone collagen C, N, and S isotope analysis conducted on BLR and VPB revealed that the two groups consumed a substantial amount of animal protein, despite being generally categorized as agricultural societies (Cheung et al., 2021). While this recent study mostly focused on evaluating dietary patterns among adults, there are some interesting observations among the subadults (age <5 years) not discussed. Figure 2 shows the bulk collagen $\delta^{13}C$ and $\delta^{15}N$ values of all humans from the two sites, plotted against the local dietary sources, as represented by mixing polygons. The bulk collagen C and N measurements from all human and faunal samples are obtained from a published report (Cheung et al., 2021)—with the exception of one new data point from BLR (BLR09) (Dataset S1). Mixing polygon is a good way to help identify possible missing source group(s) (Cheung & Szpak, 2021). Each polygon is a convex hull formed by the mean $\delta^{13}C$ and $\delta^{15}N$ values of the known major protein sources from each respective site, after adjusted for trophic enrichment factor (TEF). For the purpose of this study, collagen to collagen TEF is set to be +1.0‰ and + 3.5‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Bocherens & Drucker, 2003; Hedges & Reynard, 2007). Sources are consisted of only animals, and are grouped under four categories: wild herbivore, wild omnivore, domesticated herbivore, and domesticated omnivore. Humans sourcing the bulk of their dietary protein from these sources should fit inside each respective mixing polygon. As shown in Figure 2, excluding those located below the mixing polygons (likely due to a higher consumption of plant food in diet, which is not part of the mixing polygon), the diets of about 43% (16/37) of the individuals from BLR and 69% (20/29) of the individuals from VPB cannot be entirely explained by the sources presented. About 81% (13/16) of the subadults between the age of 0 and 5 years are located above the mixing polygons (Figure 2), indicating that subadults at these sites were consuming food sources that are from a higher TP than the adults.

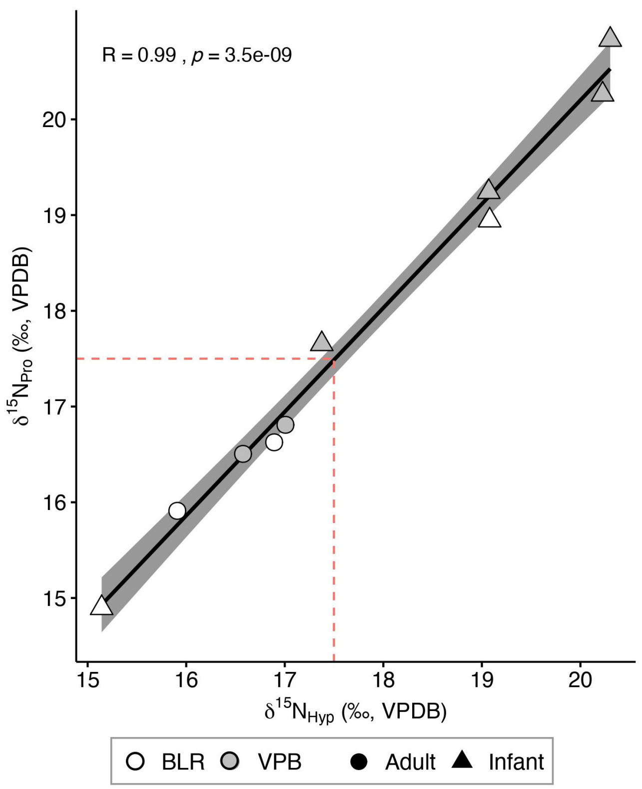

FIGURE 3 Comparison of the δ^{15}N values of proline and hydroxyproline in collagen of the 10 samples analyzed in this study

Very little is known about weaning practices in the region. The general consensus is that during the Neolithic period, animal milk and ground cereals were likely used as weaning food in continental Europe (Jay et al., 2008; Karsten et al., 2014; Stojanovski et al., 2020). If that is the case, breastfeeding individuals should be one trophic level above those of the adults. While those from BLR are located directly above the mixing polygon, and therefore could be explained by the trophic enrichment effect from breastfeeding, three of these subadults from VPB (VPB245, VPB257, and VPB258) have distinctively depleted δ^{13}C values that cannot be explained by breastfeeding. In fact, it is clear that a protein source that is relatively depleted in ^{13}C and enriched in ^{15}N is missing in the VPB mixing polygon.

The unusually low δ^{13}C values of the subadults at VPB are particularly perplexing. There are two major potential contenders for sources relatively depleted in ^{13}C: sources from a canopied area (Bonafini et al., 2013; van der Merwe & Medina, 1991) or freshwater resources (Garcia et al., 2007; Guiry, 2019). According to limited published data from the Seine and Marne valleys (Bocherens et al., 2011; Cheung et al., 2021; Drucker et al., 2018), wild games ($n = 36$) have lower mean δ^{13}C (−22.4 ± 1.1‰) and δ^{15}N values (+6.3 ± 1.8‰) than domesticated animals ($n = 45$; δ^{13}C: −21.8 ± 1.0‰; δ^{15}N: +7.7 ± 1.2‰), while freshwater resources (only available from the Seine, $n = 11$) have lower mean δ^{13}C (−24.7 ± 2.0‰) and higher mean δ^{15}N values (+8.9 ± 1.7‰) than all terrestrial animals. This suggests that freshwater resources were more likely responsible for the unusual pattern observed among the VPB subadults.

