Dentin isotopic reconstruction of individual life histories reveals millet consumption during weaning and childhood at the Late Neolithic (4500 BP) Gaoshan site in southwestern China

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Abstract

Here we present results of a pilot project that measured δ^{13}C and δ^{15}N values in bone collagen (ribs and femora) as well as dentin serial sections to examine individual dietary life histories at a Late Neolithic (4500 BP) site known as Gaoshan Ancient City (高山古城) located on the Chengdu Plain in Sichuan Province, China. The isotopic data of the bones indicate that humans consumed C_3-based foods, which corresponds to the dominance of rice agriculture in this region. However, the isotopic data of the dentin serial sections of five individuals display much more positive δ^{13}C values than those of the bones, strongly suggesting that millets (a C_4 crop) contributed substantially to human diets during the weaning process and early childhood. Furthermore, the isotopic profiles of dentin sections of the first molars and canines demonstrate that the cession of weaning was individually variable and completed between ~2.5 to 4 years of age. While limited in scope, this pilot study offers new evidence of millet consumption during human growth and development even though individuals relied on rice exclusively as adults. Moreover, our study provides another perspective with which to rethink the role that millets played during the development and spread of millet agriculture to the south of China in terms of cultural exchange and migration.

Keywords: Serial sampling; Carbon and nitrogen isotope analysis; Weaning; Millet; Chengdu Plain

1. Introduction

Over the last 40 years, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratio analysis of bulk collagen from human and animal bones has become a routine method to investigate a wide range of archaeological questions relevant to diet, evolution, mobility, social hierarchy, breastfeeding and weaning patterns, disease, nutritional stress, agriculture and animal domestication (e.g. Lee-Thorp, 2008; Schoeninger, 2014; Reitsema, 2013; Tsutaya & Yoneda, 2014; Britton, 2017). However, it has only been relatively recently that sampling methodologies have advanced to examine individual specific biographies in detail using stable isotope ratio analysis (Fuller et al., 2003; Eerkens et al., 2011; Montgomery et al., 2013; Lamb et al., 2014; Beaumont & Montgomery, 2016; Laffoon et al., 2018). For example, human life histories at the individual scale can be reconstructed based on the comparison of isotopic data of bones with different turnover rates (e.g. femora and ribs) to those of bulk teeth (Sealy et al., 1993; Sealy et al., 1995; Richards et al., 2002; Pollard et al., 2012; Lamb et al., 2014). In addition, the serial sampling of dentin has emerged an active topic of research that is used to obtain individual life histories on weaning practices, dietary variation, physiology and migration with a high degree of chronological resolution (Fuller et al., 2003; Eerkens et al., 2011; Eerkens & Bartelink, 2013; Montgomery et al., 2013; Burt & Amin, 2014; Sandberg et al., 2014; Beaumont & Montgomery, 2016).

In China, isotopic analysis of human skeletal remains was initiated in 1984 and has made significant progress over the last 30 years with studies exploring such topics as human dietary patterns from the Late Paleolithic to historic times, the origin and spread of millet and rice agriculture and the mechanisms of animal domestication (Hu, 2018). However, most previous studies focused on the isotopic analysis of bulk collagen or dentin from bones and teeth. Apart from the work of Xia et al., (2018) which examined the offset between long bones and ribs to investigate weaning patterns and human mobility at the Boyangcheng site during the Western Zhou Period (1122-771 BC) (Fig. 1), there have been no studies focused on the reconstruction of individual life histories in China.

An increasing number of archaeobotanical studies have shown that millets, including both foxtail (*Setaria italica*) and common millet (*Panicum miliaceum*) and rice (*Oryza sativa*) were respectively domesticated in the Yellow River Valley and Yangtze River Valley by approximately 10,000 years ago (Yuan, 2002; Jiang & Liu, 2006; Lu et al., 2009; Yang et al., 2012). By ~8,000 years ago, these original areas of millet and rice agriculture expanded to include many regions in north and south China (Fig. 1) (Guo et al., 2011; He et al., 2017). Isotopic studies on bulk collagen from human bones in the middle of these two agricultural regions suggest that both millet and rice contributed to human diets, and that the human dietary shifts might have been linked to cultural dominance or human movements (Fu et al., 2010; Guo et al., 2011, 2015). Yet, these dietary fluctuations in regards to individual human diets, particularly the role that these crops played in childhood diets, remain largely unknown.

Here we conduct a study that examines human life history patterns by measuring stable isotope ratios in dentin serial sections and bones (femora and ribs) from individuals at the Late Neolithic (4500 BP) Gaoshan Ancient City site (高山古城, abbreviated as Gaoshan) located on the Chengdu Plain in southwestern China (Fig. 1). In particular, we are keen to better examine and understand breastfeeding and weaning patterns as well as childhood diets in this population that lived in a region of mixed rice and millet agriculture. This pilot project is the first application of dentin serial-sectioning to an archaeological population in China and establishes a framework to better understand the dietary practices on the Chengdu Plain during the Neolithic.

