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**Research Article**

## Investigating Dietary Patterns with Stable Isotope Ratios of Collagen and Starch Grain Analysis of Dental Calculus at the Iron Age Cemetery Site of Heigouliang, Xinjiang, China

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### Abstract

Here, we present  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results for the dietary reconstruction of nomadic pastoralists from the Iron Age (ca. 1000 BC–8 AD) site of Heigouliang. The human ( $n = 27$ )  $\delta^{13}\text{C}$  values range from  $-19.6\text{‰}$  to  $-17.0\text{‰}$  with a mean value of  $-18.5 \pm 0.5\text{‰}$ , and the  $\delta^{15}\text{N}$  results range from  $11.5\text{‰}$  to  $13.8\text{‰}$  with a mean value of  $12.4 \pm 0.6\text{‰}$ . The results indicated that animals, like sheep, were part of the predominately  $\text{C}_3$  terrestrial diet, but two individuals have values greater than  $-18\text{‰}$  that is indicative of some input of  $\text{C}_4$  foods in their diets. Because of a lack of faunal samples and to supply complementary information concerning plant consumption, teeth from four individuals were analysed for dental calculus microfossils. Starch grains were found to correspond to *Triticeae* and *Poaceae*, possibly including wheat (*Triticum aestivum*),

barley (*Hordeum vulgare*), highland barley (*H. vulgare* L. var. *nudum*), foxtail millet (*Setaria italica*) and/or common millet (*Panicum miliaceum*). At the population level, no dietary differences were detected between burial owners and sacrificial victims, but variations were found when specific tombs were analysed. In particular, individuals with bone trauma associated with armed conflict also had distinct isotopic signatures possibly suggesting that some of the sacrificial victims could have been captured warriors that were sacrificed for the burial owners. While limited, the results are some of the first from an Iron Age population from Xinjiang and contribute to our understanding of the dietary patterns of this region. Copyright © 2015 John Wiley & Sons, Ltd.

## Introduction

Formed by the Tianshan Mountains, the Gansu corridor links the steppes of Central Asia with the fertile river valleys of Central China. In the eastern portion of this mountain range is the Heigouliang cemetery site that is located in the Kumul region of the Barkol Basin in Xinjiang, China (Figure 1; Eng, 2007). This site is regarded as a typical early Iron Age pastoral site and dates to the early period of the Western Han Dynasty (ca. 500 BC–8 AD) (Ren, 2011). During this period, the eastern Tianshan area was home to a large number of human settlements with diverse cultures, reflecting increased social complexity through migration, trade and the mixing of different ethnic groups (Shao, 2007; Xi, 2014). In ancient Chinese historical texts, such as *Shiji* 'Records of the Grand History of China' (Simaqian, 104–91 BC) (Sima, 1959) and *Hanshu* 'Book of Han' (Bangu 80 AD) (Ban, 1962), the early Iron Age inhabitants across the Tianshan area were described as nomads who moved several times every year with the changing seasons in search of adequate grass and water for their livestock. In addition, these same historical sources note that there were numerous battles and conflicts among several of these nomadic pastoral communities such as the Rouzhi, Wusun, Pulei and Huns.



### Figure 1.

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Map showing the location of Heigouliang cemetery site, Xinjiang, China (▲ solid triangle), with the star symbol (★) representing the capital of China, Beijing.

To date, 52 tomb burials have been discovered and excavated at the Heigouliang cemetery, and these produced remarkably diverse archaeological features and grave goods that indicate there was social complexity, hierarchy and cultural diversity within the population (Mo, 2010). In addition, most of these tombs contain the skeletons of multiple individuals that were clearly treated differently in the manner in which they were interred, again indicating a fixed social order in the community. The burial owner or owners were always placed inside coffins that were situated in the lowest portion of the tomb. Other individuals, temporarily believed to be slaves/sacrificial victims, were usually found dismembered, and their remains scattered between the fillings of the tomb. In addition, there was great disparity in the types of grave goods that were buried with these individuals. Most of the burial owners were buried with many high-quality objects neatly arranged