δ^{34}S values are frequently used to help identify the consumption of freshwater resources in the archeological record (Nehlich et al., 2010; Privat et al., 2007). Based on our limited data from the Seine Valley, there are substantial differences between the mean δ^{34}S values of the freshwater resources (−13.4 ± 4.9‰; $n = 4$), wild games (+2.2 ± 2.1‰; $n = 14$), and domesticated animals (+7.2 ± 1.7‰; $n = 13$) (Bocherens et al., 2011; Cheung et al., 2021; Drucker et al., 2018), demonstrating that it is possible to use δ^{34}S values to help identify the consumption of freshwater resources in this context. However, note that the δ^{34}S baseline is not very well characterized in the region, therefore, in this study, δ^{34}S values will only be used as a supplementary tool to help interpret patterns observed in other isotope systems.

To test whether freshwater resources could have been used as weaning food at the two sites, we re-evaluate the published (and one new) bulk bone collagen δ^{13}C, δ^{15}N, and δ^{34}S values of all individuals from BLR ($n = 37$) and VPB ($n = 29$), focusing specifically on comparing the dietary patterns between adults and subadults. In addition, 10 individuals (6 infants and 4 adults) from the two sites are selected to conduct compound specific stable carbon and nitrogen isotope analysis. For these 10 individuals, none of the subadults were known to associate with any of the adults based on archeological evidence. Selection criteria of the subadults was first based on the degree of preservation (i.e., collagen yield), and second, individuals with the most extreme isotopic compositions in bulk collagen were preferred. Four "average" adults were selected to serve as an adult "baseline."

4 | METHODS AND MATERIALS

All individuals were aged using established osteological methods (Moorrees et al., 1963; Scheuer & Black, 2000; Schmitt, 2005), as described and reported in Thomas' report (2011). Summary biological information of all human samples involved are provided in Dataset S1. The procedure for collagen extraction, collagen quality control, and bulk collagen stable isotope measurement are reported in Cheung et al. (2021). For the selected 10 individuals, an aliquot of extracted collagen was sent to the Stable Isotope Facility at University of California, Davis for CSIA. Samples were prepared and analyzed following the procedures developed by Yarnes and Herzsage (Yarnes & Herszage, 2017). A brief description of the procedure is as follows: collagen was first hydrolyzed in HCl, and derivatized as *N*-acetyl methyl esters (NACME). The AA derivatives were then separated on an Agilent DB-35 capillary column, and analyzed in duplicates by GC/C/IRMS, using a Thermo Trace GC 1310 gas chromatograph coupled to a Thermo Scientific Delta V Advantage IRMS via a GC IsoLink II Combustion interface. Measurements are reported in "per mil (‰)" and are calibrated to VPDB for δ^{13}C and AIR for δ^{15}N, using certified standard reference materials from USGS, NIST, and the IAEA. Measurement accuracy and reproducibility was monitored by a series of internal reference materials with known isotopic compositions, including a matrix-matching material—GEL (gelatine). Total measurement error was less than 1.0‰ for both carbon and nitrogen isotope

TABLE 2 Overview of the CSIA reference collections used in this study. Only a general location is provided in this table, for more detailed description of the sites, please refer to each respective publication

	$\delta^{13}C_{AA}$			$\delta^{15}N_{AA}$		
	Site	**Period**	**Location**	**Site**	**Period**	**Location**
Terrestrial fauna	Grotte du Renne ($n = 14$)	Paleolithic	France (Jaouen et al., 2019)	Grotte du Renne ($n = 6$)	Paleolithic	France (Jaouen et al., 2019)
	Les Cottés ($n = 16$)	Paleolithic	France (Jaouen et al., 2019)	Les Cottés ($n = 4$)	Paleolithic	France (Jaouen et al., 2019)
	Köpingsvik ($n = 10$)	Mesolithic	Sweden (Webb et al., 2018)	Spy Cave ($n = 21$)	Paleolithic	Belgium (Naito et al., 2016)
	Padina ($n = 3$)	Mesolithic	Serbia (Honch et al., 2012)	Pont d'Ambon ($n = 9$)	Epi-paleolithic	France (Naito et al., 2013)
	Rössberga ($n = 5$)	Neolithic	Sweden (Webb et al., 2018)	Noyen-sur-Seine ($n = 7$)	Mesolithic	France (Naito et al., 2013)
	Durankulak ($n = 3$)	Neolithic	Bulgaria (Honch et al., 2012)			
	Visby ($n = 7$)	Medieval	Sweden (Webb et al., 2018)			
Freshwater resources	Unspecified ($n = 1$)	Modern	UK (Honch et al., 2012)	Pont d'Ambon ($n = 3$)	Epi-paleolithic	France (Naito et al., 2013)
	Spanish Lookout Caye ($n = 3$)	Modern	Belize (Larsen et al., 2012)	Noyen-sur-Seine ($n = 4$)	Mesolithic	France (Naito et al., 2013)
	Upper Mississippi River ($n = 78$)	Modern	USA (Thorp & Bowes, 2017)			
	Lower Ohio River ($n = 63$)	Modern	USA (Thorp & Bowes, 2017)			
Humans—High freshwater protein intake (HFP)	Schela Cladovei ($n = 4$)	Epi-paleolithic	Romania (Honch et al., 2012)	Noyen-sur-Seine ($n = 2$)	Mesolithic	France (Naito et al., 2013)
	Lepenski Vir ($n = 3$)	Mesolithic	Serbia (Honch et al., 2012)			
	Padina ($n = 3$)	Mesolithic	Serbia (Honch et al., 2012)			
	Hajdučka Vodenica ($n = 4$)	Mesolithic	Serbia (Honch et al., 2012)			
Humans—C_3 terrestrial	Visby ($n = 11$)	Medieval	Sweden (Webb et al., 2018)			
	Schela Cladovei ($n = 1$)	Epi-paleolithic	Romania (Honch et al., 2012)			
	Lepenski Vir ($n = 2$)	Mesolithic	Serbia (Honch et al., 2012)			
	Gomolava ($n = 3$)	Neolithic	Serbia (Honch et al., 2012)			
	Varna ($n = 3$)	Neolithic	Bulgaria (Honch et al., 2012)			
	Durankulak ($n = 5$)	Neolithic	Bulgaria (Honch et al., 2012)			

