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Preliminary attempt to distinguish the domesticated pigs from wild boars by the methods of carbon and nitrogen stable isotope analysis

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Despite great achievements in the origins of domestic pigs made by the methods of zooarchaeology and molecular biology, how to scientifically distinguish the domesticated pigs from wild boars during the early stage of pig domestication is still poorly understood. Compared to wild boar's diets which come from the natural environment, the diets of domestic pigs are more easily influenced by human feeding activities. Therefore, in principle, exploration of the dietary differences among pigs and understanding the impact on pig diets fed by humans can have great potential to differentiate between wild boars and domesticated pigs. To reveal dietary differences among pigs and distinguish the domesticated pigs from wild boars based on comparison with the diets of humans and other animals, we analyzed the carbon and nitrogen stable isotopes of human bones from Xiaojingshan Site and animal bones from Yuezhuang Site, both of which belong to Houli Culture in Shandong Province and date to about 8500—7500 years ago. The mean δ^{13}C value ((–17.8 ± 0.3)‰) and δ^{15}N value ((9.0±0.6)‰) in human collagen indicate that although millet agriculture began it was not the main subsistence strategy as millets are typical of C_4 plants and that humans made a living mainly by gathering, hunting or raising some domesticated animals. The δ^{13}C value (−16.1‰) and δ^{15}N value (6.9‰) in the bovine suggest that C_3 plants were dominant in its diet with some C_4 plants complemented. The fish has lower δ^{13}C value (−24.9‰) and higher δ^{15}N value (8.8‰) than the bovine, which is the characteristic of the isotopic values from Eurasian freshwater fish. Based on the differences in carbon and nitrogen isotope values, the pigs can be divided into three groups. A group, composed of two pigs, has low δ^{13}C values (−18.1‰, −20.0‰) and low δ^{15}N values (4.7‰, 6.0‰). B group, only one pig, has the highest δ^{13}C value (−10.6‰) and mediate δ^{15}N value (6.4‰). As for the C group, also only one pig, low δ^{13}C value (−19.0‰) and the highest δ^{15}N value (9.1‰) are observed. Previous studies on the stable isotopes from modern or ancient wild boars' bones have suggested that C_3 plants are predominated in their diets and that their δ^{15}N values are close to those in herbivores and far from those in carnivores. Based on the comparison with the isotope values from humans, the wild boars and the domestic pigs from Xipo Site in Henan 6000—5500 years ago and Kangjia Site in Shaanxi 4500—4000 years ago, we conclude that A pig group belongs to wild boars while B and C groups can be attributed to domesticated pigs.

origins of domestic pigs, early stage of domestication, differentiation of domesticated pigs from wild boars, carbon and nitrogen stable isotope analysis, millet agriculture

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The origin and domestication of domestic pig, the main meat producer for humans, have drawn great attention in academic fields[1]. So far, the main method to study the origin of domestic pigs has been the zooarchaeological method, which differentiates between wild boars and domestic pigs through morphological observations and measurements of the pig bones and teeth in archaeological sites for understanding their morphology, size and age distribution, in the combination of archaeological findings[2]. The earliest domestic pigs around the world are found in "Fertile Crescent" of western Asia, such as Cayonu site and Nevali Cori site, and dated to about 9000 a[3,4]. The earliest domestic pigs in China are evidenced in Chifeng Xinglongwa Site of Inner Mongolia, Wu'an Cishan Site of Hebei, and Xiaoshan Kuahuqiao Site of Zhengjiang, around 8200−8000 years ago[5]. Moreover, three pigs with distorted tooth ranks in Wuyang Jiahu Site of Henan, led the scholars to believe that the occurrence of domestic pigs in China can be dated back as early as 8500 a[6]. In addition, the linear enamel hyperplasia (LEH) analysis of pig teeth has been developed in recent years to try and find the differences between domestic pigs and wild boars during the early stage of domestication in Europe[7] and to discuss the possibility of whether the domestic pigs occurred in Zengpiyan Site of Guangxi, as early as 10000 years ago[8].