2. Methodology of stable isotope ratio analysis and sampling strategies

Carbon stable isotope ratios are useful for distinguishing between diets due to the fact that plants with different photosynthetic pathways, such as C_3 and C_4 plants, have different isotopic results (van der Merwe & Vogel, 1978; DeNiro & Epstein, 1978). In addition, as CO_2 in seawater is more ^{13}C-enriched, δ^{13}C values can also be used to distinguish between marine and terrestrial foods (Chisholm et al., 1982). As for nitrogen, there is a stepwise ^{15}N-enrichment along the food chain, with a 3-5‰ increase with each trophic level (DeNiro & Epstein, 1981; Bocherens & Drucker, 2003). Thus, δ^{15}N values are useful to analyze the intake of high trophic level foods, such as terrestrial, freshwater and marine resources (Schoeninger et al., 1983; Britton, 2017).

Due to the fact that bone remodels over the lifetime of an individual and that the turnover rate of different bones varies, stable isotope values of different skeletal elements reflect the average diets spanning different periods before the individual's death. For instance, the isotopic data of long bones such as femora represent the diet during the period of at least 10 years before death (Hedges et al., 2007), while those of smaller bones such as ribs represent the diet during the period of 2-5 years before death (Cox & Sealy, 1997; Parfitt, 2002). Therefore, comparison of isotopic data among diverse bone elements can reveal dietary variations and possible movements of humans between different environments.

Recently, breastfeeding and weaning practices and childhood diets have been increasingly investigated in archaeological populations (e.g. Tsutaya & Yoneda, 2014; Beaumont & Montgomery, 2016), as there is a trophic-level effect between mothers and infants. Modern studies found that there is a ^{15}N-enrichment of ~2-3‰ and a ^{13}C-enrichment of ~1‰ respectively in hair and fingernail keratin from exclusively breastfeeding infants compared to their mothers (Fogel et al., 1989; Fuller et al., 2006). With the onset of the weaning process, this isotopic offset gradually decreases as a result of the introduction of complementary foods until breastfeeding ceases completely. Thus, weaning practices and childhood diets can be revealed by investigating a large number of individuals representing a cross-section of an archaeological population (Tsutaya & Yoneda, 2014).

In contrast to bones, teeth do not remodel (Fuller et al., 2003). Thus, the isotopic analysis of tooth enamel or dentin provides dietary information during the period of tooth formation

(Beaumont et al., 2014). Human dentin starts forming at the occlusal edge of the crown and incrementally grows toward the root in oblique angles (Dean & Scandrett, 1995). Deciduous teeth start to form in utero and are completed by ~2-4 years old (Smith, 1991). In contrast, permanent teeth begin to form after birth and are completed at different ages varying from ~8 to 20 years old, depending on the type of tooth (for details see Hillson, 2014). Thus, life histories of individuals from birth to adolescence can be reconstructed through the isotopic analysis of serial sections of tooth dentin at high temporal resolution (less than 1 year). For example, the first molar begins formation soon after birth and is completed around the age of 9-10, providing a record of breastfeeding, weaning and childhood diet (Henderson et al., 2014). In contrast, the second molar forms around 3-4 years of age and is completed by the age of 15, thus recording life events from early childhood to adolescence (Beaumont & Montgomery, 2016).

3. Archaeological context

The Chengdu Plain is located in southwestern China between the upper Yellow River Valley and the middle Yangtze River Valley (Fig. 1). As a consequence, it forms a geographic passageway and cultural buffer for the northward spread of rice agriculture in the south and the southward diffusion of millet agriculture in the north (Sun, 2009; Zhang & Hung, 2010); Table 1 lists the sequence of the archaeological cultures on the Chengdu Plain.

The Gaoshan site (N 30°27'09.5", E 103°34'46.3") is located in Dayi County, Chengdu on the frontier zone between the Chuanxibei Plateau (part of Tibetan Plateau) and the Chengdu Plain (Fig. 1) and covers an area of 344000 m^2. The site was discovered in 2003 but the first formal excavation was conducted by the Chengdu Municipal Institute of Cultural Relics and Archaeology in 2015-2016 and an area of 800 m^2 was fully excavated (Liu et al., 2016). In total, 89 tombs, 86 pits, 12 ditches and a single human sacrifice pit were found, and numerous artifacts such as pottery, stone tools (e.g., adze, axes and chisels), bone tools (mainly cone-shaped tools) and jade were recovered. Although the radiocarbon dating results of the organic materials have not been finished, the typological analysis of the pottery discovered from the same stratum in the vicinity of the graves indicates that the site can be dated to the Baodun Culture Phase I Period, *i.e.*, 4500 years ago (Liu et al., 2017). Therefore, this site represents the earliest prehistoric cemetery in the Chengdu Plain to date (Liu et al., 2017).