analyses. Detailed measurements and standard deviations, including those of the control reference materials, are provided in Dataset S2. The N isotope measurements of hydroxyproline and proline correlates extremely well ($r[8] = 0.99$, $p < 0.001$; Figure 3), thus showing that all collagen analyzed are reasonably well preserved and that the isotopic measurements of the AAs are reliable (O'Connell & Collins, 2018). Correlation matrices showing the relationships between all AAs are provided in Supplementary Information Figures S1 and S2.

All statistical tests were performed using R version 3.6.0 (R Core Team, 2019) with RStudio (RStudio Team, 2018). Distribution normality was tested using a Shapiro–Wilk test. Variance equality was tested using the Levene's test. To compare the means of groups with normally distributed data (or sample size >15), unpaired Student's *t* tests were used. Wilcoxon tests were used for groups with not normally distributed data (or sample size <15). A 0.05 probability ($p < 0.05$) was considered significant, unless stated otherwise. To test whether the conventional approach to estimate weaning ages in archeological populations with δ^{15}N values is applicable to these two sites, an approximate Bayesian computation—WARN (Tsutaya, 2019), was applied to help estimate the beginning (t_1) and end (t_2) of weaning ages at BLR and VPB. WARN models for t_1 and t_2 with maximum density estimators (MDE), after taking bone collagen turnover rate into consideration. Estimations with joint probability >0.0025 were deemed valid. All R scripts used in this study are provided in the Supplementary R Script.

As no faunal samples from BLR and VPB are analyzed for compound specific isotopic compositions, baseline references are drawn from published reports (Honch et al., 2012; Jaouen et al., 2019; Larsen et al., 2012; Naito et al., 2013; Naito et al., 2016; Thorp & Bowes, 2017; Webb et al., 2018). Efforts have been made to include only European archeological materials, whenever possible. Published data from several groups of humans, broadly categorized as C_3 terrestrial consumers or high freshwater protein (HFP) consumers, are also included as comparison (Honch et al., 2012; Naito et al., 2013; Webb et al., 2018). The references for these comparing data are outlined in Table 2, and the data is provided in Dataset S3.

While it is certainly not ideal that other than the $\delta^{15}N_{AA}$ values from Noyen-sur-Seine, no CSIA data from other local fauna is available. An inter-site comparison of all faunal types (e.g., herbivores, omnivores, carnivores) showed that all fauna separate reasonably well according to their feeding habits (Supplementary Information Figure S3), regardless of their provenance or time period. Furthermore, as noted previously, one major advantage of CSIA is that this approach allows researchers to evaluate TP and baseline nutritional sources directly without the need to first characterize the local isotopic baseline. This justifies using the selected faunal CSIA data to explain the general dietary patterns of the humans. As mentioned earlier, freshwater resources are a possible contender of weaning food used in these two sites. In Europe, analyses on modern and archeological fish remains have reported δ^{13}C values ranging from −32.2‰ (Lake Aiguebelette, France) to −18.1‰ (Visby, Sweden) (Dufour et al., 1999; Webb et al., 2018). Unfortunately, all available $\delta^{13}C_{AA}$ data from archeological European freshwater resources happen to come from river systems with δ^{13}C values more elevated than those of the terrestrial systems (e.g., Visby, Sweden [Webb et al., 2018]). Therefore, modern data from the UK, US, and Belize with low $\delta^{13}C_{AA}$ values are used to depict a source with very depleted $\delta^{13}C_{AA}$ values (−27.3‰ ± 1.7‰, $n = 144$, Table 2). When comparing data extracted from modern samples with archeological samples, the Suess effect correction is often applied to modern δ^{13}C values to offset the effect of fossil fuels burning (Keeling, 1979; Olsen et al., 2006). In this study, modern data are not corrected for the Suess effect, as the magnitude of the effect is highly regional and temporal specific, and not enough is known about the regions where the modern samples have come from. Considering this limitation, this very negative $\delta^{13}C_{AA}$ baseline only serves to visualize a theoretical source with depleted δ^{13}C values,