around the coffin: weapons, tools, potteries and decorative ornaments, sometimes included exotic items. In contrast, the slaves/sacrificial victims were buried with only common items (personal objects) of poor quality that were scattered about the body (Ren, 2011). In addition, a number of these individuals show evidence of physical trauma from armed conflicts (Wang & Xi, 2009; Wei *et al.*, 2012). All this has been taken as evidence of significant differences between the two groups. Recent ancient DNA evidence supports this possibility with two dominant haplotypes Q1a\* and Q1b found in the population (Li, 2012). The Q1a\* haplotype, which originated from the northeast area of Siberia and is prevalent in northern Asian populations, was detected within both the sacrificial victims and the burial owners. While the Q1b haplotype, which is prevalent in the modern Uyghur population of Xinjiang and closely related to central Asian populations, was only found in the sacrificial victims (Li, 2012; Zhao *et al.*, 2014).

Excavations at the Heigouliang cemetery uncovered numerous farming implements and faunal remains: sheep, goat, cattle, horse and dog buried with the human skeletons. This was taken as evidence in support of the historical sources that the people buried in the tombs were pastorals that were focused on animal husbandry. Plant remains were also uncovered including what are believed to be wheat and millet grains (Xi'en Chang pers. comm.). Unfortunately, there was no systematic study of the zooarchaeological and archaeobotanical remains at this site, so it is unclear what the main diets of these individuals might have been at the Heigouliang cemetery. However, a limited isotopic study published by Zhang *et al.* (2009) of nine individuals from this site revealed the diet was mainly based on C<sub>3</sub> animal protein, but besides this work, little isotopic research has been published on Iron Age sites from the Xinjiang region (Zhang & Li, 2006; Ling *et al.*, 2013; Si *et al.*, 2013). Here, we undertake a larger and more detailed stable isotope ratio study of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) values to reconstruct the diets of the individuals ( $n = 27$ ) buried in the Heigouliang cemetery. In particular, we were keen to examine if there were different dietary patterns that could be related to social status in this population (burial owners versus the sacrificial victims). Unfortunately, very little in the way of animal bones (sheep  $n = 2$ ) could be secured for analysis at this site (most were not saved during the excavations) to use as an isotopic baseline for the human diets. Thus, the teeth from four of the humans were selected for starch grain analysis of the dental calculus to determine the types of plant foods consumed by these individuals and to help complement the lack of the faunal stable isotope results.

## Diet reconstruction with stable isotope ratios and dental calculus

Stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope analysis is a well-established and widely applied method to investigate among other things: subsistence patterns, animal husbandry strategies, health and nutrition, and social status in modern and archaeological humans and animals (e.g. van der Merwe & Vogel, 1978; Schoeninger & DeNiro, 1984; Richards *et al.*, 1998; Fuller *et al.*, 2004; Müldner & Richards, 2005; Choy *et al.*, 2012; Commendador *et al.*, 2013; Quintelier *et al.*, 2014; Cui *et al.*, 2015). Based on the principle, 'you are what you eat',  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of body tissues correspond to the general type of diet consumed, and bone collagen reflects mainly the average protein diet over the lifetime of an individual or animal (Stenhouse & Baxter, 1979; Jørkov *et al.*, 2010). The  $\delta^{13}\text{C}$  results are used to distinguish between food webs based on C<sub>3</sub> (wheat, rice, barely etc.) and C<sub>4</sub> plants (mainly millets) (DeNiro & Epstein, 1978; O'Leary, 1981; Chisholm *et al.*, 1982). Given the predominance of C<sub>3</sub> vegetation

