Stable isotope dietary analysis of the Tianyuan 1 early modern human

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We report here on the isotopic analysis of the diet of one of the oldest modern humans found in Eurasia, the Tianyuan 1 early modern human dating to ≈40,000 calendar years ago from Tianyuan Cave (Tianyuandong) in the Zhoukoudian region of China. Carbon and nitrogen isotope analysis of the human and associated faunal remains indicate a diet high in animal protein, and the high nitrogen isotope values suggest the consumption of freshwater fish. To confirm this inference, we measured the sulfur isotope values of terrestrial and freshwater animals around the Zhoukoudian area and of the Tianyuan 1 human, which also support the interpretation of a substantial portion of the diet from freshwater fish. This analysis provides the direct evidence for the consumption of aquatic resources by early modern humans in China and has implications for early modern human subsistence and demography.

archaeology | Asia | diet | fish | Late Pleistocene

Understanding human adaptations to the environment and specifically their subsistence strategies is a key part of determining the processes and nature of human evolution. In particular, the position of the Tianyuan 1 human fossil remains as one of the oldest marine isotope stage (MIS) 3 modern humans in Eurasia (1, 2) poses the question of whether there might have been changes in human dietary spectra and emphasis associated with the spread of modern human biology. There have been suggestions, based on European faunal assemblages and inferred from technological changes associated with the emergence of the Upper Paleolithic, that there was a shift in human predatory abilities and associated changes in diet. At the same time, carbon and nitrogen stable isotope analyses of both late archaic humans (Neandertals) and Upper Paleolithic early modern humans in Europe (3, 4), as well as analyses of small animal remains (5), have suggested that there was a shift to a broader dietary spectrum around the time of, or shortly after, the spread of modern humans, probably including greater emphasis on aquatic resources. Yet, analyses of western Eurasian archeological faunal remains (6, 7), organic residues (8), and human functional anatomy (9) have suggested little change in human diet or predation before the Mid Upper Paleolithic.

In eastern Eurasia, the nature of any human dietary changes that might have been associated with the emergence of modern humans is still unclear. There is evidence for human predation on and processing of medium and large ungulates at Xujiayao, Zhoukoudian-Upper Cave and Tianyuan Cave (10–12). The Zhoukoudian-Upper Cave deposits yielded the remains of freshwater carp (*Cyprinus* and *Ctenopharygodon*), plus *Arca* shells (10), and a bone harpoon point from Xiaogushan may be of a similar age (13, 14). There has been some discussion of human subsistence strategies in China during the Late Pleistocene based on the changes of lithic technology (15, 16). For example, Chen (15) suggested that there were at least 4 different human adaptive strategies in north China (Shuidonggou, Siyu, Upper Cave-Dongfang Plaza-Xiaonanhai, and Xiaogushan). Further away, Niah Cave in peninsular southeast Asia provides indications of changes in dietary breadth from the same age as Tianyuan Cave Layer III (17).

It is in this context that we present here carbon, nitrogen, and sulfur stable isotopic analysis of the Tianyuan 1 human remains and associated fauna from Tianyuan Cave. Stable isotope analysis has been proved to be useful for dietary reconstruction, because it provides direct evidence for human diets (18). In addition to the more commonly used carbon and nitrogen stable isotopes, sulfur isotope values have the potential to reveal if the principal foods were from terrestrial or freshwater ecosystems (19–21). Therefore, sulfur isotope ratios were also analyzed to assess whether Tianyuan 1 consumed significant aquatic resources.

Archeological Context. Tianyuan Cave (Tianyuandong) is located in Huangshandian Village, Zhoukoudian, Beijing (39° 28′ 29″N; 115° 52′ 17″E) and has been designated Zhoukoudian Locality 27. Four geological strata were identified in the cave sediments and the human fossil, with a predominance of derived modern human characteristics, was found in Layer III (1, 2, 22). In addition, numerous fragmentary faunal remains were found, mainly distributed in the first and third layers. Radiocarbon dating of the human femur and faunal remains in Layer III indicate that the human dates to between 42–39 calibrated years BP, making it among the oldest directly dated early modern humans in eastern Eurasia (1, 2).