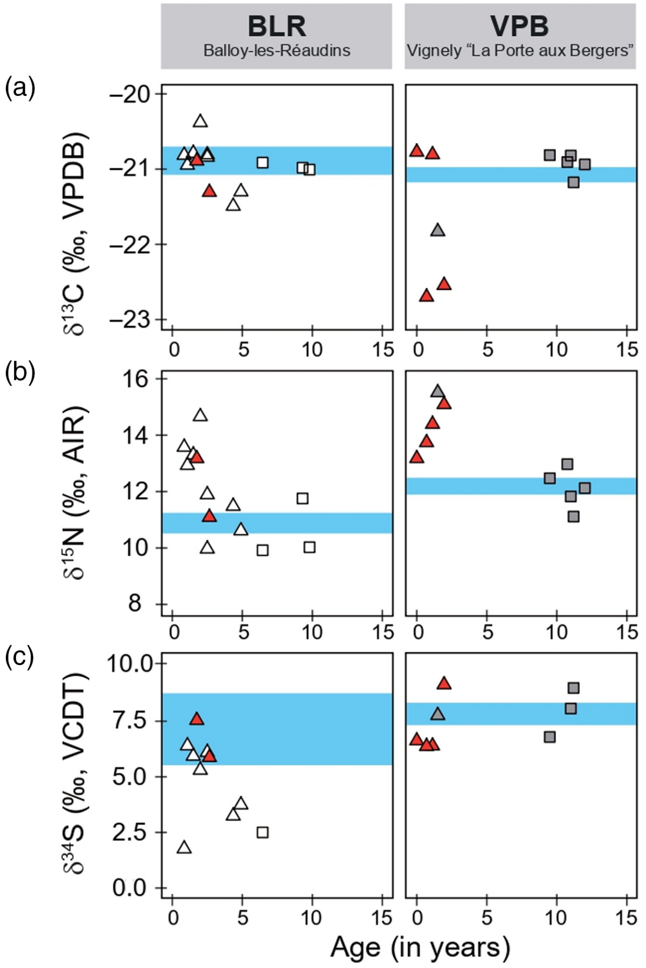

FIGURE 4 Bone collagen stable isotopic compositions of all non-adults (age < 15 years) plotted against age at death from the two sites. (a) δ^{13}C values of all non-adults; (b) δ^{15}N values of all non-adults; and (c) δ^{34}S values of all non-adults. Shaded blue area denotes the interquartile ranges of the adult values of each respective element. Red point corresponds to individuals also analyzed for compound specific isotope compositions. Triangle corresponds to subadults age between 0 and 5; square corresponds to subadults age between 6 and 15

TABLE 3 Summary statistics of bulk bone collagen $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of adults and subadults at BLR and VPB. All data are from published report (Cheung et al., 2021)

	BLR			VPB		
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Adult	-20.9 ± 0.3 ($n = 23$)	$+10.9 \pm 0.5$ ($n = 23$)	6.6 ± 2.9 ($n = 12$)	-21.1 ± 0.1 ($n = 19$)	$+12.1 \pm 0.5$ ($n = 19$)	7.7 ± 1.2 ($n = 12$)
Subadult	-21.0 ± 0.3 ($n = 23$)	$+12.0 \pm 1.5$ ($n = 23$)	4.8 ± 1.9 ($n = 12$)	-21.3 ± 0.8 ($n = 10$)	$+13.2 \pm 1.4$ ($n = 10$)	7.5 ± 1.1 ($n = 8$)

Abbreviations: BLR, Balloy; VPB, Vignely.

TABLE 4 Biological information and isotope measurements of the 10 individuals analyzed for compound specific isotope contents. TP refers to trophic positions, calculated using the formula described in Chikaraishi et al. (Chikaraishi et al., 2009; Chikaraishi et al., 2014) and illustrated in Figure 4b. Only summary results and measurements from selected amino acids are presented in this table. For the full set of results, please refer to Dataset S3

Code	Sample	Site	Element analyzed	Age category	Age (years)	$\delta^{13}C_{collagen}$	$\delta^{15}N_{collagen}$	$\delta^{13}C_{Phe}$	$\Delta^{13}C_{Gly-Phe}$	$\delta^{15}N_{Phe}$	$\delta^{15}N_{Glx}$	TP
#1	BLR04	BLR	Radius/ ulna	Infant	2–3.33	−21.3	+11.1	−28.5	+13.0	+3.0	+15.2	3.7
#2	BLR36	BLR	Cranium	Infant	0.67–2.83	−20.9	+13.2	−30.9	+14.9	+8.0	+16.9	3.3
#3	BLR45	BLR	Phalange (hand)	Adult	30–49	−21.5	+10.9	−29.7	+15.1	+5.5	+15.3	3.4
#4	BLR25	BLR	Metatarsus	Adult	25+	−21.1	+11.7	−28.3	+13.7	+10.3	+16.5	2.9
#5	VPB56B	VPB	Cranium	Infant	0	−20.8	+13.2	−28.6	+10.2	+11.1	+16.7	2.8
#6	VPB129	VPB	Rib	Infant	0.5–1.75	−20.8	+14.4	−28.6	+12.4	+10.9	+18.8	3.2
#7	VPB245	VPB	Rib	Infant	1.08–2.83	−22.5	+15.1	−30.9	+13.2	+11.0	+20.0	3.3
#8	VPB257	VPB	Rib	Infant	0.42–1	−22.7	+13.7	−30.8	+13.8	+7.0	+18.5	3.6
#9	VPB79	VPB	Phalange (hand)	Adult	30–49	−20.9	+11.2	−28.8	+15.7	+9.1	+15.4	2.9
#10	VPB153	VPB	Phalange (hand)	Adult	30–49	−21.2	+11.8	−28.7	+15.2	+7.0	+15.3	3.2

Abbreviation: TP, trophic position.

but will not be used to discuss the utilization of these sources in any specificity.

5 | RESULTS

5.1 | Bulk collagen $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values

Figure 4 shows the bulk collagen $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values plotted against the age profile of all non-adults (age < 15 years) from the two sites. Summary statistics are provided in Table 3. The interquartile ranges of all adult measurements are used as baselines to help evaluate weaning practices at each respective site. In BLR, subadults have statistically significantly elevated $\delta^{15}N$ values (Wilcoxon, $p = 0.047$) and depleted $\delta^{34}S$ values (Wilcoxon, $p = 0.028$) comparing to the adults. In VPB, subadults have statistically significantly elevated $\delta^{15}N$ values (Wilcoxon, $p = 0.035$) comparing to the adults.