of North China, it is estimated that 20% of dietary protein must come from a non-C<sub>3</sub> source in order for it to be isotopically distinguishable with a  $\delta^{13}\text{C}$  cut-off value of  $-18\text{‰}$  or greater (Pechenkina *et al.*, 2005; Barton *et al.*, 2009). In contrast,  $\delta^{15}\text{N}$  values are used to determine trophic level, because there is an approximate 3–5‰ increase associated with each ascending step in the food chain (e.g. Schoeninger & DeNiro, 1984; Reitsema, 2013). There is a growing trend of applying stable isotope ratios to archaeological sites in China to document the spread of rice, wheat and millet agriculture (Pechenkina, *et al.*, 2005; Hu *et al.*, 2006; 2008; 2009; Barton, *et al.*, 2009; Lanehart, *et al.*, 2011; Atahan, *et al.*, 2011; 2014; Liu, *et al.*, 2012; Liu & Jones, 2014) and to examine animal husbandry patterns (Ma, *et al.*, 2013; Chen, *et al.*, 2014; Hu, *et al.*, 2014). An in-depth discussion concerning the intricacies of stable isotope ratios and how they are used in paleodiet reconstruction is beyond the scope of this work, and readers should consult the following detailed reviews (Schoeninger, 1995; Lee-Thorp, 2008; Reitsema, 2013; Szpak, 2014).

In addition to stable isotope ratio measurements, the complimentary technique of microfossil analysis of dental calculus is important for paleodiet research (e.g. Dudgeon and Tromp, 2012; Buckley *et al.*, 2014; Tromp and Dudgeon, 2015). Microfossil analysis of dental calculus is a relatively new method that has been repeatedly shown to be a reliable approach to discover information about plant food consumption (Henry & Piperno, 2008; Murphy *et al.*, 2013; Horrocks *et al.*, 2014). Briefly, dental calculus forms as a consequence of the crystallisation of calcium phosphate salts within saliva adhering to bacteria that is attached to the surface of a tooth. Microfossils of food particles (e.g. starch grains, phytoliths, pollens and parasites) can be incorporated in dental calculus during calculus deposition (Lieverse, 1999; Henry *et al.*, 2014). The resulting dental calculus is highly mineralized, resistant to diagenetic changes in archaeological contexts and easily recognisable on fossil teeth. Once recovered from the calculus, microfossils can be used as a direct record for the consumption of plant food in humans and animals (White, 1997; Piperno *et al.*, 2000; Henry *et al.*, 2012; Salazar-García *et al.*, 2013; Leonard *et al.*, 2014).

## Materials and methods

### Isotopic analysis

Collagen was isolated from femora of 27 adult humans and two adult sheep (from tomb M5) at the Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, Institute of Vertebrate Palaeontology and Palaeoanthropology, Chinese Academy of Sciences, using the protocol outlined in Richards and Hedges (1999), and the reader is directed to this article for more information. All of the bone specimens represent discrete individuals, and there was no comingling of the remains. The purified collagen was measured at the Environmental Stable Isotope Laboratory, Institute of Environment and Sustainable Development of Agriculture, Chinese Academy of Agricultural Sciences. The mass spectrometer used was an IsoPrime 100 IRMS (Elementar, UK) coupled with Elementar Vario (Elementar, UK). The stable isotope ratio results were analysed as the ratio of the heavier isotope to the lighter isotope ( $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ) and reported as  $\delta$  in parts per 1000 or 'per mil (‰)' relative to the internationally defined standards for carbon (Vienna Pee Dee Belemnite) and nitrogen (ambient inhalable reservoir). In this case, the standards were sulfanilamide, IEAE-N-1 and USGS 24, and for every 10 samples, a collagen lab standard ( $\delta^{13}\text{C}$  value of  $14.7 \pm 0.2\text{‰}$  and  $\delta^{15}\text{N}$  value of  $6.9 \pm 0.2\text{‰}$ ) was also inserted in the run

for isotopic calibration. The measurement errors were less than  $\pm 0.2\text{‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Details regarding the archaeological context of samples are listed in Table 1. Because of the extremely dry climate of the Tianshan area, all of the bone samples produced sufficient amounts of collagen with excellent C:N ratios between 3.2 and 3.4 indicating that the results were indicative of good quality collagen suitable of stable isotope ratio analysis (DeNiro, 1985; van Klinken, 1999).