Another effective method to study the origin and evolution of domestic pigs is the mitochondrial DNA (mtDNA) analysis of modern or ancient domestic pigs and wild boars to reconstruct the phylogenic relationship between them. The mtDNA sequences of modern Eurasian wild boars and domestic pigs suggest that Asian and European domestic pigs were domesticated from Asian and European wild boars respectively and that there were two domestication centers[9]. However, this view has been challenged by Larson et al.[10]. Their study through the phylogenic analysis of mtDNA sequences of 686 modern wild boars and domestic pigs in the world shows that the centers for pig domestication were at least seven, not two. Moreover, the mtDNA sequences of ancient pigs in a number of archaeological sites across European continent, including wild boars and domestic pigs, suggest that the European domestic pigs, believed to come from domestic pigs in Near East, may have derived from the domestication of local wild boars[11]. Recently, the sequence analysis of 670 bp in D-loop of mtDNA from 567 modern domestic pigs and 155 wild boars across East Asia, including China, Southeast Asia and India, indicates that it is likely for wild boars in East Asia to originate in Meikong River Valley and be domesticated there and in Middle-Lower Yangtze River Valley respectively[12].

Undoubtedly some critical problems have not been well solved although great achievements to study pig domestication have been made. For example, the morphological differences between wild boars and domestic pigs are too small to differentiate between them in the early stage of domestication. Even the recently-developed LEH analysis still has some difficulty in distinguishing wild boars from domestic pigs[8,13,14]. Because the domestic pigs came from the domestication of wild boars directly at that time, there must have no obvious differences of DNA sequences between them. Apparently, more ideas and techniques are needed in order to scientifically distinguish domesticated pigs from wild boars in the early stage of domestication.

A number of studies have pointed out that the feeding on the wild animals by humans is one of essential preconditions to domesticate the animals[15,16]. The domestication of wild boars is the same too[17]. Compared to wild boar's diets coming from natural resources, the domestic pig's diets at least result from human feeding activities. Therefore, in principle, there might be dietary differences between wild boars and domestic pigs.

Carbon and nitrogen stable isotope analysis of human or animal bones in archaeological sites has been one of main methods to reveal human or animal diets[18,19]. Carbon and nitrogen stable isotopes of pig bones from the sites in Western Asia, Europe, East Asia (China and Japan) confirm that there are significant dietary differences between wild boars and domestic pigs. For instance, the δ^{15}N values of domestic pigs from Neolithic sites in Southeast Anatolia, Turkey were lower than those of wild boars because of large quantity of pulse in their diets[20]. The domestic pigs in Ryukyu Islands, Japan, had higher δ^{15}N or δ^{13}C values than wild boars in other islands, owing to different feeding patterns by humans[21,22]. The domestic pigs in Wanfabozi Site of Jilin, had higher δ^{15}N values than wild boars, which suggests that some amount of human dietary remains containing animal protein was included in their diets[23]. Similarly, the domestic pigs in European sites had higher δ^{15}N values than wild boars too[24]. So, the differentiation between wild boars and domestic pigs can be made through the identification of dietary differences among pig population to understand the impact on pig diets fed by humans. In this paper, the carbon and nitrogen stable isotopes of animal bones in the fauna dominated by pigs from Yuezhuang Site, Jinan, Shandong are analyzed and the carbon and nitrogen isotopes in human bones in Xiaojinshan Site, Zhangqiu, Shandong are combined to investigate the diets of humans and animals and try to distinguish the domesticated pigs from wild boars in the early stage of domestication.

1 Archaeological contexts

Houli Culture is the earliest Neolithic culture found in Shandong Province and dated back to 8500—7500 years ago[25]. So far, there have been many archaeological sites with the characteristic of Houli Culture, such as Houli Site, Xiaojingshan Site, Xihe Site, Qianpuxia Site, Yuezhuang Site and so on. Pollen analysis in Xiaojingshan Site, phytolith analysis in Xihe Site[25,26] and plant floatation in Yuezhuang Site[27] suggest that it was as early as in Houli Culture that primitive millet agriculture appeared[28−30]. In addition, a large number of animal remains, such as pig, dog, cattle, sheep, chicken and so on, have been found, implying that humans might have begun to domesticate these animals[26,28]. In particular, the finding of pig bones and pottery pig figures, both of which had the features of wild boars and domestic pigs, indicates that the pig domestication was still in the early stage[26].

2 Materials and methods

2.1 Sample selection

Seventeen human bones in Xiaojingshan Site and 17 animal bones in Yuezhuang Site, including pigs, cattle, deer and fish, were selected. Pig bones were small pieces that cannot be identified to be wild boar or domestic pig based on the morphological measurements.