Modeling with WARN suggested that subadults at BLR likely started weaning at 1.8 years of age, and were completely weaned by 2.0 years (joint probability of 0.033). At VPB, WARN suggested the subadults likely started weaning at 1.4 years and finished weaning at 5.3 years of age (joint probability <0.001), respectively. As only estimations with joint probability >0.0025 are deemed valid, the VPB estimation is considered invalid. Detailed results of the analysis are provided in the supplementary Information (Appendix S1).

5.2 | Compound specific isotope analysis

The summary biological information and results from selected components for the 10 individuals analyzed for compound specific isotope contents are listed in Table 4. Figure 5 shows all human data plotted against faunal data. The complete set of carbon and nitrogen isotope measurements of each AA for all data used in this study (original and previously published) is provided in Dataset S3. Carbon isotope ratios in phenylalanine ($\delta^{13}C_{Phe}$) and the difference between phenylalanine and glycine ($\Delta^{13}C_{Gly-Phe}$) are used to illustrate the sources of carbon in the diets of the BLR and VPB individuals (Figure 5a). Nitrogen isotope ratios in glutamic acids/glutamine ($\delta^{15}N_{Glx}$) and phenylalanine ($\delta^{15}N_{Phe}$) are used to highlight the TP of the BLR and VPB individuals (Figure 5b).

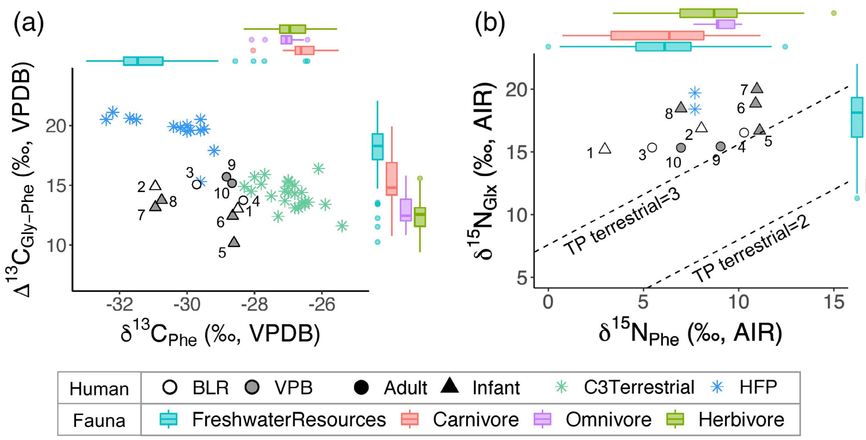

FIGURE 5 Compound specific carbon and nitrogen isotope values of humans from BLR and VPB compared with other published human and faunal data. Faunal data are shown in the boxplots; human data are shown in the biplots: (a) $\delta^{13}C_{Phe}$ versus $\Delta^{13}C_{Gly-Phe}$ values of all human groups compared with faunal data; (b) $\delta^{15}N_{Phe}$ versus $\delta^{15}N_{Glx}$ values of all human groups compared with faunal data, dashed lines illustrate the terrestrial TP, calculated using the formula described in Chikaraishi et al. (Chikaraishi et al., 2009; Chikaraishi et al., 2014). Published human data are organized according to the major sources of dietary protein consumed at the sites—C_3 terrestrial or high freshwater protein (HFP), and are denoted by the asterisks. For a key for the sample codes please refer to Table 4

As shown in Figure 5a, the $\delta^{13}C_{Phe}$ values of BLR and VPB humans shows that the individuals from BLR and VPB have obtained their dietary carbon from a source with very depleted $\delta^{13}C$ values. While the $\Delta^{13}C_{Gly-Phe}$ values of the two groups are more comparable with those exploiting mostly C_3 terrestrial resources, the range of $\Delta^{13}C_{Gly-Phe}$ values of freshwater resources is fairly large, and thus does not completely exclude the possibility that the BLR and VPB humans also consumed freshwater resources. The $\delta^{15}N_{AA}$ values are more straightforward (Figure 5b). All humans from BLR and VPB have high TP comparable to those of carnivores and freshwater resources, and similar to the two Mesolithic hunter-gatherers from Noyen-sur-Seine, a site that is less than 20 km downstream from BLR (Figure 5b).

6 | DISCUSSION

6.1 | Bulk collagen $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values

The World Health Organization (WHO) notes that breastmilk alone can no longer fully sustain the nutritional needs of a human child beyond 6 months of age (Dewey, 2003). Thus, the low probability from the VPB model with WARN, and the biologically unrealistic late t_1 estimation (1.8 years) for the BLR model suggests that the priors used in these models were inappropriate. One key assumption for WARN is that there is distinct difference between the isotopic compositions of weaning food and breastmilk, and that the onset of weaning is signified by a noticeable shift in the infant's isotopic compositions, usually in the form of a sharp decline in $\delta^{15}N$ values. This is likely true for most type of starch-based weaning food. However, the incorporation of a weaning food with high $\delta^{15}N$ values can mask the isotopic signals of the dietary shift. As mentioned earlier, the consumption of freshwater resources among subadults is suspected based on the unusual patterns in the subadult $\delta^{34}S$ values at BLR and $\delta^{13}C$ values at VPB. As freshwater resources tend to be enriched in ^{15}N, it is possible that the use of freshwater resources as weaning food have led the models to produce erroneously late weaning estimations for these two sites.