**Table 1.** Archaeological context and isotopic data of the humans and animals from the Heigouliang cemetery

Sample number	Burial number	Sex	Status	Collagen yield (%)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Carbon content (%)	Nitrogen content (%)
H1	M1:A	Female	Sacrificial victim	15.1	-18.8	12.6	46.7	16.7
H2	M1:B	Male	Burial owner	9.2	-18.5	12.5	46.2	16.8
H3	M2:A	Male	Burial owner	14.3	-18.4	12.2	46.2	16.6
H4	M2:B	Female	Burial owner	8.8	-18.5	13.3	46.7	16.9
H5	M3:A	Male	Sacrificial victim	12.1	-18.7	12.4	40.6	14.7
H6	M3:C	Female	Burial owner	18.3	-18.9	12.7	44.6	16.2
H7	M4:A	Male	Burial owner	10.3	-18.5	12.1	45.4	16.4
H8	M4:B	Female	Burial owner	18.4	-18.5	12.0	45.6	16.5
H9	M5:D	Male	Sacrificial victim	17.0	-18.4	12.2	45.9	16.6
H10	M5:E	Male	Sacrificial victim	17.3	-17.6	13.4	46.2	16.6
H11	M6:A	-	Sacrificial victim	17.0	-19.6	12.5	45.1	16.3
H12	M6:B	Male	Sacrificial victim	17.6	-17.0	12.8	45.6	16.6
H13	M6:C	-	Sacrificial victim	19.7	-18.5	13.8	45.1	16.3
H14	M7:D	Female	Burial owner	9.5	-18.6	11.8	43.8	14.5

H	M	Sex	Role	9.5	-18.6	11.8	42.6	14.5
H14	M7:B	Female	Burial owner	9.5	-18.6	11.8	42.6	14.5
H15	M8:2	–	–	15.9	-18.3	12.1	45.3	16.5
H16	M8:3	–	–	18.3	-18.2	12.0	44.5	16.1
H17	M8:D	–	–	15.1	-18.2	11.8	44.8	16.2
H18	M10:A	Female	Sacrificial victim	17.6	-18.6	11.5	47.2	17.0
H19	M10:B	Male	–	17.6	-18.4	11.9	45.2	16.4
H20	M10:01	–	–	18.8	-18.8	12.3	45.6	16.4
H21	M11	–	Sacrificial victim	15.8	-18.6	11.9	45.4	16.5
H22	M11:X	Female	Burial owner	17.1	-18.7	11.8	46.7	17.0
H23	M11:06	–	Sacrificial victim	19.4	-18.9	11.7	43.7	15.8
H24	M11:08	–	Sacrificial victim	16.2	-18.8	12.8	45.8	16.5
H25	M11:F	Male	Sacrificial victim	15.2	-18.2	13.3	46.0	16.7
H26	M15	–	–	18.0	-18.6	12.8	45.3	16.4
H27	M17	–	–	15.2	-18.6	11.5	47.9	17.3
H28	M5:a	Sheep	Animal	15.3	-18.3	7.9	45.0	16.3
H29	M5:b	Sheep	Animal	13.3	-19.0	7.5	45.6	16.3
H30	M11:D	–	Sacrificial victim	–	–	–	–	–

## Microfossil analysis of dental calculus

To better understand the dietary strategies of the Heigouliang community, four tooth samples from four separate individuals (M5:E, M6:B, M6:C and M11:D) were analysed for microfossil analysis of dental calculus. Before analysis, images of the sampled area on each tooth were taken by a stereoscopic microscope (Nikon Model C-DSS230, Nikon, Japan) to label the thickest part of the calculus. The calculus was then sampled, using a dental scalpel to pick off small flakes into a 5-ml centrifuge tube. A 10% solution of sodium hexametaphosphate (Calgon) was added to each sample to deflocculate the calculus and ease dispersal. After 10 h, the sample was placed in an ultrasonic bath for 20 min then centrifuged (3000 rpm for 10 min), and the supernatant pipetted off.

