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To cite this article: Gwenaëlle Goude, Léonie Rey, Françoise Toulemonde, Mathilde Cervel & Stéphane Rottier (2017) Dietary changes and millet consumption in northern France at the end of Prehistory: Evidence from archaeobotanical and stable isotope data, Environmental Archaeology, 22:3, 268-282, DOI: 10.1080/14614103.2016.1215799

To link to this article: http://dx.doi.org/10.1080/14614103.2016.1215799

Dietary changes and millet consumption in northern France at the end of Prehistory: Evidence from archaeobotanical and stable isotope data

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Diachronic changes of dietary human habits between the Neolithic and the Bronze Age are mainly identified through archaeological artefacts and archaeozoological and archaeobotanical studies. This paper aims to demonstrate the importance of a multi-disciplinary approach for palaeodietary studies and to identify the food changes between Neolithic and Bronze Age human groups in northern France. These changes are probably linked to the introduction of new crops, such as millet, and the use of stable isotope analysis on bones and teeth proves to be an effective method for assessing the role of this specific cereal in the diet and the economy. Stable isotope analyses were performed on bone and tooth collagen and apatite from eight humans and five domestic animals from a Late Bronze Age site (LBA; Barbuise; 15th–13th c. BC; Aube). The studied corpus is compared with isotopic data from human and animal bones from a nearby Neolithic site (Gurgy; 5th mill. BC; Yonne) and regional Neolithic to Iron sites located in northern France. Moreover, Barbuise data are supplemented by information from an important archaeobotanical study carried out on 21 LBA and Early Iron Age sites in the region. Neolithic and LBA human collagen isotopic ratios ($\delta^{13}C$, $\delta^{15}N$) differ statistically, as do those of some animals. Carbon isotopic ratios of human apatite corroborate collagen results indicating the consumption of ^{13}C enriched food by LBA humans and animals compared to Neolithic samples. The high number of occurrences of plant remains in the Bronze Age settlements near the site points to the consumption of C_4 plants, such as millet, and would account for these results.

Keywords: Stable isotope, Archaeobotany, Millet, Neolithic, Bronze Age, France

Introduction

Archaeological contexts

Ancient diet studies efficiently document the socio-cultural changes that have occurred during the main Prehistoric transition phases. Stable isotope analysis is now recognised as an essential method to track individual/human group subsistence strategies. One of the numerous examples is the Mesolithic–Neolithic transition, which has been extensively assessed since the reference works of Tauber (1981) and Schoeninger *et al.* (1983). The strong decrease in the bone carbon isotope ratio observed between Mesolithic and Neolithic humans along the Atlantic coast indicates a new food acquisition pattern linked to the arrival and development of agriculture and herding (Richards 2003; Richards and Hedges 1999; Richards and Schulting 2003; Schulting and Richards 2002), and recent data from archaeological artefacts corroborate these studies (Cramp *et al.* 2014). On the other hand, a less contrasted pattern was proposed for some inland areas, where freshwater resources played a significant role in the subsistence economy at the beginning of the Neolithic while cereal cultivation was not clearly attested (Bonsall *et al.* 2000; 2004; Boric *et al.* 2004; Nehlich *et al.* 2010).

Protohistorical periods are also of major interest to archaeologists and anthropologists as new trade exchanges (e.g., salt), the material economy (e.g., metallurgy), migrations and crops, followed by demographic increase and the development of chiefdoms and hierarchy (e.g., Earl and Kristiansen 2010; Guilaine 2007; Harding 2000; Kristiansen and

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Rowlands 1998; Rottier *et al.* 2012) probably had an impact on diet. Diachronic changes in human habits between the Neolithic and the Bronze Age are well documented in the archaeological record (e.g., Harding and Fokkens 2013; Tarrête and Le Roux 2008). Environmental modifications, climatic deterioration between 1550 and 1350 BC (Magny 2004; Magny *et al.* 2007), during the transition between the Middle and Late Bronze Age (LBA), also prompted Bronze Age populations to adapt to different cultivation conditions (Richard *et al.* 2007). In Western Europe, human groups occupied new territories from the beginning of the Neolithic (end of the 6th to the beginning of the 5th mill. BC), basing their subsistence economy on a new way of life including animal herding and plant cultivation. Neolithic societies settled and exploited these environments more intensively during the middle Neolithic (5th–4th mill. BC). During the end of the Neolithic and the transition to the Bronze Age (3rd mill. BC), long distance trade began to develop and human groups profoundly changed their social organisation (Besse 2007; Guilaine 2007; Kristiansen 1998; Rowlands 1998). Other modifications are also evidenced by the advent of metallurgy, leading mainly to different trading practices according to raw material availability (copper, then tin in particular) and the place where they were worked (Harding and Fokkens 2013). It seems that, during the Neolithic, most of the exchanges took place step by step, strengthening relationships with neighbours. On the other hand, at the end of the Neolithic and during the Bronze Age, trade exchanges increased and covered wider areas more quickly. Archaeological records from the second half of Bronze Age substantiate this pattern, particularly with regard to the domestication of horses (Harding and Fokkens 2013).

This study focuses on the south-eastern part of the Paris Basin, which remained a major cultural intersection throughout these four millennia, due to the merging of the Danube and Mediterranean neolithisation routes and of the North-Alpine and Atlantic complexes. This cultural crossroads then shifted towards the South along the Rhône valley. Cultural exchanges thus changed over time and could possibly also have modified dietary patterns. In France, diachronic studies have rarely been conducted using stable isotope analyses on human bones and teeth. Moreover, the comparison of archaeobotanical and anthropological data is still under-developed in this region. Our paper aims to assess the potential dietary changes between the Neolithic and Protohistoric periods in relation with the spread of millet and to document the role of this crop in the Bronze Age economy in northern France. The study focuses on a LBA site, Barbuise, by using stable isotope analysis. Isotopic data of Barbuise are compared to available data sets from (1) a Neolithic regional site, Gurgy, and (2) Neolithic to Iron Age sites located in the northern half of France.

The LBA funerary complex of Barbuise

The funerary group of Barbuise and La Saulsotte (Aube, France), located in the south-eastern part of the Paris Basin, includes the most important human bone collection (ca. 150 burials) dated to the beginning of the LBA (15th–12th c. BC) in the northern half of France. This funerary group is part of a cultural system with more than 30 funerary sites discovered in a confined area. In this area, funerary practices appeared to diversify during the transition between the Middle and the LBA. Cremations became more frequent while burial types were more and more diversified (Rottier *et al.* 2012). Besides cremations, two main tomb categories have been described: one with a long pit where the deceased is lying down and one with a shorter-sized pit where the deceased is vertically crouched (Rottier 2004). Despite the variability of these practices, all the burials are managed in similar and standardised ways with bone removal after the burial (Rottier 2009, 2011). Bone removals could have been performed while the corpse was rotting or when decomposition was finished (Rottier 2012, 2016). Up to now, this funerary practice is known only in the southeast of the Paris basin (Rottier *et al.* 2012). This phenomenon leads to a severe lack of skeletal elements, particularly for pelvis and cranium bones, sometimes restricting biological studies. Moreover, immature individuals are often missing from the site. The identification of "local" versus "non-local" individuals is proposed according to the grave goods (several publications detail the different funerary items; Rottier 2015; Rottier *et al.* 2012), and the bone morphometric study did not differentiate biological groups (Rottier *et al.* 2012). The archaeological records indicate an allochthonous origin for some of the material with central European influences. Long distance trade and contacts are well known for this period, including the widespread exchange of goods and raw materials. The archaeo-anthropological study carried out at Barbuise highlighted a possible foreign origin for some of the individuals inhumed here (Rottier *et al.* 2012).