Stable Isotope Analysis as a Dietary Indicator. Carbon ($^{13}C/^{12}C = \delta^{13}C$) and nitrogen ($^{15}N/^{14}N = \delta^{15}N$) isotope values of mammal bone collagen are related to the isotope ratios of foods consumed (23, 24). In humans, the carbon and nitrogen isotope values indicate the sources of dietary protein over many years of life (25–27). Carbon isotope values indicate if the main source of dietary protein was from marine or terrestrial resources and can distinguish between the consumption of C_4 and C_3 photosynthetic pathway plants (or, in the case of omnivores or carnivores, animals that consumed C_3 or C_4 plants) (28, 29). Numerous studies indicate that bone collagen $\delta^{13}C$ and $\delta^{15}N$ values are enriched by ≈1.0‰ and ≈3–5‰, respectively, from herbivores to carnivores in the same food web (23, 24, 28, 30, 31).

Sulfur is found in only one amino acid in mammalian bone

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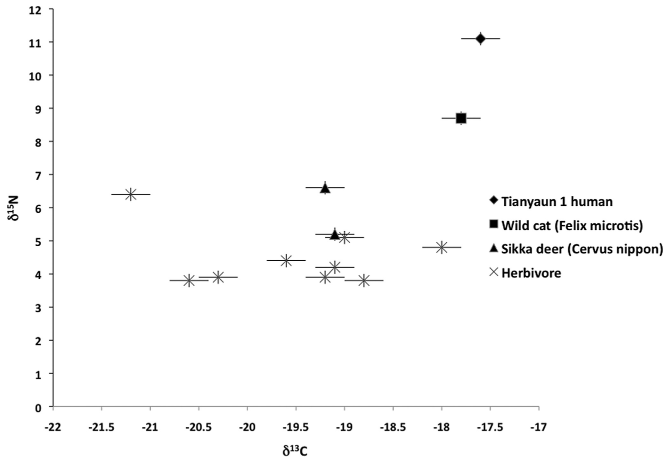

Fig. 1. Scatter plot of $\delta^{15}N$ vs. $\delta^{13}C$ values for the human skeleton and the faunal remains from Tianyuan Cave. Black diamond: Tianyuan 1; gray square: wild cat; gray triangles: sikka deer; open triangles: herbivores. Error bars indicate measurement errors.

collagen, methionine (32). Because methionine is an essential amino acid for humans, it must be obtained through the consumption of methionine-containing proteins from either plants or animals. Sulfur isotope ratios ($^{34}S/^{32}S = \delta^{34}S$) in plants and animals are ultimately derived from soil sulfur, which can come from the underlying bedrock or be deposited as rainfall (33). Sulfur isotope values of terrestrial animals are usually 5–10‰, while marine organisms have a relatively constant value of ≈20‰ (34). There is a slight (≤1‰) fractionation between dietary methionine and human bone collagen sulfur isotope ratios (20). Therefore, sulfur isotope analysis, analogous to strontium isotopes (35), can be used as a geographical indicator, especially for identifying individuals from coastal areas (where the $\delta^{34}S$ value is dominated by marine sulfur from sea spray) in inland locations (19, 36). The relevant aspect of sulfur isotopes is that, in freshwater environments, organisms often have sulfur isotope values that are distinct from the local terrestrial values, usually caused by the bacterial fractionation of sulfur in freshwater ecosystems (37, 38). Therefore, it can be used as an indicator of freshwater food consumption, if it can be established that the freshwater system has distinct sulfur isotope values from the local terrestrial ecosystem. Because sulfur is present only in one amino acid, the amount of sulfur in bone collagen is low, ≈0.2%; therefore, relatively large samples of collagen (≈10 mg) are required for a single $\delta^{34}S$ measurement using continuous-flow isotope methods (39, 40).