At BLR, a downward trend in the $\delta^{34}S$ values of the subadults similar to that of the $\delta^{15}N$ values can be observed across the age profile (Figure 4C). As mentioned earlier, the $\delta^{34}S$ baseline of freshwater resources at the Seine is very depleted (−13.3‰ ± 4.9‰) comparing to those of the wild (+2.2‰ ± 2.1‰) and domesticated animals (+7.2‰ ± 1.7‰) (Bocherens et al., 2011; Cheung et al., 2021; Drucker et al., 2018). As the adults have mean $\delta^{34}S$ value similar to those of the domesticated animals, the pattern in the subadults could be explained by an increase in proportion of foodstuff from either wild animals, or freshwater resources among the subadults. Note that the $\delta^{34}S$ baseline of the freshwater resources is significantly more negative than those of the terrestrial sources, a small amount of protein from the freshwater systems could strongly shift an individual's S isotopic composition. It is therefore more likely that a small proportion of freshwater protein, rather than a large proportion of wild games, was included in the diets of the subadults.

At VPB, the $\delta^{34}S$ values of the subadults present a different pattern than that observed at BLR. Unfortunately, no faunal remains from the freshwater system have been recovered to verify the S baseline at the Marne River. However, three of the youngest subadults have substantially depleted $\delta^{13}C$ values that cannot be explained by any terrestrial sources presented in the mixing polygons (Figure 2). For $\delta^{15}N$ values, subadults who should have begun the weaning process (age >1 year) continued to show highly enriched $\delta^{15}N$ values (Figure 4b), suggesting high trophic level foodstuff was fed to these subadults as weaning food. Therefore, both $\delta^{13}C$ and $\delta^{15}N$ values of the subadults suggested that freshwater resources were likely consumed by the subadults at VPB.

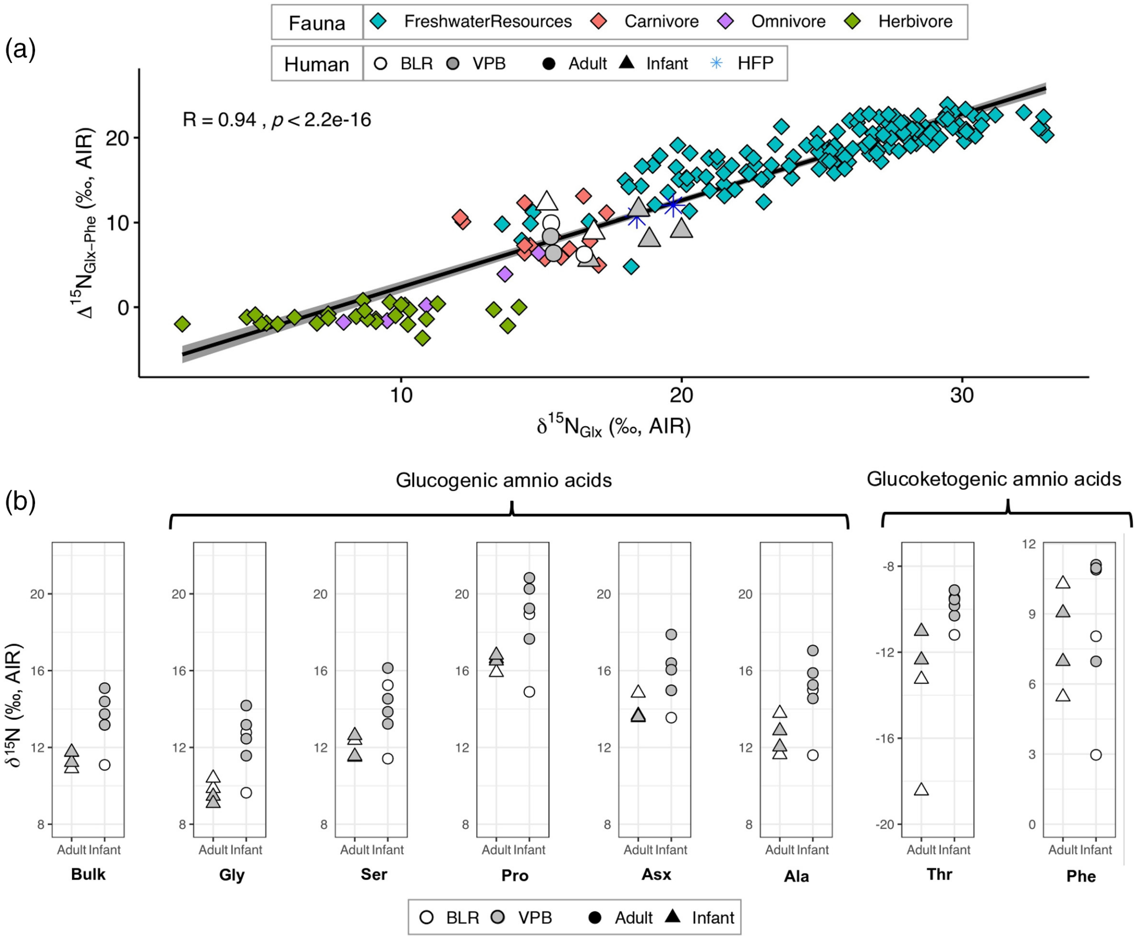

FIGURE 6 Comparison of the δ^{15}N values of different amino acids. (a) $\delta^{15}N_{Glx}$ versus $\Delta^{15}N_{Glx-Phe}$ values of all humans compared with faunal data; (b) Comparison of the δ^{15}N values of bulk collagen versus other amino acids in all adults and infants from BLR and VPB. BLR, Balloy; VPB, Vignely