The archaeobotanical data

A recent archaeobotanical synthesis of 272 settlement sites and 375 Bronze Age occupations in France (Bouby *et al.* in press) reported that millet cultivation expanded greatly on French territory during the LBA (the species involved are broomcorn millet – *Panicum miliaceum* – and to a lesser extent foxtail millet – *Setaria italica*). This phenomenon is

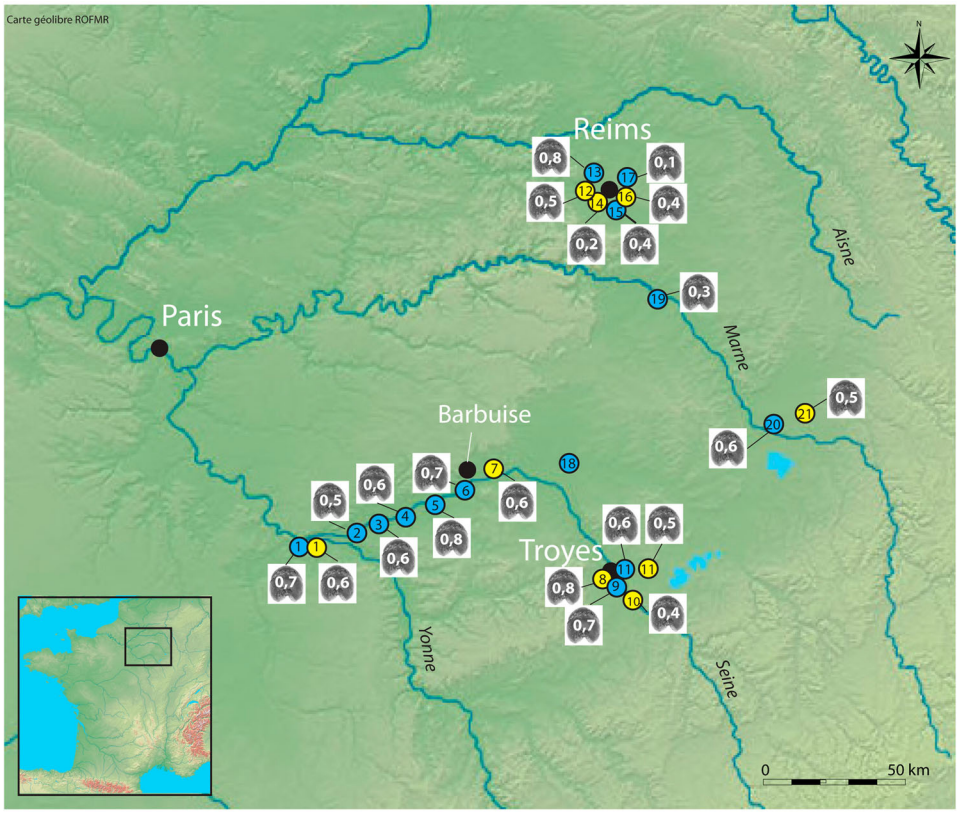
particularly remarkable and precocious in north-eastern France. This was most probably enhanced by cultural influences and exchanges within the north-alpine complex, linking during the Middle and the LBA this part of the present French territory to central Europe. Millet cultivation is already well developed in central Europe during the middle Bronze Age, and may have spread from this area to other parts of Europe during the end of the Middle/beginning of the LBA (Stika and Heiss 2013).

Broomcorn millet is noted occasionally on the French territory from the Early Bronze Age (EBA) onwards, but gaps in the data for this period and for the subsequent Middle Bronze Age do not allow for a full understanding of how and exactly when during the Bronze Age this cereal gained economic significance. In north-eastern France, in an area of less than one hundred kilometres around the site of Barbuise, an archaeobotanical study conducted on 21 LBA and Early Iron Age settlements (Ferrage-Toulemonde 2013; Toulemonde *et al.* forthcoming) has shown that during this period (1350–460 BC), millet, and especially broomcorn millet, had real economic significance and definitely contributed widely to the human diet. Food production was based on a highly diversified spectrum of cultivated species, including ten cereals (different species of barley, wheat and millet), four pulses and three oil plants. Staple crops were mainly cereals. Hulled barley (*Hordeum vulgare*) was the most important, but millet also played an important role in production. All but one of the 21 analysed settlements yielded broomcorn millet, sometimes in large quantities and with high ubiquity (Fig. 1): caryopses of millet were found in 57% of the 421 analysed samples, and represented 23% of the total of the remains (compared to 79% of the samples and 24% of the remains for barley). Millet was particularly frequent and abundant in the upper Seine valley, where the LBA cemetery of Barbuise is located, since the beginning of the LBA. So even if archaeobotanical study was not performed directly on vegetal remains from Barbuise, we can assume that millet was cultivated by the inhabitants of Barbuise, as this cereal was grown everywhere else in the area.

Stable isotope analysis and diet: a brief review

In bone tissues, the recorded stable isotope ratios are linked to ingested food and water with a differentiation between collagen, which is mainly formed from the protein in the diet and apatite carbonate, derived from blood CO_2, which reflects all of the consumed energy (i.e., carbohydrates, fats and the rest of the protein not used for collagen; Ambrose and Norr 1993). At the base of the food chain, plants have specific $\delta^{13}C$ and $\delta^{15}N$ ratios according to climatic and edaphic conditions (e.g. Ambrose and De Niro 1989; Mariotti 1982), photosynthetic pathways (Farquhar *et al.* 1989) and species (Virginia and Delwiche 1982). In particular, this study focuses on $\delta^{13}C$ which is different in C_3 plants (such as wheat and barley) and C_4 plants (such as maize and millet), allowing for differentiation between the consumers of these plants located far from coastal environments (e.g. Ambrose *et al.* 2003). Herbivore bone collagen registers the $\delta^{13}C$ of the plant consumed, with an enrichment of ca. 5‰ and the difference between the bone collagen of a prey and predator (i.e., herbivore–carnivore) is ca. 0–1‰. The nitrogen isotopic ratio is also an environmental marker and is mainly used for differentiating marine vs. terrestrial environments (Schoeninger and DeNiro 1983) and detecting the trophic level of an individual, as the difference observed between prey-predator bone collagen is ca. 3–5‰ (see up to 6‰) (Bocherens and Drucker 2003; O'Connell *et al.* 2012). The study of both $\delta^{13}C$ and $\delta^{15}N$ in human bone collagen thus provides information on the environment exploited, preferentially consumed plant types and the relative quantity of ingested animal protein, provided that the range of values defining the local environment based on animal and/or botanical isotopic data is known (e.g. Herrscher and Le Bras-Goude 2010).

In human bone and tooth, apatite carbonate is derived from blood CO_2 and reflects bulk energy, including carbon from non-protein sources and the remaining carbon from amino acids not used to synthesise protein (Ambrose and Norr 1993; Krueger and Sullivan 1984). Apatite $\delta^{13}C$ is enriched by ca. 9·4‰ compared to the diet (Ambrose *et al.* 1997). Contrary to collagen, $\delta^{13}C$ in apatite carbonate provides information on the low protein sources consumed, such as cereals or molluscs, for example (Ambrose and Norr 1993). The combination of both collagen and apatite isotope ratios adds a wider perspective to the study of the human diet as proposed by Ambrose *et al.* (1997; 2003) and by Kellner and Schoeninger (2007), particularly regarding the consumption of C_4 resources. The measurement of oxygen isotopic ratios in apatite carbonate has often been used to track the geographic origin and mobility of humans (e.g. Iacumin *et al.* 1996). Indeed, $\delta^{18}O$ in ground water sources is linked to different geographic parameters such as latitude, altitude and temperature (Longinelli 1984). For example an individual living in a mountainous area would show a lower $\delta^{18}O$ ratio than a person living in a coastal environment (e.g. Knudson 2009). However, several factors highlight the difficulties in effectively using this proxy in archaeological human bone tissue from European areas: apatite carbonate preservation, lack of discriminating $\delta^{18}O$ ratios in a wide area of the European