Morphological studies on human skeletons, skulls in particular, suggested that the individuals were attributed to diverse geographical origins (Liu et al., 2016). Large quantities of plant and animal remains were recovered, including rice (*Oryza sativa*), coix (*Coix lacryma-jobi* L.), foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*), cocklebur (*Xanthium sibiricum Patrin ex Widder*), cowpea (*Vigna unguiculata* (Linn.) *Walp*.), as well as pigs, dogs, deer and fish, among which rice and pigs were the most common (Liu et al., 2016). As a result of phytolith analysis at the Baodun type site of the Baodun Culture, ~22 km away from the Gaoshan site, it was suggested that rice was the most dominant crop during the late Neolithic period, but that this was supplemented with millet agriculture (Table 1) (Guedes et al., 2013). Unfortunately, no systematic archaeobotanical and archaeozoological studies have been published for the Gaoshan site to date.

4. Materials and Methods

4.1 Materials

Here, 12 individuals from the Gaoshan site were sampled for isotopic analysis. For each individual, demographic information including age estimation and sex, was determined by Dr. Haibing Yuan from the Center for Archaeological Science, Department of Archaeology, Sichuan University using standard methods (Ubelaker, 1999; White & Folkens, 2005; Zhu, 2004) and listed in Table 2. The skeletal elements sampled included femurs, ribs and teeth and the sampling information is also presented in Table 2.

4.2 Collagen preparation and isotopic measurements

The collagen from all tooth and bone specimens was prepared at the Key Laboratory of Vertebrate Evolution and Human Origins of the Chinese Academy of Sciences, Institute of Vertebrate Palaeontology and Palaeoanthropology, Chinese Academy of Sciences, according to the modified protocols outlined in Method 2 by Beaumont et al., (2013) for teeth and by Richards & Hedges (1999) for bones respectively. Surface debris was removed mechanically and rinsed in an ultrasonic bath for further cleaning. The crown of each tooth was dissolved in acid solution rather than removed directly, considering the poor preservation of the teeth. Then, samples (bones and teeth) were demineralized in a 0.5 M HCl solution at 4 ºC for ~2 weeks, with the acid refreshed every 1-2 days, rinsed by deionized water until neutrality and soaked in a 0.125 M NaOH solution at 4 ºC for ~20 hours. After being rinsed to neutrality again, the bone and tooth remains were processed separately. For teeth, the secondary dentin was removed (if necessary) and the primary dentin was cut into cross sections transversely at 1 mm intervals by hand with a sterile scalpel. The series of teeth sections and bone residues were gelatinized at 70 ºC in pH = 3 HCl solution for 24 hours, and all remains were frozen and freeze-dried to produce collagen.

Approximate ~0.5-1 mg of extracted collagen was weighed and the elemental contents and stable isotope ratios of carbon and nitrogen in the collagen were measured in an IsoPrime-100 IRMS coupled with an Elementar Pyro Cube elemental analyzer at the Archaeological Stable Isotope Laboratory, Department of Archaeology and Anthropology, University of Chinese Academy of Sciences. The stable isotope ratios were expressed as δ in parts per thousand (per mil or ‰) relative to the international standards for carbon (VPDB) and nitrogen (AIR) respectively. The international standards, including Sulfanilamide, IAEA-600, IAEA-N-2, IAEA-CH-6, USGS 40 and USGS 41, were used for elemental and isotopic calibration as well as one collagen lab standard (δ^{13}C value of

-14.7±0.2‰ and $\delta^{15}N$ value of 7.0±0.2‰). The uncertainty of the $\delta^{13}C$ and $\delta^{15}N$ values is less than or equal to ±0.2‰. All sample information as well as the elemental and isotopic results are listed in the Supplementary Tables 1-3.

Unfortunately, only 8 of the 24 (33%) bone samples including 2 ribs and 6 femora, and 5 of the 12 (42%) teeth (1 canine, 2 first molars and 2 second molars) from five individuals, produced good quality collagen with atomic C:N between 2.9-3.6 (DeNiro 1985), nitrogen concentrations (%N) above 4.8% and carbon concentration (%C) above 13% (Ambrose 1990). In addition, it should be noted that the crown or the root parts of teeth were sometimes lost due to occlusal surface wear to some degree. Therefore, the estimation of the approximate age of the serial sections was made from the starting point of the cementum-enamel junction according to Beaumont & Montgomery (2015) (Supplementary Table 3). The five teeth were reclassified as Subjects A to E (Supplementary Table 2).

5. Results and discussion

5.1 Isotopic Results

As seen in Fig. 2 and Supplementary Table 1, the $\delta^{13}C$ values of the human bone collagen range from –19.6‰ to –18.0‰ with a mean value of –18.8±0.6‰ (n=8), indicating these individuals consumed predominately C_3-based diets. The $\delta^{15}N$ values range from 9.5‰ to 11.1‰ with a mean value of 10.3±0.6‰ (n=8). In addition, one individual (M86) had paired rib and femora results, and as these $\delta^{13}C$ and $\delta^{15}N$ values were nearly identical, this indicates that the short-term average diet for the last 2-5 years before death (rib) did not differ much from the longer-term average diet represented by the femur.