Results

Isotopic Food Web at Tianyuan Cave. The $\delta^{13}C$ and $\delta^{15}N$ values of all of the samples from Tianyuan Cave are plotted in Fig. 1. The herbivores, including the 2 sikka deer and the 9 unidentified herbivores, have $\delta^{15}N$ values of 4.7 ± 1.6‰, plotting in the expected range for herbivores from this time period (41). The herbivore mean $\delta^{13}C$ value of −19.5 ± 0.9‰ suggests that C_3 plants were generally dominant in their diets. However, the relatively large range of $\delta^{13}C$ values (−18.0–−21.2‰) may indicate that some individuals (i.e., those with $\delta^{13}C$ values ≥−19‰) may have consumed a relatively small amount of C_4 plants, such as wild millet, which resulted in the more positive $\delta^{13}C$ values. The sikka deer have higher $\delta^{15}N$ values than most of the unidentified animals, suggesting that more ^{15}N-enriched C_3 plants were included in their diets.

The 4‰ enrichment of $\delta^{15}N$ between the mean herbivore $\delta^{15}N$ value and the carnivore (wild cat; Table 1) is well within the expected enrichment factor for predator-prey species. The difference of $\delta^{13}C$ value (1.7‰) between the wild cat and the mean herbivore $\delta^{13}C$ value is close to the expected 1‰ carbon isotopic fractionation in the food web, and may also indicate a minor input of herbivores that consumed C_4 plants.

Carbon and Nitrogen Isotope Values of Tianyuan 1. Tianyuan 1 has a $\delta^{13}C$ value close to that of the wild cat, which may indicate a similar diet, with a similar carbon isotope fractionation between prey and consumer, and indeed also the potential consumption of some C_4 foods. However, the human $\delta^{15}N$ value (11.1‰) is much higher than that of the wild cat (8.7‰) and all of the herbivores. It is, therefore, unlikely that herbivores were the sole source of protein in the diet of this human.

The $\delta^{15}N$ values of animals in aquatic ecosystems (marine and freshwater) are generally higher than those in terrestrial ecosystems due to their longer food chains (3). Therefore, we suggest that the human nitrogen isotope value can best be explained by the consumption of freshwater foods and perhaps terrestrial animals that consumed C_4 vegetation. No freshwater fish bones were found in Tianyuan Cave (22); however, fish remains have been found at sites near Tianyuan Cave, including Zhoukoudian-Upper Cave (10) and the early Neolithic Donghulin site (42), implying that there should have been freshwater resources available around Tianyuan Cave. Carbon isotope

Table 1. Isotope samples and resultant data for the human and fauna from Tianyuan Cave and fish sulfur values from Donghulin

	Layer	C%	N%	S%	C/N	C/S	N/S	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Tianyuan 1 (*Homo sapiens*)	3	43.3	15.5	0.19	3.3	623	223	−17.6	11.1	4.1
Sikka deer (*Cervus nippon*)	3	41.2	14.7		3.3			−19.2	6.6	
	3	40.9	14.3		3.3			−19.1	5.2	
Wild cat (*Felis microtis*)	1	43.7	16.0	0.20	3.2	587	215	−17.8	8.7	7.6
unidentified herbivore	1	42.1	15.4		3.2			−18.8	3.8	
	1	42.1	15.3		3.2			−18.0	4.8	
	1	43.1	15.9	0.18	3.2	639	235	−21.2	6.4	7.2
	2	41.8	15.0		3.2			−19.2	3.9	
	2	43.6	16.0	0.20	3.2	572	209	−20.6	3.8	7.9
	2	41.6	15.0		3.2			−20.3	3.9	
	3	42.0	15.3		3.2			−19.0	5.1	
	3	42.3	15.4		3.2			−19.6	4.4	
	3	42.0	15.2		3.2			−19.1	4.2	
Donghulin fish	NA	26.0	8.5	0.13	3.6	385	108	NA	NA	5.6
	NA	39.9	12.9	0.32	3.6	336	93	NA	NA	5.3

NA, not applicable.