1-Ville-St-Jacques "Le Fond des Vallées"/"Le Bois d'Echalas"; 2-Balloy "La Haute Borne"; 3-Jaulnes "Le Bas des Hauts Champs-Ouest ;4-Noyen-sur-Seine "Le Nord du Bois du Chêne" ; 5-Villiers-sur-Seine "Le Gros Buisson" ;6-Pont-sur-Seine "La Gravière" ; 7-Périgny-la-Rose "Pampeleine" ; 8-St-André-les-Vergers "Echenilly" ; 9-Pont-Ste-Marie "Rue Fernand Jaffiol";10-Bréviandes "Zac St Martin" ; 11-Buchères, Moussey, St-Léger-Près-Troyes "Parc Logistique de l'Aube"; 12-Thillois "Les Terres Soudées"; 13-Thillois "La Croix Rouge";14-Bezannes "La Bergerie"; 15-Cormontreuil "Les Grands Godets"; 16-Reims "Zac Croix Blandin" ; 17-Bazancourt-Pomacle "Sur les Petits Poissons"; ;18-Plancy-l'Abbaye "Saint Martin" ;19-St Martin-sur-le-Pré "Rue des Castors"; 20-Matignicourt-Goncourt "Les Brouillards" ; 21-Thiéblemont-Farémont "RN4 Echangeur".

X Late Bronze Age settlement (1350 - 800 BC) (X= n° of settlement)

X Early Iron Age settlement (800 - 460 BC)

X Cultivation of broomcorn millet. X = Ubiquity = proportion of contexts with millet (max = 1)

territory and the significant increase in $\delta^{18}O$ ratios in "anthropic" beverages (e.g. tea) and cooked products (e.g. soup), compared to ground water (Brettell *et al.* 2012). For these reasons, the $\delta^{18}O$ ratio will not be included in the analysis of our data.

Samples and analyses

Our study focused on both human and animal remains from the LBA of Barbuise. The analyses of different animal bones allowed us to define a range of values for a specific environment and a known diet and thus to interpret the human data (e.g. Goude and Fontugne 2016). Isotopic analyses were performed on eight human bones and teeth (five females and three males; due to skull removals in most of the burials, there were only few individuals available with both bone and teeth; Rottier *et al.* 2012), as well as five animals (two pigs, two horses and one dog). Sex determination was performed by using morphoscopic and morphometric methods (Bruzek 2002; Murail *et al.* 2005) and age at death was estimated by using bone maturation. In order to (1) compensate for the small sample size of animal remains studied and (2) attempt diachronic comparisons, data from other sites located in the centre and north of France were also used (Neolithic animal remains: Balasse *et al.* 1997; Balasse 1999; Balasse *et al.* 2000; Bocherens *et al.* 2000; Neolithic to Iron Age human and animal remains: Goude *et al.* 2014; Herrscher *et al.* 2013; Le Bras-Goude *et al.* 2011; Fig. 2). For humans, both bone and M2 root were selected. Due to permanent remodelling, bone tissues provide information on the last years of life. The remodelling is faster during infancy and strongly diminishes with the passing years (Valentin 2003). The LBA samples only come from adults, thus the isotopic signal recorded in bone represents average food consumption during adulthood. On the other hand, the M2 root mainly grows during childhood and the apex closes during adolescence (ca. 6–14 years old; Schaefer

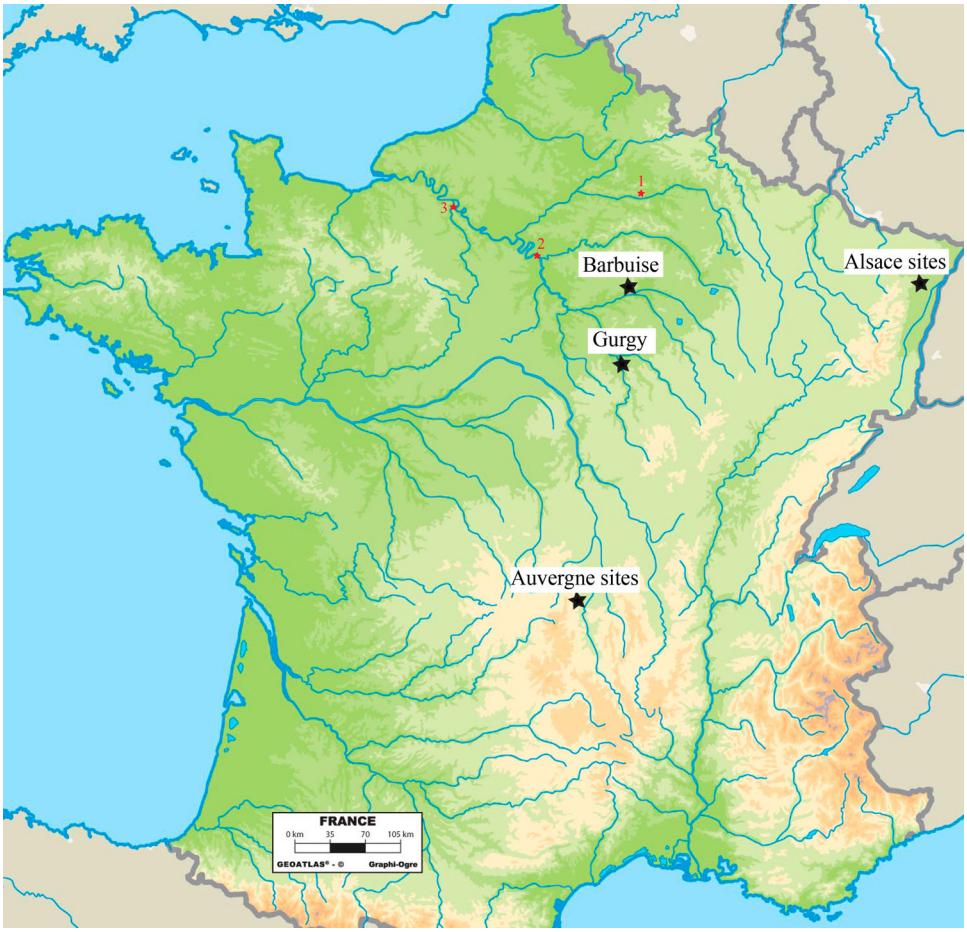


Figure 2. Location of the archaeological sites mentioned in the study. 1 – La Villette (Bocherens *et al.* 2000); 2 – Bercy and 3 – Maizy/Cuiry (Balasse, 1999, Balasse *et al.* 2000).

et al. 2009) and does not remodel afterwards. The isotopic ratios recorded in this root thus indicates mean food consumption during these growth years and provides data on an earlier period in life compared to bone (Tables 1 and 2). With this double sampling strategy we expect to detect dietary changes between these two periods of life, particularly for individuals considered as "non-local".

Collagen extraction and apatite purification were performed at the LAMPEA laboratory (UMR 7269, Aix-en-Provence, France). Bone and tooth collagen extraction follow the methods elaborated by Longin (1971) and Bocherens (1992). Material was first cleaned with a sandblaster and ground (0·7 mm). Bone and root powder were demineralised in a strong acid solution (HCl; 20 min, room temperature), filtered and the remaining residue was put in a NaOH solution (for 20 h, at room temperature), and filtered again. The remaining residue was solubilised in a HCl solution (pH 3; 17 h, 100°C) and filtered with an Ezee-Filter® device. The clean solubilised collagen was frozen, freeze-dried (min 48 h) and then analysed by EA-IRMS (Europa Scientific 20–20 IRMS, Iso-Analytical Ltd, UK) with reproducibility below 0·1‰ for C and N stable isotope ratios. LBA bone and root apatite were purified following protocols commonly used for archaeological material (Balasse *et al.* 2002; Silence and LeGeros 1991). The clean material was more finely ground than for collagen. The powder was put in a sodium hypochlorite solution (24 h, room temperature) and rinsed several times by centrifugation. The remaining residue was left in an acetic acid solution (1 h, room temperature) and rinsed several times by centrifugation. The remaining residue was frozen, freeze-dried (min 2 h) and the carbonate was then analysed using a Kiel IV Carbonate Device coupled to DeltaV Advantage IRMS (SSMIM Muséum National d'Histoire Naturelle, UMR 7209, Paris, France) with a measurement error of 0·03‰ for C. Collagen extraction yield, elemental content, stable isotope ratio, C/N ratio as well as information on surplus $CaCO_3$ measured on LBA samples are available in Tables 1 and 2.