2.2 Collagen preparation

The bone collagen was prepared according to the protocol in the paper written by Jay et al.[31]. The contaminants in inner and outer surface of bones were taken away mechanically. Some bones were weighed and soaked in 0.5 mol/L HCl at 4℃. Every 3 or 4 days, the fresh acid was added until the bones were soft and no bubbles came out. The remains were centrifuged, washed into neutrality with distilled water and rinsed in HCl (pH=3) for 48 h at 70℃ to gelatinize the bones. Then after simple filtration, the remains were centrifuged in Millipore Amicon Ultra-4 filter and the solution with molecular weight higher than 30 KD was collected. Afterwards, the solution was frozen overnight and freeze-dried for two days to get the collagen. The collagen was weighed in order to calculate the collagen yield rate which is equal to the collagen weight divided to the bone weight.

In general, the bone samples, including humans and animals, were poorly preserved and only 16 of 34 samples (10 humans, 6 animals) can be extracted to get the collagen with low yield rate. The site name, location, species and collagen yield rate of the bones from which the collagen was extractable were listed in Table 1.

2.3 Measurements of carbon and nitrogen stable isotopes

Collagen was weighed and put into Thermo Finnigan DELTA plus XL combined with elemental analyzer to get the contents of carbon and nitrogen and the stable isotope ratios. The carbon and nitrogen isotope ratios were expressed as $\delta^{13}C$ value (relative to V-PDB) and $\delta^{15}N$ value (relative to AIR) with the precision of 0.1‰ and 0.2‰ respectively. Most of samples were run by duplicate. The contents of carbon and nitrogen and their stable isotope ratios were also listed in Table 1.

3 Results and discussions

3.1 Contamination of bone samples

One of important preconditions for stable isotope analysis is that the chemical compositions of human or animal bones (stable isotope ratios and elemental concentrations) should retain their *in vivo* biological features after long-term burial and not be affected and contaminated by external surroundings[32,33]. Therefore, identification of contaminated bones, selection of well-preserved bones and exclusion of the poor-preserved become critical before stable isotope analysis.

In brief, the bone samples were poorly preserved and collagen can only be extracted from 16 of 34 samples. Moreover, the collagen yield rate with average value of (0.9±0.6)% is much lower than that from modern bones (20% collagen)[34], indicating that most of collagen was degraded during burial process. However, the remaining collagen can also possibly retain its own biological features. Ambrose[34] suggested that if the carbon and ni-

Table 1 The properties and locations of samples and all measurement data

Site name	Species	Location	Collagen (%)	C content (%)	N content (%)	C:N	δ^{13}C (‰)	δ^{15}N (‰)
Xiaojinshan	human	M14	0.9	38.1	13.6	3.3	−17.4±0.03	8.1±0.03
Xiaojinshan	human	M2	1.3	44.5	16.1	3.2	−17.9±0.06	9.2±0.01
Xiaojinshan	human	M16	1.0	37.8	13.4	3.3	−17.5±0.04	8.6±0.14
Xiaojinshan	human	M20	1.8	43.9	15.7	3.3	−18.0±0.14	9.4±0.11
Xiaojinshan	human	M1	0.3	39.2	13.4	3.4	−17.4±0.16	8.1±0.25
Xiaojinshan	human	M19	0.4	30.6	10.8	3.3	−18.2	9.8
Xiaojinshan	human	M21	0.7	42.3	15.1	3.3	−17.9±0.06	9.2±0.09
Xiaojinshan	human	M17	0.3	33.8	12.1	3.3	−17.4	9.1
Xiaojinshan	human	M15	0.5	42.2	14.9	3.3	−17.8±0.08	9.4±0.15
Xiaojinshan	human	M4	1.3	36.6	13.3	3.2	−18.2	9.0
Yuezhuang	fish	H61	0.1	36.9	12.2	3.5	−24.9	8.8
Yuezhuang	pig	H193	0.1	39.6	14.5	3.2	−18.1	4.7
Yuezhuang	pig	H61	1.8	43.3	15.8	3.2	−19.0±0.07	9.1±0.03
Yuezhuang	pig	H82	2.1	42.6	15.4	3.2	−10.6±0.11	6.4±0.05
Yuezhuang	pig	H172	0.8	41.5	14.8	3.3	−20.0	6.0
Yuezhuang	cattle	H189	0.2	41.0	14.2	3.3	−16.1	6.9

trogen content in collagen were located in the range of 15.3%—47% and 5.5%—17.3% and their atomic ratio was in the range of 2.9—3.6 the collagen can be regarded as good. According to these criteria, the extractable collagen in this study has carbon content (average (39.6±3.8)%), nitrogen content (average (14.1±1.5)%) and C/N atomic ratio (average 3.3±0.1) located in the range of good collagen, making them suitable for stable isotope analysis.