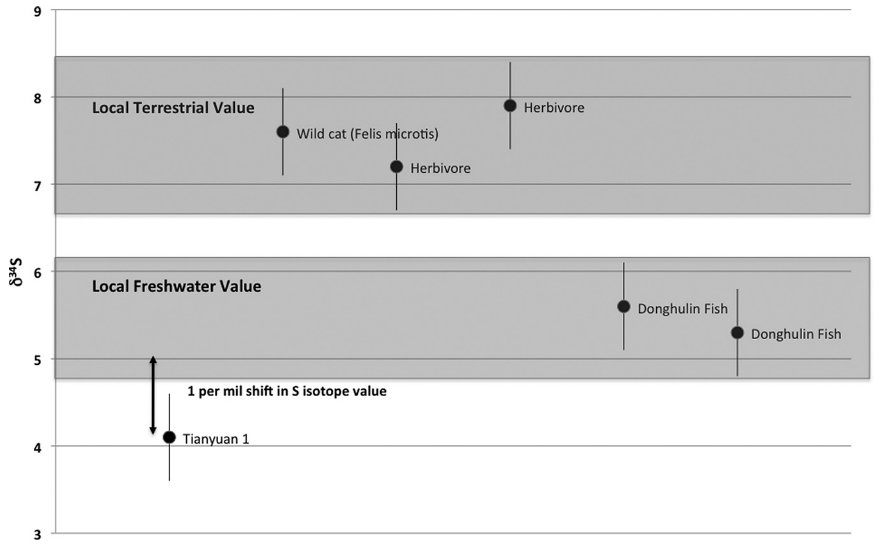

Fig. 2. Sulfur isotope values of Tianyuan 1, 3 terrestrial animals from Tianyaun Cave, and 2 fish from Donghulin. Error bars indicate measurement errors.

values of freshwater fish can be quite variable, and relate to the local geology (43). Therefore, the carbon isotope value may indicate the consumption of freshwater fish, and not herbivores as discussed above. Indeed, European Mesolithic freshwater fish consumers from the Iron Gates gorge area of the Danube have more positive δ^{13}C values than local herbivores, and are similar to the Tianyuan 1 human value (44). Yet, without contemporary fish from Tianyuan Cave we cannot at present use the carbon isotope values as additional support for the interpretation of the human δ^{15}N values as indicating freshwater fish consumption.

Sulfur Isotope Values of Tianyuan 1 and the Fauna. The sulfur isotope values of the Tianyuan Cave terrestrial animals (Table 1; Fig. 2) are similar and average 7.6 ± 0.4‰. The average sulfur isotope value of fish from Donghulin is 5.5‰. The sulfur isotope value of Tianyuan 1 (4.1‰) is substantially lower than those of the few terrestrial animals providing data, yet it matches well with the local freshwater sulfur value, especially considering that there is a 1‰ decrease between diet and consumer collagen sulfur isotope values (20). These data further support the inference that the human dietary resources were not derived solely from the terrestrial environment, and indeed were likely to have been substantially from freshwater sources.

Discussion and Conclusion

Carbon, nitrogen, and sulfur isotope analyses of bones from the Tianyuan 1 early modern human and the associated animals in Tianyuan Cave and the Donghulin site indicate that the human most likely obtained a substantial portion of its protein from a freshwater ecosystem, probably from freshwater fish. These data provide the earliest direct evidence of significant freshwater food exploitation by modern humans in Eurasia, even though it has been suggested (3, 4) that it may have occurred at approximately the same time period in Europe based on the relatively high δ^{15}N values of some early Upper Paleolithic humans.

There is nonetheless increasing evidence for human exploitation of fish remains and maritime coastal resources from earlier, Middle Paleolithic/Middle Stone Age, sites in western Eurasia and Africa (3, 6, 45–47). In this context, it was not the exploitation per se of aquatic resources that was unusual in the Tianyuan 1 early modern human, but the evidence for sufficient year-round freshwater fish consumption to register in its bone collagen isotopic signature. It may well be that this evidence is an additional reflection of increasing population size at this time period, additional pressure on food resources, and the associated acquisition of difficult to access small animals (terrestrial and aquatic) by human populations (*cf.*, refs. 5 and 48). This time period is also when modern humans first appeared across most of Eurasia (1, 17, 49), a process [whatever the phylogenetic dynamics may have been (1, 50)] that must have involved substantial population increases to produce the relatively rapid dispersal of modern human biology. The stable isotopic analysis of the Tianyuan 1 may, therefore, provide insight into both MIS 3 human demography and the subsistence dynamics of early modern humans in eastern Eurasia.