To compare the isotopic ratios of the different datasets, only non-parametric exact tests were applied (Mann–Whitney U exact test; two-sided test, 0·05 threshold; Statistica 9.1®). This version of non-parametric test is particularly recommended for very small sample size. Moreover, to solve problems linked to multiple comparisons, we applied False

Table 1 Anthropological, archaeological and stable isotope data of the LBA bone samples from Barbuise

Lab code	Species	Sex	Origin	%N	%C	C/N	Yield (mg/g)	$\delta^{15}N_{AIR\ (‰)}$	$\Delta^{15}N_{b\text{-}t}$	$\delta^{13}C_{V\text{-}PDB\ (‰)}$	$\Delta^{13}C_{b\text{-}t}$	$CaCO_3$ exc.	$\delta^{13}Cap_{\ V\text{-}PDB\ (‰)}$	$\Delta^{13}Cap_{b\text{-}t}$	%C4 diet
GDF 1444 o	*Human*	*M*	*Non local*				*2·3*					X	*−10·8*		
GDF 1445 o	Human	F	Local	14·4	40·7	3·3	41·3	11·5	0·7	−19·1	0·5	X	*−10·5*		
GDF 1455 o	Human	M	Non local	14·6	39·5	3·1	31·7	10·1	−1·8	−19·0	0·3		−12·3	−1·1	22·3
GDF 1293 o	Human	F	Local	14·2	40·1	3·3	24·2	9·8	−0·5	−18·8	0·6		−10·5	−0·2	35·7
BPV 93 38 A o	*Human*	*M*	*Local*	*1·9*	*7·3*	*4·4*	*22·8*	*8·9*		*−22·2*		X	*−11·7*		
BPV 93 38 B o	Human	F	Local	11·7	32·4	3·2	12·6	9·3	−0·3	−18·4	−1·1	X	*−10·1*		
BPV 93 91 A o	Human	F	Local	11·9	32·4	3·2	63·3	9·3	−2·4	−18·9	0·3	X	*−11·6*		
LBB 95 142 o	*Human*	*F*	*Local*				*3·7*					X	*−10·7*		
GDF 91 13 f	Pig			14·3	39·9	3·2	25·1	6·6		−19·8		X	*−11·1*		
BPV 93 47 27 f	*Pig*						*2·4*					X	*−11·3*		
BPV 91 13 f	Horse			13·3	36·9	3·2	21·3	6·1		−21·7			−11·3		
GDF 94 101 f	Horse			15·0	44·4	3·4	25·6	7·2		−22·3		X	*−11·8*		
BPV 94 101 f	Dog			11·1	31·1	3·3	37·6	7·7		−19·0		X	*−10·1*		

Percentage of C_4 resources in total diet was estimated according to the calculation of Schwarcz (1991) and Ambrose *et al.* (1997; 2003). Data in italic were not used for the discussion.

Table 2 Anthropological, archaeological and stable isotope data of the Late Bronze tooth samples from Barbuise

Lab code	Species	Sex	Origin	%N	%C	C/N	Yield (mg/g)	$\delta^{15}N_{AIR\ (‰)}$	$\Delta^{15}N_{b\text{-}t}$	$\delta^{13}C_{V\text{-}PDB\ (‰)}$	$\Delta^{13}C_{b\text{-}t}$	$CaCO_3$ exc.	$\delta^{13}Cap_{\ V\text{-}PDB\ (‰)}$	$\Delta^{13}Cap_{b\text{-}t}$	%C4 diet
GDF 1444 d	*Human*	*M*					*5·2*					X	*−11·0*		
GDF 1445 d	Human	F		13·5	37·9	3·2	39·0	10·8		−19·7			−10·4		33·7
GDF 1455 d	Human	M		10·2	27·6	3·1	110·1	11·8		−19·3			−11·2		29·7
GDF 1293 d	Human	F		14·6	39·1	3·1	87·2	10·3		−19·4			−10·2		34·2
BPV 93 38 A d	Human	M		13·9	37·3	3·1	60·6	9·2		−19·6		X	*−12·0*		
BPV 93 38 B d	Human	F		10·2	28·3	3·2	20·4	9·6		−17·3		X	*−11·0*		
BPV 93 91 A d	Human	F		14·1	39·0	3·2	165·3	11·7		−19·2			−10·6		26·7
LBB 95 142 d	Human	F		14·9	40·6	3·2	75·8	9·4		−18·2			−10·2		33·0

Percentage of C_4 resources in total diet was estimated according to the calculation of Schwarcz (1991) and Ambrose *et al.* (1997; 2003). Data in italic were not used for the discussion.

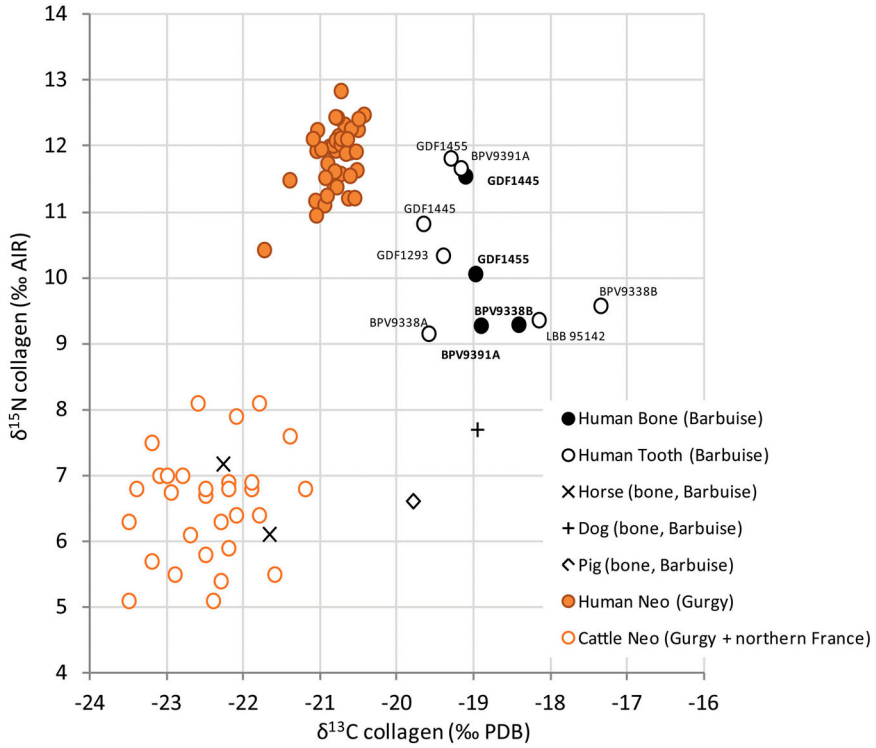


Figure 3. C and N stable isotope ratios of human and animal bones/teeth from Barbuise (this study), Gurgy (Rey 2013) and other northern sites (Balasse 1999; Balasse *et al.* 1997).

Discovery Rate correction (FDR; Benjamini and Hochberg 1995; Benjamini and Yekutieli 2001; R Core Team 2016) to *p*-values obtained for pairwise comparisons.