3.2 Human diets and the importance of millet agriculture in human livelihood

Archaeological findings indicate that humans began to cultivate millet crops during Houli Culture. As millet crops are typical of C_4 plants, carbon and nitrogen isotope analysis of human bones can not only reconstruct human diets but also explore how millet agriculture developed and what its importance in human livelihood was.

The δ^{13}C value of modern Chinese millets is about −11.7‰[35]. Influenced by the fossil combustion in industrial era, the δ^{13}C value of the atmosphere in prehistory is about 1.5‰ more positive than that now[36]. So, the δ^{13}C value of millets in Chinese Neolithic should be around −10.2‰. When foods are absorbed by humans or animals and converted into the body components, carbon isotopes will be fractionated. For example, 5‰ and 1‰ enrichments from diets to collagen and from diets to muscles are observed respectively[19,37]. If the impact of trophic level on carbon isotopes (about 1‰ enrichment along the rise of trophic level) is omitted and 100% millets in human (or animal) diets are assumed, their δ^{13}C values in bone collagen might be equal to −5.2‰.

Figure 1 is the scatter plot of carbon and nitrogen isotopes of human bones from Xiaojingshan Site. The δ^{13}C values of all samples are located in the range of −18.2‰—−17.4‰ with the average of (−17.8±0.3)‰. Obviously, in comparison with humans consuming 100% millets (−5.2‰), the δ^{13}C values of humans from Xiaojingshan Site are more negative, indicating that millets only occupied a small portion in human diets and that most of human diets came from C_3-based foods. In order to estimate the percentage of millets in human diets, −26.5‰[19] and −10.2‰ are used to stand for the δ^{13}C values of C_3 foods and millets respectively. According to the simple two-end member mixing model[38,39], the contribution of millets to human diets is only 23%, strongly suggesting that millet agriculture during Houli Culture was still primitive. Compared to the δ^{13}C values, the δ^{15}N values scatter more loosely (8.1‰—9.8‰), which may imply that substantial differences of meat resources existed in human diets. Moreover, the combination of δ^{13}C and δ^{15}N values shed light on the fact that millet agriculture was still not important in human livelihood and that human made a living by gathering, hunting or feeding on some quantity of domestic animals.

3.3 Animal diets

The δ^{13}C and δ^{15}N values of six animals from Yuezhuang Site, including cattle, fish and pig, are plotted in Figure 2. The average and standard deviation of δ^{13}C and δ^{15}N values from Xiaojingshan Site are also plotted

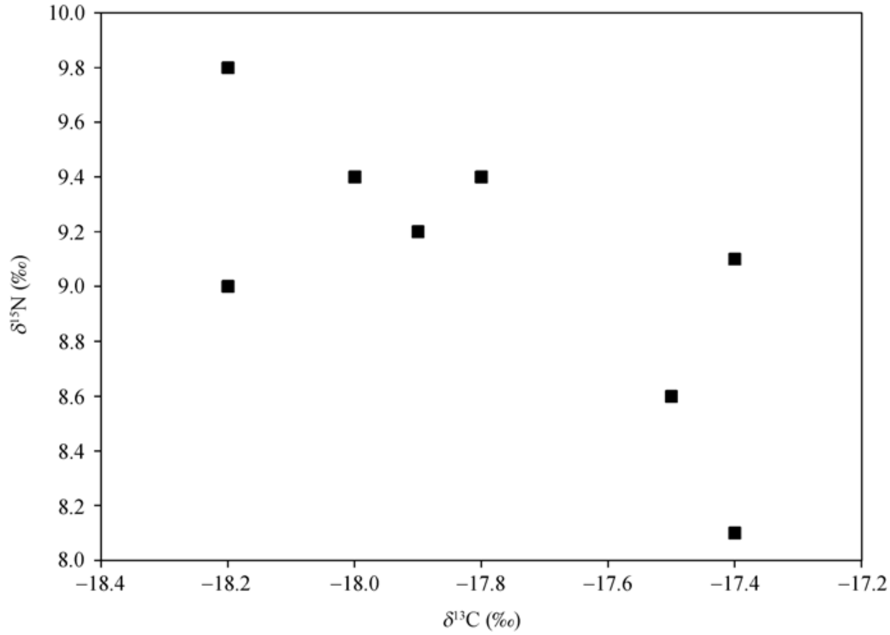

Figure 1 Scatter plot of δ^{13}C and δ^{15}N values in human collagen. Only 8 points can be seen in the figure because the values of M14 and M1 and the values of M2 and M21 are exactly the same.