It was then rinsed twice with distilled water. The remaining sample was mounted on a microscope slide in a 1:2 glycerin/water solution and examined under a compound light microscope equipped with a set of polarising lenses (Nikon Eclipse LV100POL, Nikon, Japan) at 200 times magnification. Each microfossil was counted and photographed at 500 times magnification, and compared with a reference collection of microfossils from modern plants in order to identify it to family, genus or species level where possible. The types and unique forms of the microfossils were compared with modern references, and published literature was also consulted (Piperno *et al.*, 2004; Barton, 2007; Ge, 2010).

## Results and discussion

### Human and faunal isotope data

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results of the humans are listed in Table 1 and plotted in Figure 2. The  $\delta^{13}\text{C}$  values range from  $-19.6\text{‰}$  to  $-17.0\text{‰}$  with a mean value of  $-18.5 \pm 0.5\text{‰}$ . This indicates the diet was predominately  $\text{C}_3$  terrestrial. However, there were two individuals with values greater than  $-18\text{‰}$  that is indicative of some input of  $\text{C}_4$  foods in their diets (Pechenkina *et al.*, 2005; Barton *et al.*, 2009). The  $\delta^{15}\text{N}$  results range from  $11.5\text{‰}$  to  $13.8\text{‰}$  with a mean value of  $12.4 \pm 0.6\text{‰}$ . These values are in agreement with the results ( $\delta^{13}\text{C} = -18.6 \pm 0.6\text{‰}$ ;  $\delta^{15}\text{N} = 13.3 \pm 0.6\text{‰}$ ) from the only other published Iron Age site (Dongheigou) in the eastern Tienshan region (Ling *et al.*, 2013). The two sheep had a mean  $\delta^{13}\text{C}$  of  $-18.7\text{‰}$  and a mean  $\delta^{15}\text{N}$  value of  $7.7\text{‰}$  that indicate that the diet of these animals was nearly entirely  $\text{C}_3$  terrestrial. It is worth noting that the isotopic spacing between the sheep and humans is  $4.7 \pm 0.6\text{‰}$ , and this is within the expected 3–5‰, indicating that domesticated animals, like sheep, were consistent as dietary items for these individuals. This is in agreement with the findings from the archaeological excavations that concluded that, in addition to cattle and dogs, many sheep and goat skeletons were found inside pottery beside the burial owners (Xi'en Chang pers. comm.).



Figure 2.

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$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of humans and animals from the Heigouliang cemetery.

### Starch grain analysis of dental calculus

Because of a lack of fauna results and to supply complementary information concerning plant consumption, teeth from four individuals (M5:E, M6:B, M6:C and M11:D) were analysed for microfossils in dental calculus. A total of four different kinds of microfossils were recovered from the dental calculus of the tooth samples belonging to M11:D and M11:E, including phytoliths (Figure 3a), parasite (Figure 3b), pollen (Figure 3c and d) and starch grains (Figure 3e–g). Here, we mainly focus on the identification of the starch grains because they play a significant role in revealing the types of plants consumed. A total of 14 starch grains were extracted from the four teeth. Six of these were readily identified to two different plant taxa according to diagnostic

morphological features (Yang *et al.*, 2005; Ge, 2010; Yang & Jiang, 2010; Wan *et al.*, 2012; Ma *et al.*, 2014).



### Figure 3.

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Images of the microfossils recovered from the dental calculus of the four selected individuals at the Heigouliang cemetery, Xinjiang, China. (a) Phytolith; (b) parasite; (c and d) pollen; (e1–e4) a starch grain consistent with some members of the *Triticeae*; (f1, f2, g1, g2) starch grains most likely from the caryopsis of a member of the *Poaceae* (a–d, e1, e3, f1 and g1: transmitted light, 500×, scale bars = 20 μm; e2, e4, f2 and g2: cross-polarised light, 500×, scale bars = 20 μm).