Results and discussion

The LBA diet

The collagen quality was checked according to several criteria and particularly the C/N ratio (Ambrose 1990; DeNiro 1985). Among the 21 LBA samples, four had a very low collagen extraction yield (<10 mg/g) and were not analysed; one sample gave poor %C, %N and C/N results and was excluded from our corpus (in italics in Tables 1 and 2; Ambrose 1990; DeNiro 1985; van Klinken 1999). The data set is thus composed of seven teeth, five human and four animal bone collagen samples. The preservation of the apatite carbonate is difficult to assess, in particular for human bone and dentin samples. Infrared analyses (FTIR) were attempted, most of the time using the crystallinity index (CI; e.g., Weiner *et al.* 1993). However, although FTIR gives promising results to detect well vs. poorly preserved bone structures (Lebon *et al.* 2010, 2011; Salesse *et al.* 2014), the correlation with the preservation of the *in vivo* stable isotope ratio has not been demonstrated to date (e.g., Beasley *et al.* 2014; Hu *et al.* 2008; Salesse *et al.* 2014). All the 21 LBA bone and dentin carbonate apatite gave isotopic results, but several samples displayed carbonate surpluses during IRMS analysis. This surplus can be considered as potentially contaminating (Zazzo 2001). Our interpretation is thus only based on the rest of the available samples: five human teeth, two human bones and one animal bone. Moreover, we can observe that samples with a low collagen yield all have carbonate surpluses (Tables 1 and 2). These observations are consistent with the study by Hu *et al.* (2006), in which apatite is considered to be more degraded when collagen is poorly preserved.

LBA animal ratios range from −22·3‰ to −19·0‰ for carbon and from 6·1‰ to 7·7‰ for nitrogen ($n = 4$). LBA animals from Barbuise show different isotopic ratios according to the species. Pig and dog, both omnivorous animals, have higher $\delta^{13}C$ compared to horses (comparison cannot be statistically tested due to the very small sample size). On the other hand, $\delta^{15}N$ is similar for the three species. Equids from Barbuise show similar $\delta^{13}C$ to Neolithic herbivores ($n = 33$) from northern France ($P < 0{\cdot}05$; Fig. 3). The horse diet is made up of grass but they can also be fed with hay, straw or grain, and in any case with open environment resources (e.g. King and Gurnell 2005; Mihlbachler *et al.* 2011). Taking into account their ethology and their isotopic similarities with Neolithic domestic species (Balasse 1999; Balasse *et al.* 1997, 2000; Bocherens *et al.* 2000), it is relevant to assume that horse isotopic ratios provide a baseline for LBA C_3 plant consumers in this region. At Barbuise, the difference between pig/dog and horses

$\delta^{13}C$ ranges from 2·2‰ to 3·0‰, implying that pig/dog analysed consumed ^{13}C enriched food compared to horses. The intake of animal protein in the diet of pig/dog does not seem a relevant hypothesis to explain this difference, as pig and horses show identical $\delta^{15}N$ and the $\delta^{15}N$ difference between dog and horses is only 1·0‰. Considering the region and the period, the only ^{13}C enriched food is a C_4 plant: millet; local/regional comparisons will further support this hypothesis (cf. § Diachronic comparisons).

LBA human collagen isotopic ratios range from −19.7‰ to −17·3‰ for carbon and from 9·2‰ to 11·8‰ for nitrogen ($n = 12$; Tables 1 and 2). LBA bone and tooth apatite $\delta^{13}C$ ratios range from −12·3‰ to −10·2‰ ($n = 8$; Tables 1 and 2). Combined with collagen data, the carbon isotopic ratio in apatite has often been used to assess the trophic level of animals (Ambrose *et al.* 2003; Hedges 2003; Lee-Thorp *et al.* 1989), and to estimate the contribution of marine *vs.* terrestrial or C_3 *vs.* C_4 food in the whole human diet (Ambrose *et al.* 1997, 2003; Froehle *et al.* 2012; Kellner and Schoeninger 2007; Schwarcz 1991). The apatite-collagen spacing model ($\Delta^{13}C_{ap\text{-}coll}$) helps to estimate the relative proportion between C_3/C_4 protein vs. C_3/C_4 low-protein food; a $\Delta^{13}C_{ap\text{-}coll}$ greater than 4·4‰ demonstrates a protein intake depleted in ^{13}C compared to the overall diet (Ambrose *et al.* 2003). Using the Schwarcz (1991) and Ambrose *et al.* (1997; 2003) equation, we proposed a percentage of C_4 plant in the overall diet of each individual of Barbuise: $\%C_4 = (-25 - (\delta^{13}C_{ap} - 9{\cdot}4)) / 15*100$ (Tables 1 and 2). Estimations range from ca. 22% to 36%. According to the authors (*ibid.*), at least 20% of C_4 food in the diet is required in order to be detected in the collagen isotopic signature. Our apatite data are just over this limit which partially justifies the low human $\delta^{13}C_{coll}$ compared to other populations for which diet is mainly based on C_4 plants (Tafuri *et al.* 2009). The application of the Kellner and Schoeninger regression lines (2007) and the combination of collagen $\delta^{15}N$ with $\Delta^{13}C_{ap\text{-}coll}$ indicate a major protein intake from a C_3 environment (Fig. 4) and a bulk diet including a significant portion of low-protein C_4 food (Fig. 5). In this case, the more relevant dietary pattern for LBA humans would be the consumption of C_3-feeding animals (possibly horses; cattle and caprines are not analysed in this study) and different cereals with millet.

LBA intra-individual comparison

Due to poor sample preservation, intra-individual comparisons, specifically for "non-local" subjects, do not conform to our expectations. Only five humans yielded both tooth and bone collagen results and two for apatite. Considering the type of data, intra-individuals, only paired difference test (Wilcoxon) can be performed; however, the limited sample size does not allow using it and no statistical comparison can be made. We thus attempted to understand potential variability between M2 root dentin (marking mainly childhood) and bone (marking adulthood) looking at the Fig. 6. Discussion in this case is speculative but few observations worth to be pinpointed. The more intriguing individual is the "non-local" GDF 1455, on which an increase of 1·7‰ for $\delta^{15}N$ and 1·1‰ for $\delta^{13}C_{ap}$ is observed between childhood and adulthood. The diet of this subject could have changed, with a higher animal protein intake and more C_4 plants (or animals fed with C_4 plants), during the last years of life. The new diet could be linked to mobility and a new living place as suggested by funerary evidence. The female BPV 9391A shows a $\delta^{15}N$ decrease, suggesting a lower animal protein intake during adulthood. This is a shared feature (i.e. dietary modification) with the "non-local" male but no archaeological evidence supports this observation.

Diachronic local comparison

The necropolis of *Les Noisats* at Gurgy (also called Gurgy; Yonne, France) is located ca. 76 km south of Barbuise. Gurgy is the biggest Neolithic site studied and available for a diachronic local comparison with Barbuise, including 128 individuals found in different types of burials (Rottier *et al.* 2007; Rottier *et al.* 2005). The necropolis was used and expanded during the 5th millennium BC in an archaeologically well-documented geographical area (Chambon *et al.* 2013). Funerary practices are homogeneous and seem to be linked to the location of the site, at the intersection between Danubian and Mediterranean cultural influences (Rivollat *et al.* 2015). However, the necropolis cannot be exclusively associated with one of the archaeological cultures known in the region (Cerny and Chassey; Chambon *et al.* 2013). The men, women and children buried in the necropolis do not display distinct anatomical traits and cannot thus be clustered into different groups. Moreover, a first study of dental microanatomy and occlusal tooth wear confirms the apparent homogeneity of these individuals and appears to corroborate the hypothesis of a similar dietary pattern for all of them (Le Luyer *et al.* 2014; Rey *et al.* 2013). The long duration of the necropolis (one millennium) and the apparent similarity of the individuals buried there contrast with the archaeological record (diversity of goods and funerary structures) attesting to multiple peripheral contacts. This necropolis could be defined as a "homogeneous mosaic" in which the different cultural influences would have been integrated by a population and then absorbed into their own territory. At Gurgy, stable isotope