6.2 | Compound specific isotope analysis

The $\delta^{13}C_{Phe}$ values of all BLR and VPB humans fall within the range of those of freshwater resources and HFP consumers (Figure 5a). Three individuals (#2, #7, #8) in particular have $\delta^{13}C_{Phe}$ values completely outside of the expected C_3 terrestrial range. Thus, it is very likely that freshwater resources were utilized at these two sites. The patterns in the $\Delta^{13}C_{Gly-Phe}$ spacings are harder to explain. As mentioned above, higher $\Delta^{13}C_{Gly-Phe}$ spacings are expected in high aquatic protein consumer. The BLR and VPB humans have $\Delta^{13}C_{Gly-Phe}$ values more similar to terrestrial protein consumers than to HFP groups. However, given that terrestrial sources with extremely low $\delta^{13}C_{Phe}$ values are rare, freshwater resources are still the most appropriate explanation for the unusually low δ^{13}C values in the subadults. There are two possible reasons for the unexpectedly lower $\Delta^{13}C_{Gly-Phe}$ values: (i) the baseline $\delta^{13}C_{Gly}$ at both rivers (Seine and Marne) are unusually depleted, or (ii) the two groups mostly exploited lower trophic resources (i.e., smaller fish) from the rivers. Note that the HFP humans in Figure 5b have significantly higher $\delta^{15}N_{collagen}$ values ($+15.7 \pm 1.6$‰) than those from BLR and VPB (11.8 ± 1.3‰), therefore the relatively lower $\Delta^{13}C_{Gly-Phe}$ values could indeed reflect the consumption of lower trophic level freshwater resources at BLR and VPB. More baseline data from the two rivers are required before more concrete conclusion can be reached. Moreover, most subadults appear to have even lower $\Delta^{13}C_{Gly-Phe}$ values than those of the adults (Figure 5a). This distinction is even more marked amongst the VPB individuals, where the adults and subadults have completely non-overlapping $\Delta^{13}C_{Gly-Phe}$ values (subadults: +10.2‰ − +13.8‰; adults: +15.2‰ − +15.7‰). Thus, we argue that at the two sites, a foodstuff with distinctively lower $\delta^{13}C_{Phe}$ and $\delta^{13}C_{Gly}$ values, likely freshwater resources, were consumed by both adults and subadults. The subadults likely consumed a higher proportion of such foodstuff than the adults, thus explaining why the adults are positioned in between the subadults and other C_3 terrestrial communities in Figure 5a.

As noted by the recently published study on BLR and VPB (Cheung et al., 2021), the bulk collagen δ^{15}N values of individuals from these two sites suggested the groups consumed a considerable amount of animal protein. This is corroborated by compound specific isotope data from the current study, where all examined humans from

BLR and VPB have TP within the ranges of carnivores and freshwater resources, and are comparable to the two Mesolithic hunter-gatherers from Noyen-sur-Seine (Figure 5b). To ensure the enriched $\delta^{15}N_{AA}$ values of these individuals actually reflect higher TP but are not artifacts of other factors, $\delta^{15}N$ values of several AAs are further examined. For agrarian societies, one concern is that intensive manuring could raise the $\delta^{15}N$ values of the soils and plants, and subsequently pass on the enrichment along the food chain (Bogaard et al., 2013; Fraser et al., 2011; Szpak et al., 2012). An experimental study has shown that the consumption of manured plants should not affect one's TP as reflected in their $\Delta^{15}N_{Glx-Phe}$ values (Styring et al., 2014). Figure 6a compares the $\Delta^{15}N_{Glx-Phe}$ values with $\delta^{15}N_{Glx}$ values of humans and animals with different feeding behaviors. The positive correlation reflects the trophic level effect. As shown in Figure 6a, all individuals from BLR and VPB, especially the subadults, clearly clustered with the carnivore and freshwater resources, suggesting that the enriched $\delta^{15}N_{collagen}$ values among the subadults indeed reflected their higher TP, and were not due to the consumption of heavily manured plants. Another concern is that higher $\delta^{15}N$ values in young children could be caused by nutritional stress related to weaning (Kinaston et al., 2009; Pearson et al., 2010). There is little direct work done on the effect of catabolism in the $\delta^{15}N_{AA}$ values in human bone collagen. However, a recent study reported that in southern elephant seals, intense catabolism can cause increases in the $\delta^{15}N$ values of many glucogenic AAs, most notably in glycine (Gly), serine (Ser), proline (Pro), and aspartic acid (Asx) (Lübcker et al., 2020). An exception is that of for Ala, as it is associated with the glucose-alanine cycle, where muscles are broken down to produce glucose during periods of nutritional stress (Mizock, 1995). As shown in Figure 6b, a comparison of the bulk and compound specific $\delta^{15}N$ values between the subadults and adults from the two sites do not show any sign of catabolism. Note that there are some fundamental differences in the two studies in terms of species and tissues involved. In particular, keratin (whiskers) is expected to turnover much quicker and therefore registered a stronger catabolic response comparing to bone collagen. Therefore, this comparison is rudimentary and is only provided for general informational purposes. Nevertheless, based on the associated anomalies observed in $\delta^{13}C$ (VPB) and $\delta^{34}S$ (BLR) values, as well as the magnitudes of differences in isotopic compositions between adults and subadults, it is quite likely that the elevated $\delta^{15}N$ values in subadults were dietary related rather than caused by nutritional stress or consuming intensively manured crops (Hatch, 2012; Hobson et al., 1993; Szpak et al., 2019; Treasure, Church, & Gröcke, 2016). Considering that the Cerny people are known to cultivate several varieties of wheat and barley (Bakels, 1999; Bakels, 2009), it is safe to assume individuals from BLR and VPB likely consumed a considerable amount of plant foods and did not have a carnivore-like diet. The high TP of these individuals thus connotes the consumption of some freshwater resources, instead of having a highly carnivorous diet.