Compared to the bones, the isotopic data of the dentin serial sections display a wider range of $\delta^{13}C$ (–19.6‰ to –15.0‰) and $\delta^{15}N$ values (9.0‰ to 14.9‰). It can also be seen in Fig. 3 that the isotopic data of all the teeth, except subject B (M86), fluctuate to some degree indicating that the diets of these individuals varied during the period of tooth formation. In addition, there are isotopic variations between the tooth serial sections (points) and the bones (solid or dash lines) in Fig. 3, indicating that some dietary changes such as breastfeeding and weaning occurred during this period. This will be discussed in more detail in the following sections.

5.2 Reconstruction of individual weaning patterns and life histories

Since both rice and millets were cultivated in the region of the Gaoshan site, it is reasonable to infer that the C_3 isotopic signatures mainly reflect the consumption of rice while the C_4 isotopic signatures are the result of millets (Fig. 3; Supplementary Tables 2-3). The $\delta^{13}C$ values of bones (ribs and femora) show that large amounts of C_3-based foods were consumed, indicating the dominance of rice agriculture in human diets from this point of view. The high and diverse $\delta^{15}N$ values suggest that animal protein from terrestrial and/or freshwater resources dominated these diets, which is evident through the existence of faunal remains at this site (see the archaeological context). It is highly unfortunate that these faunal remains could not be studied for isotopic analysis, as this would have permitted a more detailed reconstruction of dietary practices at Gaoshan. In general, the five teeth can be divided into two general age categories. One is the canine and first molars (Subjects A, D, E in Fig. 3), representing the period of childhood including the breastfeeding and weaning period. The other (Subjects B, C in Fig. 3) is the second molars, representative of the period of childhood to young adulthood (>14 years old).

Subject A (Burial M6), is a female about 30 years of age. The δ^{13}C values of dentin serial sections range from -17.6‰ to -15.0‰ with an average of -16.8±0.7‰ (n=15). The δ^{15}N values of the dentin serial sections range from 9.9‰ to 14.9 ‰ with an average of 11.5±1.4‰ (n=15). The δ^{15}N values show a gradual and steady decrease from 2.5 to 4 years of age as a result of weaning (Fig. 3), and then remain slightly elevated compared to the rib until about the age of 7. Interestingly, the δ^{13}C values display an increase from ~2.5-3 years of age and then a gradual decrease toward the rib over the length of the tooth. As this ^{13}C-enrichment occurs during the weaning process and is ~3‰ compared to the adult dietary signatures of the rib, this indicates that this individual consumed some C_4 dietary items (millets) during the period of weaning and early childhood.

Subject B (M86), is a female approximately 30-40 years old. The δ^{13}C values of tooth sections range from -17.5‰ to -16.8‰ with an average of -17.1±0.2‰ (n=15). The δ^{15}N values of tooth sections range from 10.5‰ to 10.9 ‰ with an average of 10.7±0.1‰ (n=15). These values are slightly higher than the rib (-18.1‰) and femora (-18.3‰) in Fig. 3. This isotopic difference between the dentin and the bones demonstrates that this individual consumed more C_4 foods (millets) and possibly animal protein during childhood and adolescence compared to later life. In addition, it can be observed that the diet of Subject B was fairly constant over the period of study (Fig. 3).

Subject C (M87) is a male and aged to approximately 20-30 years old. The δ^{13}C values of the tooth sections range from -19.6‰ to -17.8‰ with an average of -18.3±0.5‰ (n=19). The δ^{15}N values of the tooth sections range from 10.4‰ to 11.4‰ with an average of 10.9±0.3‰ (n=19). The δ^{13}C values of the serial sections are elevated compared to the femora but display a decrease toward a more C_3 based diet between ~12-17 years of age. The δ^{15}N values of the serial sections are also generally elevated compared to the femora but display more variability than the δ^{13}C results. This could indicate that there were periods of different amounts of animal protein consumption or possibly even nutritional stress as this has been noted to cause elevated δ^{15}N values in humans (Fuller et al., 2005; Mekota et al., 2006). However, between ~15-17 years of age there is an increase in δ^{15}N values that is paired with a decrease in δ^{13}C values, and this dual isotopic shift likely represents some type of dietary change at this age toward increased consumption of rice or other C_3 foods.

Subject D (M38) is a male with an approximate age of 30-40 years old. The δ^{13}C values of the tooth sections range from -18.2‰ to -19.3‰ with an average of -18.9±0.4‰ (n=12). The δ^{15}N values of the tooth sections range from 10.0‰ to 12.0‰ with an average of 11.0±0.5‰

(n=12). The $\delta^{15}N$ values of the tooth sections continuously decrease until the age of 3.5 years as a result of the weaning process, increase gradually and become stable around the age of 4.5 years. As the $\delta^{15}N$ values of the tooth serial sections intersect with the femora by 2.5 to 3 years of age this is likely when Subject D stopped breastfeeding. In contrast, the $\delta^{13}C$ values increase during the weaning period, especially after the cessation of breastfeeding between ~4-5 years of age, and this likely reflects a weaning and early childhood diet that contained millets. However, after the age of 5 there is a very gradual decrease in $\delta^{13}C$ values and a diet based more on C_3 foods such as rice.