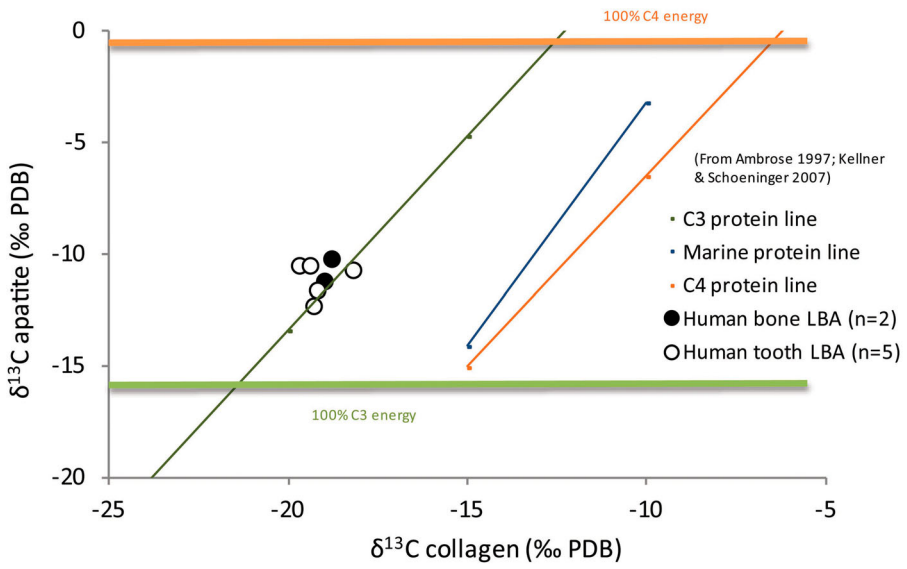


Figure 4. Comparison of C stable isotope ratios from collagen and apatite from human teeth and bones of Barbuise according to the model of Kellner and Schoeninger (2007).

ratios are available for 40 human bones (12 females, 20 males, 5 undetermined >15 years old and 3 children between 5 and 14 years old), and one *Bos* sp. bone (Rey 2013; Rey *et al.* 2013). Neolithic human collagen isotopic ratios range from −21·1‰ to −20·4‰ for carbon and from 10·9‰ to 12·8‰ for nitrogen (n = 40; Rey 2013; Rey *et al.* 2013). The *Bos* sp. from Gurgy show consistent isotopic ratios ($\delta^{13}C = -23{\cdot}0‰$; $\delta^{15}N = 6{\cdot}7‰$) with those from domestic herbivores recorded in northern France: from −23·6‰ to −21·2‰ for carbon and from 5·1‰ to 8·1‰ for nitrogen (for bovines and caprines, n = 36; Balasse 1999; Balasse and Tresset 2002; Balasse *et al.* 1997, 2000; Bocherens *et al.* 2000; Goude and Fontugne 2016). The study carried out by Rey (2013; Rey *et al.* 2013) indicate that Neolithic humans had high animal protein intake and/or significant ^{15}N enriched protein consumption ($\Delta^{13}C_{\text{human-cattle}}$ of ca. 2·0‰ and $\Delta^{15}N_{\text{human-cattle}}$ of ca. 5·0‰), such as freshwater fish or young domestic herbivores. No archaeozoological evidence from Gurgy site currently preferentially points to one or the other of these hypotheses. Milk exploitation and calf slaughtering is confirmed on the Neolithic sites of Balloy (Tresset 1990), Bercy, Cuiry and Maizy (Fig. 1; Balasse 1999; Balasse and Tresset 2002; Balasse *et al.* 1997, 2000) and could partly explain the human values registered at Gurgy (consumption of young domestic animals high in the trophic web). However, freshwater fish is also a resource to consider. Freshwater food consumption in the Paris Basin was shown for the Mesolithic by analysing amino acid isotopic ratios of human bone from Noyen-sur-Seine (Naito *et al.* 2013). Moreover, this hypothesis was also proposed for the

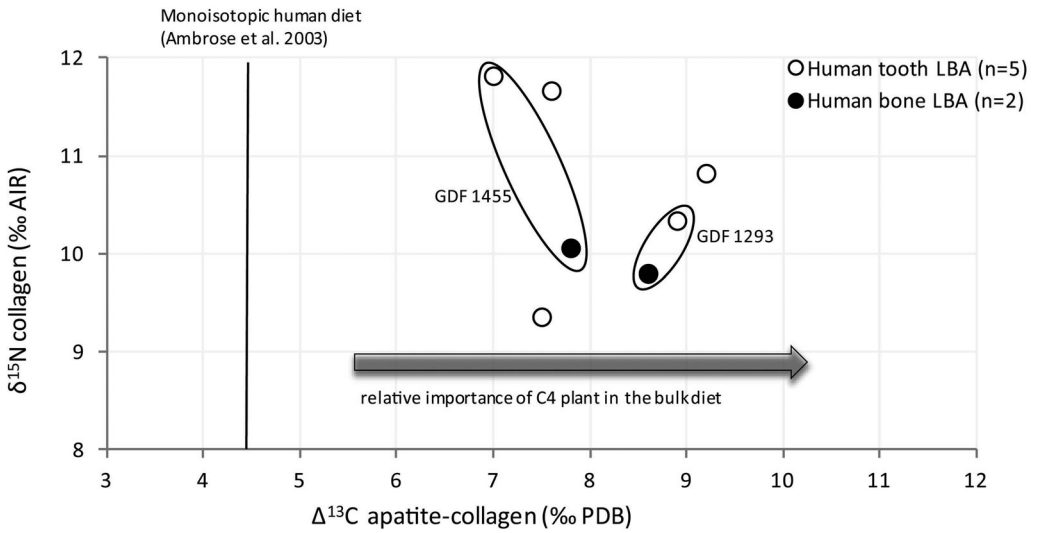


Figure 5. Comparison of N stable isotope ratios in collagen and C stable isotope ratios of collagen-apatite spacing human teeth and bones of Barbuise, according to the model of Ambrose *et al.* (1997; 2003).

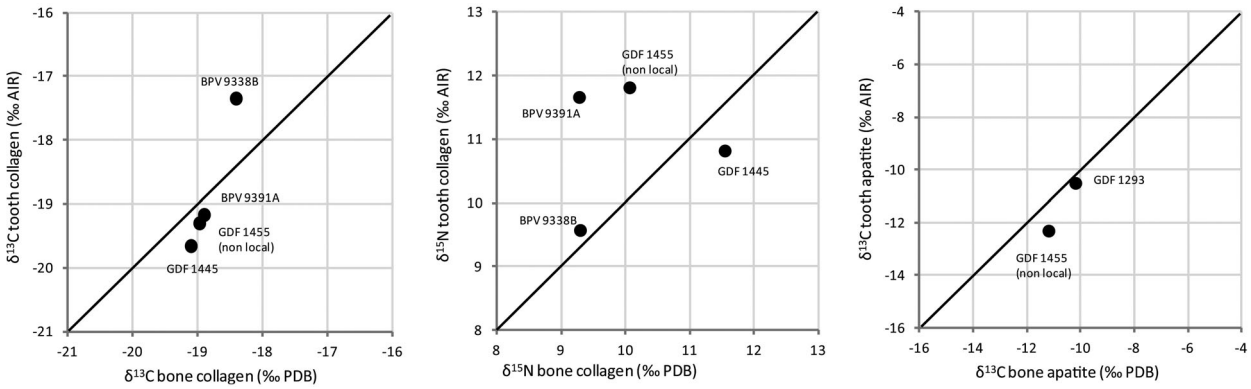


Figure 6. Comparison of stable isotope ratios (collagen and apatite) between bone and teeth for each individual.