Materials and Methods

Materials. Although most of the Tianyuan Cave animal bones are very fragmentary, 31 animal species could be identified in the faunal assemblages with a possible 6 additional species (51). However, 18 of these species are rodents or lagomorphs. Most of macromammal species are herbivores, and carnivores are rare and fragmentary (22, 51). As a result, 13 bones were selected for stable isotopic analysis (Table 1). A sample of bone, that had been removed from the diaphysis of the right humerus for radiocarbon dating, was used for the Tianyuan 1 human isotopic analysis. For the faunal remains, 2 samples of sikka deer (*Cervus nippon*) from Layer III were included, plus 9 samples of unidentified herbivore (most likely *C. nippon*), 3 from each of Layers I, II and III. In addition, a sample was taken from a Layer I wild cat (*Felis microtis*) given the dearth of carnivores in Layer III. Kruskal–Wallis analysis of the herbivore carbon and nitrogen isotopic values across the 3 Layers of Tianyuan Cave (Table 1) provided *P* values of 0.299 and 0.119, respectively; the values are, therefore, pooled in the comparisons.

To establish a local sulfur signal for freshwater ecosystems in this area we also sampled fish from the nearby site of Donghulin, an Early Neolithic site, dating to 11–9 kyBP (42). This site is much later than Tianyuan Cave, but analogous to strontium isotope analysis (35), the sulfur isotope signals from a region relate to the local geology and so remain constant through time. Therefore, in the absence of fish from Tianyuan Cave, sulfur values from the fish from the Donghulin site can be used as a proxy for the local freshwater sulfur isotope values.

Analytical Methods. All of the samples were prepared by using a modified Longin method (52) with the addition of an ultrafiltration step (53). The procedure for collagen extraction was as follows: The samples were cleaned mechanically by powder abrasion, rinsed in 0.5 M HCl at 4 °C and refreshed for several days to completely decalcify the bones. The samples were then rinsed to neutrality in deionized water and gelatinized in HCl (pH 3) at 70 °C for 48 h. The residues were ultrafiltered to isolate the fraction with a molecular weight >30 kDa. Finally, the residues were frozen and lyophilized.

Carbon, nitrogen, and sulfur contents and δ^{13}C, δ^{15}N, and δ^{34}S values of collagen were determined through EA–IRMS [Thermo-Finnigan Delta Plus V

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(ThermoFisher, Inc.) coupled with a Heka elemental analyzer (HekaTech, Inc.)]. Stable isotope ratios are expressed relative to the VPDB standard for carbon, atmospheric N (AIR) for nitrogen, and Vienna Canyon Diablo Troilite for sulfur, using the delta (δ) notation in parts per thousand (‰). Each sample was run in duplicate, and an internal standard was measured with each set of 10 samples. Measurement errors on the δ^{13}C and δ^{15}N values are $\pm$0.2‰, and $\pm$0.5‰ for the δ^{34}S measurements. The contents of carbon, nitrogen, and sulfur, and the values of δ^{13}C, δ^{15}N, and δ^{34}S of all samples, are listed in Table 1. All samples discussed below have an average C content of 42.3 $\pm$ 0.9%, an average N content of 15.3 $\pm$ 0.5%, and atomic C/N ratios in the range of 3.2–3.3, similar to those in modern bones (41% C content, 15% N content, and 2.9–3.6 C/N ratio) (54, 55), suggesting that all samples retained their in vivo isotopic signatures. In addition, the atomic C/S and N/S ratios of 6 samples (1 from the human, 3 from mammals, and 2 from fish) are within the range of modern collagen (40).

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