Subject E (M40) is an indeterminate sexed individual that died about the age of 12-15 years old. The $\delta^{13}C$ values of the tooth sections range from -17.9‰ to -15.7 ‰ with an average of -17.0 ±0.9‰ (n=10). The $\delta^{15}N$ values of the tooth sections range from 10.0‰ to 12.8‰ with an average of 10.9±1.1‰ (n=10). The dentin $\delta^{15}N$ values are elevated from about 2.5 years of age and then decrease and reach the femora value at approximately 3.5 years of age, which is the approximate time for the cessation of breastfeeding. Between ~2-2.5 years of age the $\delta^{13}C$ values display an increase of ~1‰, and this isotopic signature indicative of the consumption of millet, remains elevated throughout the weaning process or up to the age of 3.5 years. If this individual was transitioning from breastfeeding to a C_3 based diet such as rice, then we would expect the $\delta^{13}C$ values to drop first before the decline in $\delta^{15}N$ values (Fuller et al., 2006). However, from the age of 3.5 to 4.5 years old there is a decrease of ~2‰ in $\delta^{13}C$, and this occurs after the $\delta^{15}N$ values have stabilized. This indicates that there was consumption of millets during the weaning and early childhood diet of Subject B.

Based on this very limited pilot study, these individuals stopped breastfeeding between ~2.5 to 4 years of age at the Gaoshan site, and these results are similar to our previous study at the Western Zhou Dynasty site of Boyangcheng (Fig. 1) in Anhui Provence (Xia et al., 2018). Furthermore, the role of millet consumption during early childhood as related to males and females can be observed to a very limited extent. In Fig. 4, the females (Subjects A and B) have higher $\delta^{13}C$ values than the males (Subjects C and D). This suggests that females preferred or were given more access to millets during weaning and childhood, but we must clearly emphasize that this inference is speculative give the small number of sexed individuals studied. However, what is highly interesting is the fact that the $\delta^{13}C$ analysis of the dentin serial sections revealed that millet was consumed during the weaning process and early childhood, a discovery that would have remained invisible if only the bone collagen were studied. Thus, this small project clearly demonstrates the importance and application of using dentin serial sections to archaeological collections in China.

5.3 Importance of millet agriculture on the Chengdu Plain with mixed millet and rice farming

Archaeobotanical studies at the Guiyuanqiao site (Phase I, 5100 to 4600 BP) located on the Chengdu Plain near Gaoshan indicate that millet agriculture, including broomcorn millet and foxtail millet, likely originated from the Chuanxibei Plateau (Guedes & Wan, 2015). During the Baodun period (4500 to 3700 BP), rice agriculture took over as the main crop in this area with westward cultural expansion from the Yangtze River Valley (Guedes et al., 2013). Our isotopic results suggest a preference for C_3-based foods, which is in accordance with this subsistence strategy relying on rice. It is argued, however, that millet farming in the uplands was an important supplement for rice farmers to maintain dietary diversity and that this double-crop system was beneficial for encouraging population growth, facilitating expansion into new territory and the development of social complexity in this area (Guedes, 2011). Here, our pilot study, based on the isotopic analysis of dentin sections as well as bones, provides important new evidence for elucidating the importance of millet agriculture during human growth and development.

Modern nutritional research indicates that millets provide healthy and diverse nutrients for humans including proteins, fat, thiamine, cellulose and minerals (Ravindran, 1991; Zhang et al., 2007). In particular, foxtail millet contains a hypoallergenic protein which is quite suitable for pregnant women and infants (Ravindran, 1991; Saleh et al., 2013). Thus, porridge made from millets is easily digested and considered an ideal food for infants and children (WHO, 2009). Today even in northern China, pregnant women and babies still consume millet (foxtail) gruel (Li, 1986; Zhang et al., 2007). However, this practice is rare in southern China where rice is the staple food (Zhang et al., 2007). Thus, it is quite likely that the habit of consuming millets during childhood at the Gaoshan site originated from the Ganqin region and the Chuanxibei Plateau in the north, and that this practice was maintained for long time even though rice agriculture was dominant in this region. Additionally, the possible dietary differences observed between males and females during their childhoods in this study imply that the females were influenced more by the millet-based agriculture than males. This dietary difference between males and females needs to be investigated in much greater detail in the near future to better understand the social behavior and social complexity in this region.

6. Conclusion

Since ~8000 years ago, the co-existence of millet agriculture and rice agriculture created a vast overlapping area between the Yellow River Valley and Yangtze River Valley of China. Our pilot study based on the isotopic analyses of serial sections of dentin as well as bone collagen at the Gaoshan site suggests that millets substantially contributed to human diets during the period of human growth and development, especially during the weaning process and early childhood, despite the fact that the main form of agriculture in this region was rice. Unfortunately, due to the poor preservation of the bone and tooth samples in this study, only a limited number of samples were available for isotopic analysis. Still our findings present a new perspective with which to rethink the role that millet agriculture played during the processes of cultural exchange and human movement in the Late Neolithic on the Chengdu Plain of China.