The first type of starch grains was characterised as having a flat and circular form with centric and closed hila on their stationary surface. The length of the major axis is between 10.4 and 38.9 μm, while the length of the minor axis is between 8.8 and 36.4 μm. Some of these starch grains have visible lamellae and craters, but no obvious fissures were present in the front view (Figure 3e1 and e2). However, these starch grains were observed to have an oval shape with a longitude fissure from the side view after compression and rotation (Figure 3e3 and e4). When these starch grains were compared with the modern reference collections (Figure 4a1–a4), they were consistent with *Triticeae*, including wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and/or highland barley (*H. vulgare* L. var. *nudum*), and so on (Ge, 2010; Li *et al.*, 2010; Yang and Jiang, 2010; Ma *et al.*, 2014).



### Figure 4.

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Images of starch grains from modern references of *Triticeae* and *Poaceae*. (a1–a4) Starch grain from the *Triticum aestivum*; (b1, b2, c1 and c2) starch grains from *Setaria italica*; (a1, a3, b1 and c1: transmitted light, 500×, scale bars = 20 μm; a2, a4, b2 and c2: cross-polarised light, 500×, scale bars = 20 μm).

The second class of starch grains had a polygonal shape, with centric and open hila, radial fissures and no lamellae (Figure 3f1, f2, g1 and g2). The length of the major axis was between 13.3 and 18.5 μm, and the length of the minor axis was between 12.1 and 16.4 μm. In general, starch grains with polygonal shapes are usually characteristic of seeds from the grass family, *Poaceae*. Comparison with modern millet references (Figure 4b1, b2, c1 and c2) found these starch grains were consistent with foxtail (*Setaria italica*) and common millet (*Panicum miliaceum*) (Yang *et al.*, 2005; Wan *et al.*, 2012).

These findings are in agreement with the archaeological excavations at Heigouliang as well as

other sites such as Kuisu and Ranjiaqu around the Barkol Basin that found carbonised grains of wheat and barley as well as grain-grinding tools (Wang, 1983; Zhang, 1997). According to  $^{14}\text{C}$  dating results, these sites have an age of 2800 BP, indicating that grains like wheat and barley were widely cultivated in the Barkol region before the Heigouliang period (He, 2007). Additionally, the discovery of the millet starch grains in the dental calculus indicates that the two individuals that have  $\delta^{13}\text{C}$  values greater than  $-18\text{‰}$  were consuming these millets, and this is in agreement with the recovery of millet grains from the site. In addition, the remains of foxtail and common millet, as well as processed millet cakes, have also been found at the nearby Iron Age site of Wubao in Kumil, and this indicates that millets were widely available for early Iron Age populations that inhabited the eastern Tienshan region (He, 2007). However, it is not known if these millets were grown at Heigouliang because the cold and dry climate of the Barkol Basin is unsuitable for the growth of this crop (Lü *et al.*, 2000). Thus, millets may have been imported from other regions or only grown during the summer months.