In Figure 5b, most of the subadults (with the exception of #5, which is a perinate and likely died before registering a strong breastfeeding signal) have higher TP (3.2–3.7) than those of the adults (2.9–3.4). While the high TP of certain subadults, for example of #8 (age: 0.4–1 years; TP: 3.6) likely reflected some breastfeeding effect, similarly high TP of slightly older subadults, such as #1 (age: 2–3.3 years; TP: 3.7) likely reflected the consumption of some freshwater resources as transitional weaning food. A modern study of species richness at the Seine River basin documented at least 30 species of fish in the area (Oberdorff et al., 1993). The biodiversity of the region prior to the intensive anthropogenic perturbations over the past several centuries would likely be even greater. Fish meat is an excellent source of high-quality protein, other parts of the fish, such as liver and brain, also contain a host of other essential nutrients for human growth (Gil & Gil, 2015). Thus, this easily digestible and high-quality protein is an excellent and obvious food option for both adult and young children in riverine communities.

The results from this study have revealed valuable insights into the diets and weaning practices of the Cerny communities (BLR and VPB). However, the bulk collagen isotopic patterns of the two sites contradict one another. Particularly at BLR, as $\delta^{13}C$ values from freshwater resources from the site has already confirmed a very depleted C baseline in this part of the Seine, yet no individual from BLR has particularly depleted $\delta^{13}C$ values. *Au contraire*, the $\delta^{13}C_{Phe}$ values of all individuals from BLR and VPB readily indicate a diet consisting of a protein source that is very depleted in ^{13}C. This shows that bulk collagen isotope measurements are not as sensitive as compound specific isotope measurements, and that they can be obscured by factors that are not necessarily related to diets. Nonetheless, our research has shown that during the Middle Neolithic period, the Cerny communities utilized freshwater resources, even fed protein from the freshwater systems, possibly smaller fish, to weaning children. In fact, the initial assessment that the two groups consumed more animal proteins than other contemporaneous agrarian groups (Cheung et al., 2021) was probably misguided. Based on the CSIA results from this study, it is more likely that the relatively higher $\delta^{15}N_{collagen}$ values observed across the two groups were due to their utilization (both adults and subadults) of freshwater resources, attested by the high TP of all individuals as shown in Figure 5b. More importantly, our results have stressed the importance of combining CSIA of both carbon and nitrogen, especially when evaluating the consumption of freshwater resources with little to no baseline data. In this particular case study, looking at $\delta^{13}C_{AA}$ values alone could lead to the mistaken conclusion that all the infants were in a lower TP than the adults; while the $\delta^{15}N_{AA}$ values alone would not be able to characterize the source of dietary protein in the diets of these two groups.

7 | CONCLUSIONS

Other than identifying the possible exploitation of freshwater resources at the two Cerny sites, especially among the subadults, there are several significant implications to the findings of this study. First, our results have revealed that bulk collagen $\delta^{13}C$ and $\delta^{15}N$ values are not always able to identify the consumption of freshwater resources. While $\delta^{34}S$ values can be of help, the local S baseline can vary greatly between different freshwater systems, and therefore should only be used when the local S baseline is well understood. Compound specific isotopic patterns, however, are less geographically dependent and more directly related to diets, especially when both C and N isotope profiles are considered, and therefore can help identify the consumption of freshwater resources more effectively.

This leads to the second implication: due to our inability to reliably detect freshwater resources consumption in past diets, it is possible that protein/lipid-based weaning food played a bigger role in past agricultural communities than previously thought, as demonstrated in this case study. In fact, ethnographic studies have shown that fish has been fed to weaning children in some cultures (Table 1). A handful of studies have also detected the possible use of freshwater fish as weaning food in past societies (Nehlich et al., 2011; Waters-Rist, 2019). Moreover, comparing to freshwater resources, the consumption of other protein/lipid-based weaning food such as meat or meat broth, can be even harder to detect isotopically. This calls for caution when making assumption regarding the composition of weaning food in the past.

The third implication of this study is related to the general assumption that weaning diet would be isotopically similar to adult diet. Adults have a higher daily caloric requirement, and likely feed from a much wider range of food sources than weaning children do. Therefore, if a high-protein, high-trophic food, for example fish, is used as weaning food, even a small quantity of it could strongly sway the isotopic profile of the child, potentially creating the impression that weaning activities began much later than it did. Thus, the conventional approach of using the mean $\delta^{15}N_{collagen}$ value of adults to help estimate weaning age is only applicable in communities where diets across all age groups are highly homogenous and largely plant-based. When working on prehistoric communities, it is important to consult all available archeological/historical evidence to gain a better understanding of what kind of resources could have been used as weaning food, and adjust the weaning baseline accordingly.

CSIA has proved to be a powerful analytical tool for palaeodietary reconstruction, especially in helping to distinguish between source groups with overlapping isotopic ranges and poorly understood baselines. Nevertheless, despite the small sample size and lack of local faunal baseline data, by combining multiple lines of stable isotope evidence, especially those of $\delta^{13}C_{AA}$ and $\delta^{15}N_{AA}$, our study has provided illuminating insights into how freshwater resources may have been utilized by these two Middle Neolithic communities.

AUTHOR CONTRIBUTIONS

Christina Cheung: Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (lead); software (lead); visualization (lead); writing – original draft (lead). **Estelle Herrscher:** Methodology (supporting); project administration (supporting); resources (equal); supervision (equal); visualization (supporting); writing – review and editing (supporting). **Aline Thomas:** Funding acquisition (lead); project administration (lead); resources (equal); supervision (equal); writing - review and editing (supporting).

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data used in this study are provided in the Supplements (Datasets S1 to S3), and all R scripts used are provided in S4.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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