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Table 1. The sequence of Late Neolithic and Ancient Shu Cultures on the Chengdu Plain according to Wan & Lei (2013).

Cultures	Age	Typical sites	Agriculture	References
Guiyuanqiao Culture	5100-4600 BP	Guiyuanqiao Phase I	Millet	(Guedes & Wan, 2015; Jiao et al., 2013)
Baodun Culture	4600-4000 BP	Baodun, Manchengcun, Guchengcun, **Gaoshan** etc.	Mixed rice and millet	(Guedes et al., 2013)
Sanxingdui Culture	4000-3100 BP	Sanxingdui	Mixed rice and millet	(Sun, 2009)
Shierqiao Culture	3100-2600 BP	Shierqiao, Jinsha	Dominant rice	(Sun, 2009)

Table 2. Sample information including age estimation, skeletal elements and sex.

Subject	Burial	Age at death (years)	Sex	Tooth/Bone Type	Approximate age of development (in years)
A-L	-	-	-	Femora	~10 years before death
A-L	-	-	-	Ribs	~2-5 years before death
A	M6	~30	Female	RC_1	0.9-13
B	M86	~30-40	Female	RM^2	2.5-15.5
C	M87	~20-30	Male	RM_2	2.5-15.5
D	M38	~30-40	Male	RM_1	0.3-10
E	M40	~12-15	?	RM_1	0.3-10
F	K1	~35-40	Female	RM_2	2.5-15.5
G	M8	~20-30	Female	RM_1	0.3-10
H	M25	~5	?	LM_1	0.3-10
I	M42	~13-15	?	RM_1	0.3-10
J	M47	~8	?	RC_1	0.9-13
K	M77	~20-25	Female	RM_1	0.3-10
L	M85	~40-45	Female	RI_1	0.6-8.5

Note: Age estimation of development for each tissue is according to Cox & Sealy, (1997); Parfitt, (2002); Hedges et al., (2007); Beaumont & Montgomery, (2015).

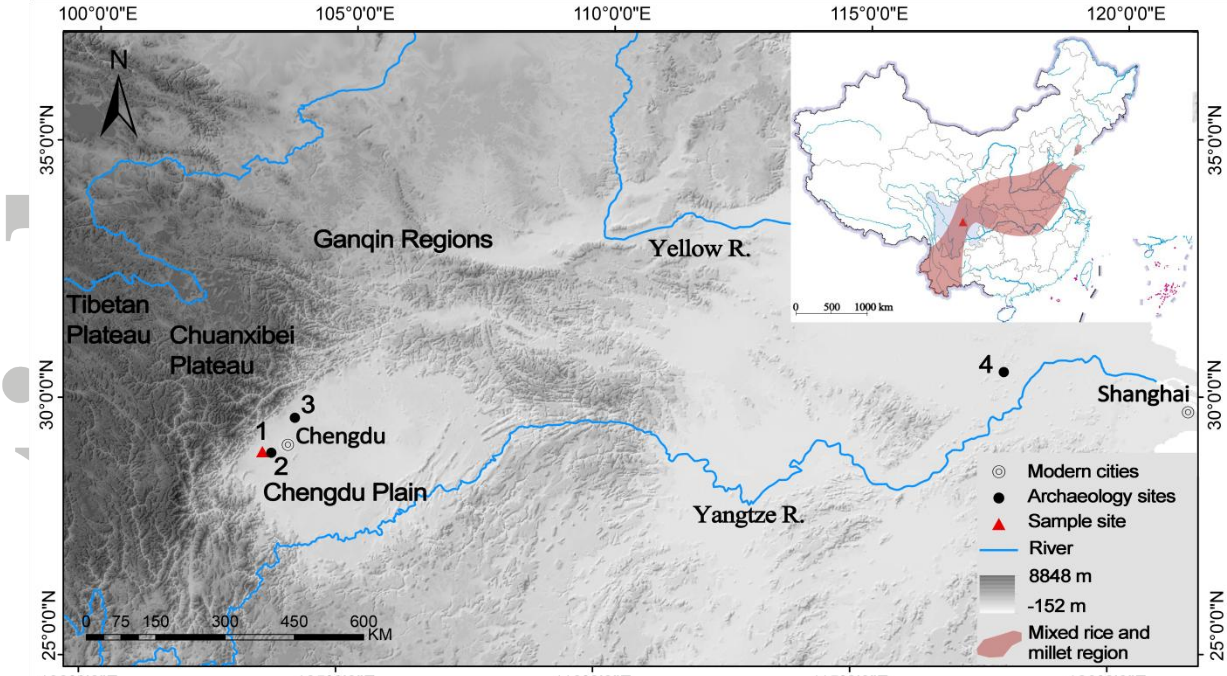

Fig. 1. Location of main sites mentioned in the text: (1) Gaoshan; (2) Baodun; (3) Guiyuanqiao; (4) Boyangcheng. The mixed rice and millet farming region is illustrated in red shadow which was modified from He et al., (2017).