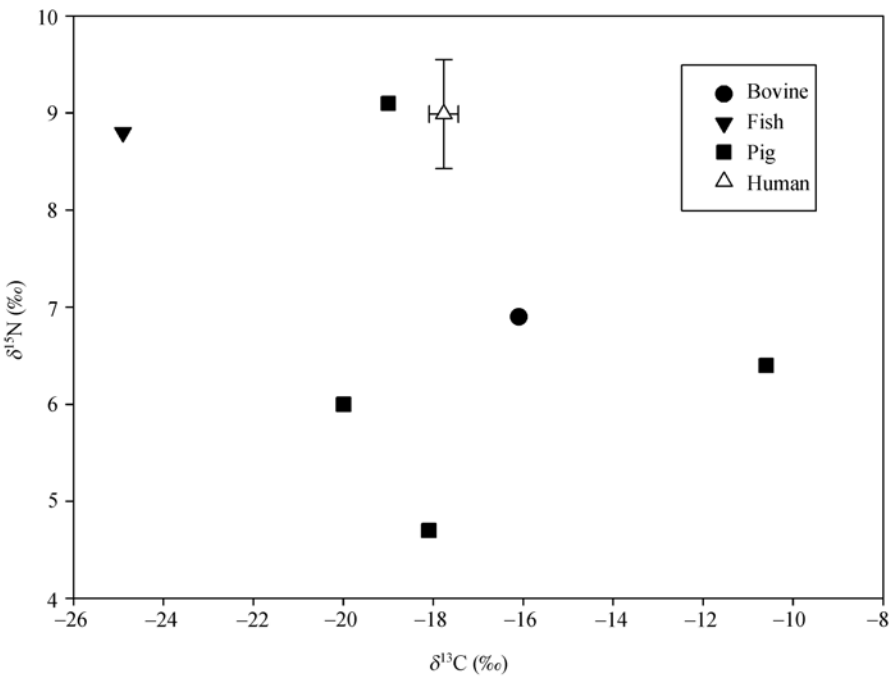

Figure 2 Scatter plot of δ^{13}C and δ^{15}N values in animal collagen and standard deviation plot of δ^{13}C and δ^{15}N values in human collagen.

in order to reveal the dietary relationship between humans and animals more clearly. The δ^{13}C value of the cattle is −16.1‰, showing that C_3 plants were dominant in its diet with some C_4 plants supplemented. The C_4 plants might be possible from natural C_4 plants in Northern China, such as grasses, sedges and chico[40], or the contribution from millet agriculture. The δ^{15}N value of the cattle (6.9‰), as a typical herbivore, can stand for the δ^{15}N value of the herbivores during Houli Culture to some extents. The fish has significant lower δ^{13}C (−24.9‰) and higher δ^{15}N values (8.8‰) than the cattle, which is the isotopic characteristic of freshwater fish in Eurasia[41]. In terms of the differences of δ^{13}C and δ^{15}N values among pigs, they can be divided into three groups.

A group, composed of two pigs, have low $\delta^{13}C$ (−18.1‰ and −20.0‰) and $\delta^{15}N$ values (4.7‰ and 6.0‰), suggesting that plant foods were dominant in their diets. B group, only one pig, has the highest $\delta^{13}C$ value (−10.6‰) and mediate $\delta^{15}N$ value (6.4‰), which shows that C_4 plants were the main component in the diet. Although the $\delta^{13}C$ value (−19.0‰) in C group, only one pig too, is low, the highest $\delta^{15}N$ value (9.1‰) is observed, which is located in the range of human $\delta^{15}N$ values (average (9.0 ± 0.6)‰). This result indicates that the pig in C group had a similar trophic level to humans and their relationship was very close and that the pig might consume mainly the human leftover or excrements containing much animal protein.