contemporaneous human group of Pontcharaud (Auvergne, Fig. 1), where the vicinity of streams allows for the easy acquisition of such food (Goude *et al.* 2013) and for any Neolithic sites in Germany and Belgium (e.g., Bocherens *et al.* 2006; Bösl *et al.* 2006; Dürrwächter *et al.* 2006). Furthermore, the significant amount of fish remains found in several Villeneuve-Saint-Germain (a nearby Neolithic culture) sites in the Paris Basin, and the seasonal catch, indicate that fishing could have provided a significant source of protein during the warm period, in addition to farm produce (Clavel 2009; Clavel and Arbogast 2007). LBA human isotopic ratios are statistically different from Neolithic ratios for both C and N ($P < 0{\cdot}01$). LBA human bone collagen show higher $\delta^{13}C$ ($\mu = -18{\cdot}8‰$, 0·3 SD; $n = 5$) and lower $\delta^{15}N$ ($\mu = 10{\cdot}0‰$, 0·9 SD) than Neolithic ones, distinctly visible in Fig. 3. In the same way as for animals, LBA human protein intake seems to include a significant part of ^{13}C enriched food, such as millet grain and/or animals fed with millet (as is the case for the pig from Barbuise).The isotopic data set leads us to propose the following first diachronic scheme in this region: a mixed Neolithic human diet with a significant amount of terrestrial animal protein and probably freshwater resources and a mixed LBA human diet with less animal protein intake and increased cereal consumption, including C_4 plants.

Diachronic regional comparison

Additionally to Gurgy, several Neolithic to Iron Age sites have been recently investigated by stable isotope analysis in the northern half of France and hold our attention for regional comparisons (Goude *et al.* 2013; Herrscher *et al.* 2013; Le Bras-Goude *et al.* 2011). This area focused our interest due to the availability of human/animal remains and to the presence of millet archaeological charred seeds indicating that C_4 plant commonly circulated from East at the end of Prehistory (e.g., Cabanis *et al.* 2010; Ferrage-Toulemonde 2013). Although we identified few millet eaters during the Iron Age (IA) in Alsace (Goude *et al.* 2014), Barbuise is, for the moment, the only LBA site in this area where human/animal remains were enough preserved and where local archeobotanical study was well detailed to document the both cultivation and consumption of this new cereal at its arrival. Thus, the comparison between Barbuise and other Protohistoric sites, located in the area of the spread of millet, appeared obvious to understand the possible dietary changes implied by this new crop. Barbuise isotopic data were compared with different regional sites (Fig. 2): Auvergne (Middle Neolithic and EBA) and the Alsace region (Late Neolithic and IA; Goude *et al.* 2013, 2014 Herrscher *et al.* 2013; Le Bras-Goude *et al.* 2011), as well as the Neolithic sites of Bercy (4th mill. cal. BC; Balasse *et al.* 1997), La Villette (4th mill. cal. BC; Giligny 2005) and Cuiry/Maizy (Balasse 1999; Balasse *et al.* 2000; Bocherens *et al.* 2000) for animals (Fig. 7). As the geographical location of sites can lead to environmental isotopic variations in plants (e.g., Amundson *et al.* 2013; Goude and Fontugne 2016; Tieszen and Boutton 1989), domestic animals are thus first compared. Neolithic cattle from northern France, Alsace and Auvergne regions show the lowest $\delta^{13}C$ ratios (from −23·5‰ to −21·2‰, $n = 39$). IA and EBA cattle have $\delta^{13}C$ ratios within a similar range (−21·4‰ to −20·8‰, $n = 6$) as the LBA horses; these values can be considered as a local range base for C_3 herbivores in the demarcated area. Neolithic, EBA and IA pigs from northern France, Auvergne and Alsace have similar $\delta^{13}C$ ratios: from −21·0‰ to −20·1‰ ($n = 16$); the LBA pig data are slightly higher (−19·8‰). One of the most striking results concerns dogs (Fig. 7). Neolithic and EBA dogs have lower $\delta^{13}C$ ratios (21·0‰ to −20·0‰, $n = 5$) than the LBA Barbuise dog (−19·0‰). Neolithic dogs show the highest $\delta^{15}N$ ratios (from 9·1‰ to 10·0‰) compared to the LBA (7·7‰) and EBA ones (7·6‰ and 8·7‰). The higher carbon isotopic Barbuise pig and dog ratios, as well as the low $\delta^{15}N$ dog ratio, can be fairly well linked to the consumption of human food or refuse, including

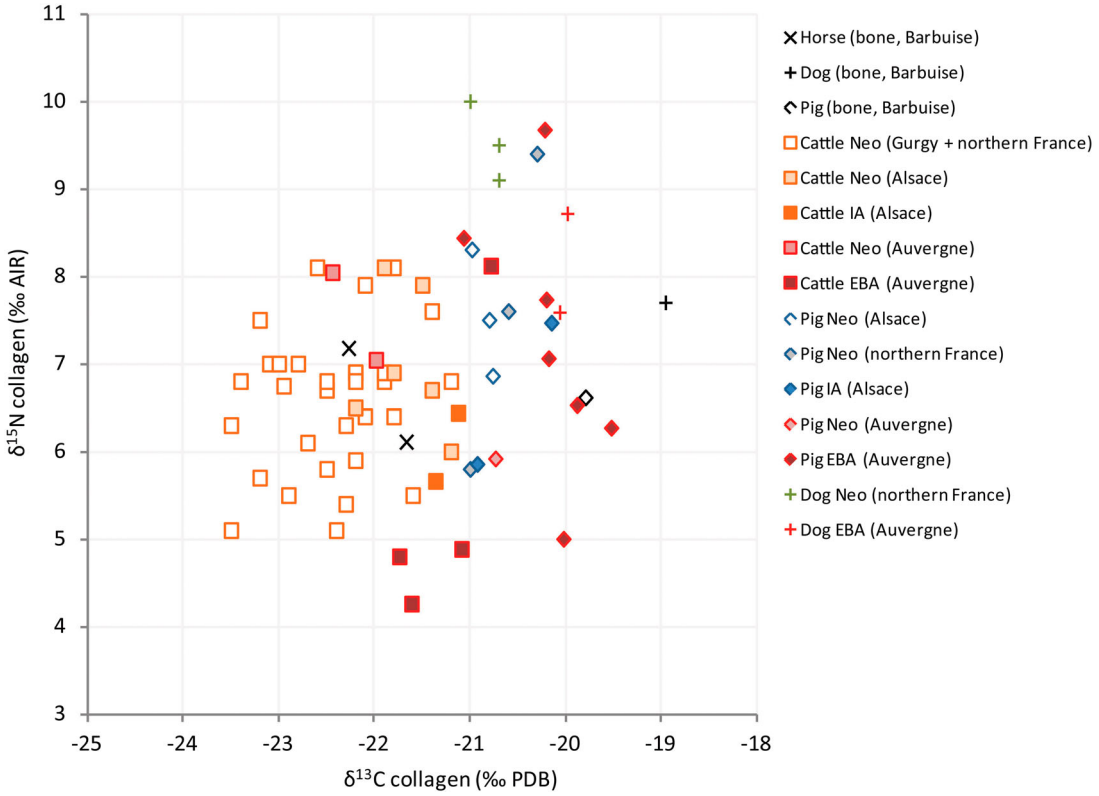


Figure 7. C and N stable isotope ratios of animal bones Barbuise (this study), Gurgy (Rey 2013) and other Neolithic, Early Bronze Age (EBA) and Iron Age (IA) sites in northern France (Balasse 1999; Balasse *et al.* 1997; Goude *et al.* 2014).