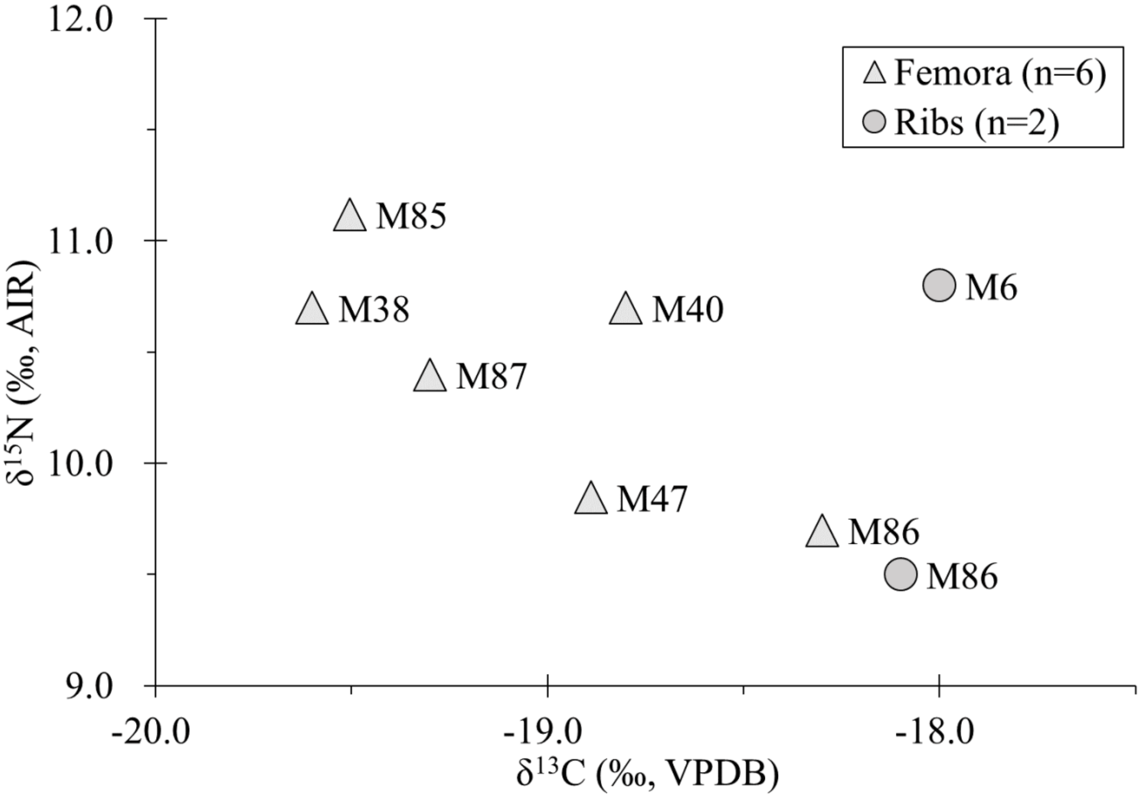

Fig. 2. Scatter plot of δ^{13}C and δ^{15}N results of bone samples.