3.4 Scientific differentiation between wild boar and domestic pig

The domestication of wild animals by humans contains two parts, *i.e.*, biological domestication and cultural domestication[42−44]. In biological domestication, the breeding and development of some of wild animals are controlled and selected intentionally, which makes them under human control. Then, these animals deviate gradually from wild species and great differences in morphological characteristics and DNA sequences between the domesticated and the wild occur accordingly. It is well known that the changes of morphological characteristics and DNA sequences can not be completed in a short time but over one thousand or even thousands of years[8]. On the other hand, the cultural domestication is that humans control the habitat and diets of animals and make the wild animals under human cultural control which may change the animal behaviors in short time and have them be connected with human societies closely. Obviously, exploration of whether the cultural domestication occurred, in other words, identification of whether the wild animals were in human cultural control, is the key to understanding animal domestication.

Studies on the behaviors of modern wild boars have demonstrated that they belong to omnivores and mainly eat the plant roots and stems, fruits, grasses, insect larva and carrion, especially underground plant stems[45−47]. Carbon and nitrogen stable isotope analyses of modern or ancient wild boars in Western Asia[20], Europe[24], Japan[21,22] and China[23] indicate that their $\delta^{13}C$ values are of strong C_3 signal and $\delta^{15}N$ values are close to those of herbivores such as deer, sheep and cattle and far from those of carnivores such as fox and wolf. Therefore, the pigs in A group in this study with similar $\delta^{13}C$ and $\delta^{15}N$ values to wild boars are attributed to wild boars.

The occurrence and development of primitive agriculture laid a great economic foundation for humans to feed the wild boars and domesticate them so that the feeding with agricultural by-products became one of preconditions to produce domesticated pigs[14,17]. Archaeological findings and stable isotope analysis of human bones suggest that millet agriculture occurred during Houli Culture and was one of subsistence strategies in human livelihood. Meanwhile, the development of millet agriculture created the condition for human to domesticate the pigs. Although the pig in B group from Yuezhuang Site has similar $\delta^{15}N$ value to that of the bovine, its high $\delta^{13}C$ value implies that plenty of C_4 plants were included in its diet which may correlate closely with the feeding of millet by-products such as stems and hulls. In addition, its $\delta^{13}C$ value is also close to those of domestic pigs in Xipo Site (−7.4‰, −7.7‰) during Yangshao Culture 6000—5500 years ago and those of domestic pigs in Kangjia Site (−11.5‰, −11.8‰, −7.5‰) during Longshan Culture 4500—4000 years ago[35]. Based on the above evidence, we believe that the pig in B group was domesticated. As for the pig in C group, its $\delta^{13}C$ value is close to that in A group, but its $\delta^{15}N$ value is similar to those of humans ((9.0±0.6)‰) in Xiaojingshan Site, higher than those of domestic pigs during Yangshao Culture (7.5‰, 8.0‰) and close to those of domestic pigs during Longshan Culture (7.8‰, 9.6‰, 8.7‰)[35], suggesting that its diet resulted from human food remains or excrements. So, the pig in C group can also be regarded as domesticated pig.

4 Conclusions

The results of carbon and nitrogen isotope analyses of human bones from Xiaojingshan Site and animal bones from Yuezhuang Site during Houli Culture indicate that although millet agriculture occurred, it was not yet of great importance in human livelihood and that human mainly relied on gathering, hunting or feeding on domesticated animals. C_3 plants dominated in the bovine diet with some C_4 plants supplemented. Fish has low $\delta^{13}C$ and high $\delta^{15}N$ values, characteristic of freshwater fish in Eurasia. In terms of the differences of δ^{13}C and δ^{15}N values, pigs can be divided into three groups. i.e., A group with low δ^{13}C and δ^{15}N values, B group with high δ^{13}C and mediate δ^{15}N values and C group with low δ^{13}C and high δ^{15}N values. In comparison with the stable isotope values of humans, wild boars and domestic pigs during Yangshao Culture and Longshan Culture, A group is attributed to wild boars while B and C group belong to domesticated pigs.

Finally, though the differentiation between wild boar and domestic pig in the early stage of domestication seems successful by stable isotope analysis in this study, the poorly-preserved bone samples and low sample numbers available for analysis, especially pig samples, greatly restrict the effectiveness and precision in distinguishing domestic pig from wild boar. Therefore, more studies should be undertaken in the future, such as stable isotope analyses of modern or ancient wild boars and humans and fauna in archaeological sites. On the basis of the above studies, the dietary differences among pigs can be identified. Moreover, in combination with the stable isotope values of humans and other animals, the differentiation of domesticated pigs in the early stage of domestication can be made, which can shed light on the origins and feeding patterns of domestic pigs in China as well as the relationship between pigs and humans.

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