a certain amount of ^{13}C enriched protein, such as millet and/or animals fed with millet. A diachronic comparison of the human data is presented in Fig. 8 (immature > three-year-old and adults). Neolithic individuals from Gurgy and Late Neolithic (LN) specimens from the Alsace region display isotopic ratios ranging from −21·7‰ to −19·5‰ for carbon and from 9·7‰ to 13·2‰ for nitrogen ($n = 76$). In the Centre of France, the isotopic range for Middle Neolithic and EBA individuals varies from −20·9‰ to −19·2‰ for carbon and from 8·6‰ to 13·0‰ for nitrogen ($n = 107$). IA specimens from Alsace ($\delta^{13}C$: −20·5‰ to −20·0‰; $\delta^{15}N$: 7·8‰ to 10·5‰, $n = 4$) differ statistically from Neolithic individuals from the same site ($P < 0·05$; Goude *et al.* 2014; Fig. 8). These differences, with lower $\delta^{15}N$ and higher $\delta^{13}C$, have been described either as a climatic difference between the two periods or as a shift to a diet with less animal protein and a higher cereal intake, including millet, for IA humans (Goude *et al.* 2014). The presence of few millet archaeobotanical remains in Alsace and the lack of archaeological and environmental data pointing towards significant climatic change tend to back up the second hypothesis. This pattern is similar when comparing LBA humans from Barbuise and Neolithic subjects from Gurgy. A statistical comparison between Barbuise humans (bone collagen) and the other Neolithic sites located in the northern and centre of France highlights significant isotopic differences, particularly for carbon (Alsace-Neolithic vs. LBA: $P < 0·01$ for $\delta^{13}C$ and $P < 0·05$ for $\delta^{15}N$; Auvergne-Neolithic vs. LBA: $P < 0·01$ only for $\delta^{13}C$). Similar results emerge from the comparison of EBA humans from Auvergne ($P < 0·01$ only for $\delta^{13}C$) and also for IA individuals from Alsace with LBA human bone data from Barbuise ($P < 0·05$ for both $\delta^{13}C$ and $\delta^{15}N$). Even though the carbon isotopic ratios recorded at Barbuise are lower than more commonly recorded data in other millet eaters (e.g., Tafuri *et al.* 2009, Varalli *et al.* 2014), the diachronic and local comparisons of bone collagen results strongly support dietary change during the LBA period, probably linked to increases in millet crops (Jacob *et al.* 2008). By Looking at the few EBA sites already studied in France (Herrscher, in press; Herrscher *et al.* 2013), stable isotope data do not demonstrate that C_4 plant was included in the daily diet. The same assessment was also proposed in Italy (Varalli, 2015). However, in Italy, the recent studies performed on North, Centre and Liguria, have demonstrated a significant impact of C_4 plants in protein intake at the Early-Middle Bronze Age transition (Tafuri *et al.* 2009; Varalli *et al.* 2013, 2016). For these areas, C_4 plants/animal fed with C_4 plants became important food sources, particularly at the LBA-IA transition (Varalli *et al.* 2012–2013). This contrast reflects either an heterogeneous diffusion of millet crop

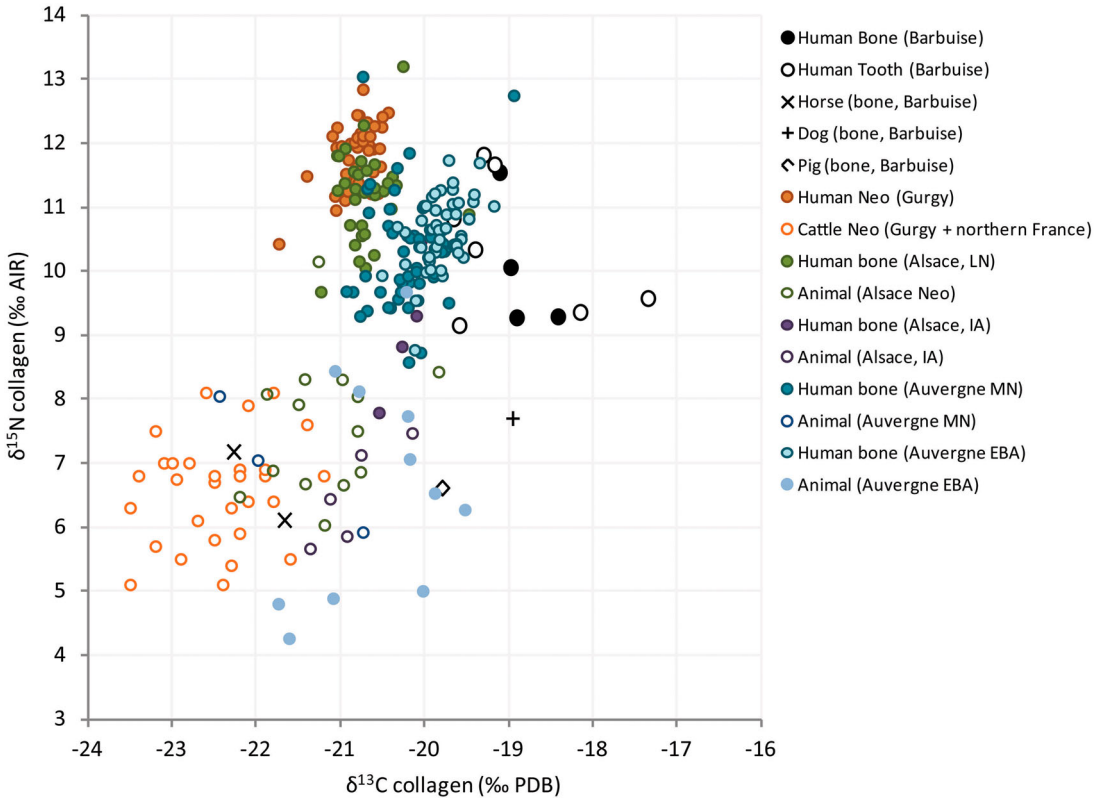


Figure 8. Human and animal C and N stable isotope ratios from Neolithic to Iron Age in northern and Centre of France (this study, Balasse 1999; Goude *et al.* 2014; Herrscher *et al.* 2013; Rey 2013). LN: Late Neolithic; IA: Iron Age; MN: Middle Neolithic; EBA: Early Bronze Age.

through France and less impact of such plant in diet and/or a crucial lack of Bronze Age and IA isotopic data in French regions concerned by its diffusion.

Conclusion

Diachronic comparisons of human and animal stable isotope ratios, as well as archaeobotanical studies performed in the Paris Basin and surrounding areas, support the archaeological hypothesis on dietary and food economy changes since the LBA. For the first time in France, this study highlights millet consumption by LBA humans (14th c. BC) using stable isotope analysis and illustrates the importance of local comparisons for detecting dietary modifications. Broomcorn millet may have spread from Asia to Europe during the Bronze Age (earliest findings in Europe and Caucasus, some of which are listed in Hunt *et al.* 2008, are currently under revision by AMS radiocarbon dating and the first results did not yield dates prior to 1606–1414 cal BC; Hajnalova *et al.* 2013; Motuzaite-Matuzeviciute *et al.* 2013). The diffusion of millet as a crop occurred at different rates (Lightfoot *et al.* 2013; Motuzaite-Matuzeviciute *et al.* 2015; Stevens *et al.* 2012). It is widely accepted that millet was extensively cultivated during the LBA in the Paris Basin but we still do not know exactly when it was introduced and became an established crop. Settlement sites are very scarce in the area for the Early and Middle Bronze Ages, and archaeobotanical data are even more scant. Considering the lack of archaeobotanical data for these periods (only a few settlements), it is now essential to conduct new isotopic studies on Early/Middle Bronze Age human and animal remains to record the advent of millet in the region and its role in diet.

Acknowledgements

This work was funded by the Agence Nationale de la Recherche – Investissements d'avenir – ANR-10-LABX-52 (LaScArBx, projet DHP) and the University of Bordeaux. Archaeobotanical studies received a grant from the Direction de l'Architecture et du Patrimoine from the Ministry for Culture and Communication. We also thank S. Cabut for the statistical expertise (UMR 7269 LAMPEA, Aix en Provence, France) and J. Ughetto and M. Balasse (SSIMM MNHN UMR 7209, Paris, France) for their availability and services.

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