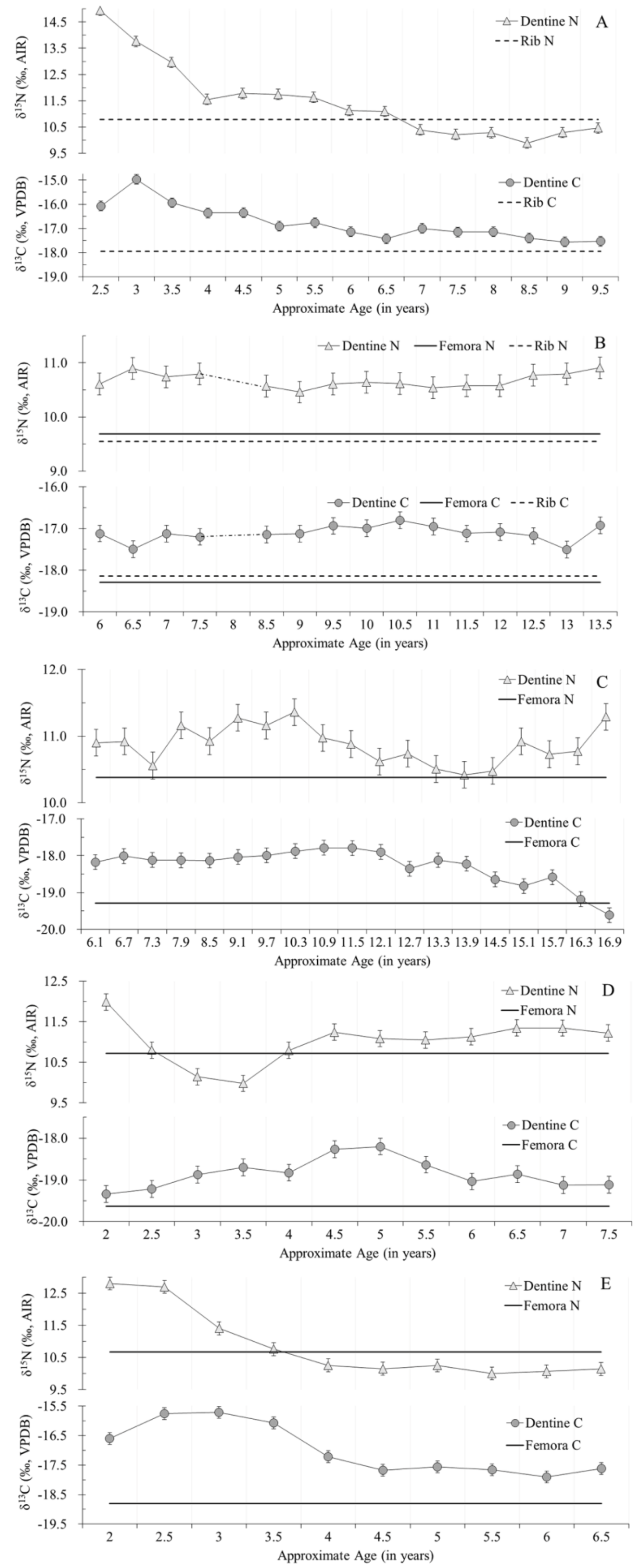

Fig. 3. A-E, Isotopic profiles of serial sections in five teeth. Note: Solid lines denote $\delta^{15}N$ or $\delta^{13}C$ values of the femora, and dotted lines denote the $\delta^{15}N$ or $\delta^{13}C$ values of the ribs.

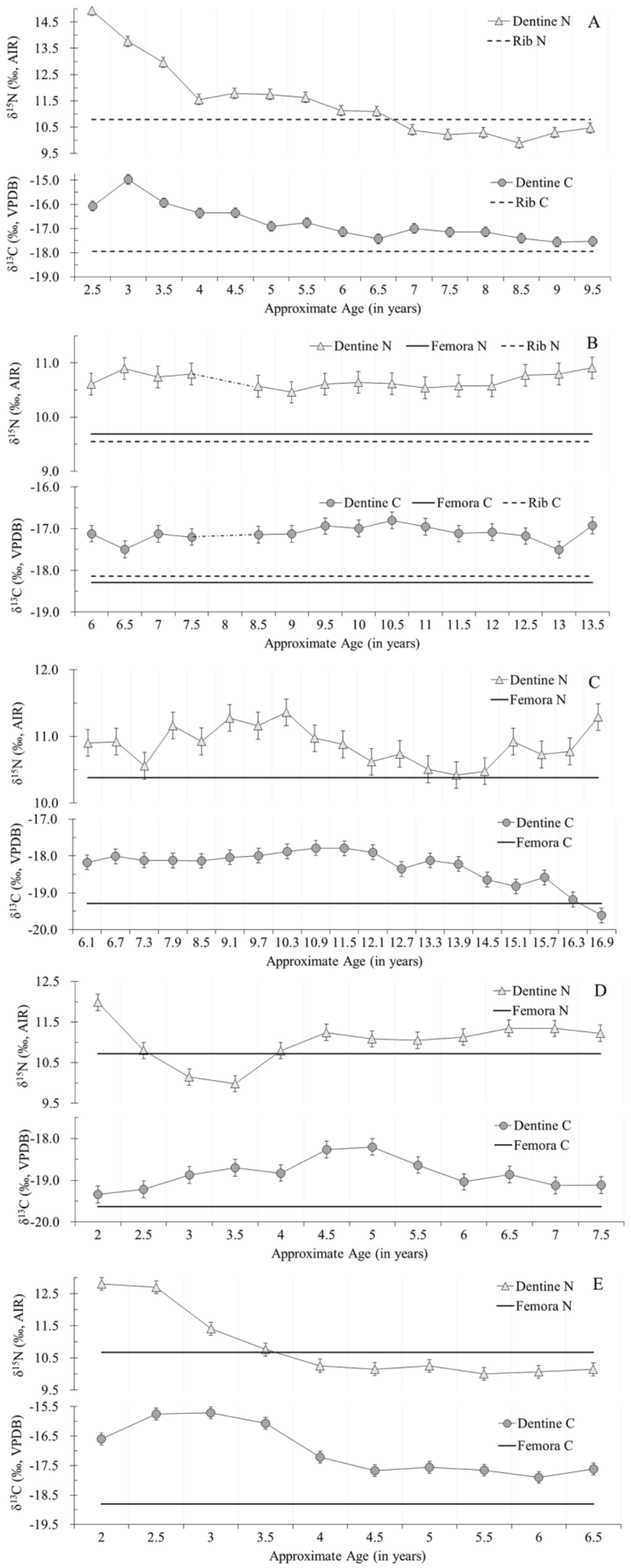

Fig. 4. Comparison of the $\delta^{13}C$ values in the dentin serial sections of Subjects A, B, C and D.