## Diet and social status at Heigouliang

We expected the possibility to find isotopic differences between the burial owners and the sacrificed individuals. However, a *t*-test revealed that there were no differences for either the  $\delta^{13}\text{C}$  ( $p = 0.316$ ) or the  $\delta^{15}\text{N}$  ( $p = 0.385$ ) for the population as a whole with both classes of individuals having nearly identical results (Figure 2). We then proceeded to examine individual tombs to determine if isotopic differences could be correlated with social status. However, the only complete tomb that we had available in which both the burial owner and the slaves/sacrificial victims were present was M11 (Figure 5). The burial owner (M11:X) was a female buried inside of a wooden coffin with numerous high-status grave goods including 17 pieces of pottery including one with an entire skeleton of a baby sheep, two silver earrings, a shell and stone necklace, a bronze drill, a bronze sword, four bronze bells, an iron knife, two bronze mirrors and 16 sheep heads placed inside the coffin. In contrast, the grave goods of the four slaves/sacrificial victims (M11, M11:06, M11:08 and M11:F) included only broken pottery and a broken bronze drill, and these individuals were found at different levels in the burial matrix and were dismembered. This is an indication of differences in social status based on grave goods and the burial treatment of these individuals. However, the isotopic results did not show a clear distinction between these individuals. The burial owner (M11:X) had  $\delta^{13}\text{C}$  ( $-18.7\text{‰}$ ) and  $\delta^{15}\text{N}$  ( $11.8\text{‰}$ ) values that were nearly identical with the two slaves/sacrificial victims M11 ( $\delta^{13}\text{C} = -18.6\text{‰}$ ;  $\delta^{15}\text{N} = 11.9\text{‰}$ ) and M11:06 ( $\delta^{13}\text{C} = -18.9\text{‰}$ ;  $\delta^{15}\text{N} = 11.7\text{‰}$ ). This could suggest that these two individuals possibly lived with the owner for many years before her death because they all have similar diets. However, the other two individuals (M11:08 and M11:F) that were buried with the burial owners both have elevated  $\delta^{15}\text{N}$  ( $12.8\text{‰}$ ,  $13.3\text{‰}$ ) results. It was determined that one of these slaves/sacrificial victims was a male. This might suggest that these individuals were captured warriors or immigrants that would agree with the historical literature concerning this region. However, additional isotopic studies such as sulfur and strontium stable isotope ratios are needed (Beard & Johnson, 2000; Richards *et al.*, 2001; 2003; Bentley, 2006).



Figure 5.

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$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the individuals buried in tombs M6 and M11 at the Heigouliang cemetery, Xinjiang, China.

Additional support for the possibility that the slaves/sacrificial victims might have been captured warriors is found in tomb M6 (Figure 5). In tomb M6, four individuals were discovered, but only three of these could be examined as the fourth skeleton (the burial owner) was lost after excavation (Figure 5). Analysis of the physical anthropology of the three slaves/sacrificial victims (individuals M6:A, M6:B and M6:C) was able to determine that two of these individuals suffered from bone trauma associated with armed conflict (M6:A was too poorly preserved to study) (Wei *et al.*, 2012). In particular, M6:B was found to have two different injuries consistent with stab wounds from sharp weapons (possibly knife or sword) on both the frontal and occipital regions of the cranium that did not show any traces of regrowth (Figure 6a and b). This indicates that these injuries were likely the cause of death for M6:B. The analysis of the M6:C skeleton revealed this individual had chop wounds on the distal side of the right supraorbital margin that were different from the injuries of M6:B, but that these showed traces of regrowth indicating that the individual survived for some period after this injury (Figure 6c). These findings are possible evidence that these individuals were warriors that were captured in battle and used as human sacrifice for the burial owner. The stable isotope results could support this possibility as these three slaves/sacrificial victims all show different stable isotope ratios, with M6:B having the most  $\text{C}_4$ -based diet ( $\delta^{13}\text{C} = -17.0\text{‰}$ ), and M6:A having the most  $\text{C}_3$ -based diet ( $\delta^{13}\text{C} = -19.6\text{‰}$ ) compared with the entire population studied at the Heigouliang site. In addition, while M6:C displays an average  $\delta^{13}\text{C}$  value ( $-18.5\text{‰}$ ), this individual has the highest  $\delta^{15}\text{N}$  value ( $13.8\text{‰}$ ) of the entire Heigouliang dataset. Given the fact that this individual was buried next to the female burial owner, it might be possible that individual M6:C was a captured war prisoner that might have been held with the Heigouliang inhabitants for a longer period (wounds allowed to heal) before his ultimate sacrifice. However, given the small sample size, this is speculative, and more individuals, additional isotopic measurements (sulfur and strontium) and ancient DNA analysis are needed to test this possibility.



## Figure 6.

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Images of identified physical trauma on the bones of individuals from M6 [(a) identified stab wound on the occipital bone of the cranium of individual M6:B; (b) identified stab wound on the frontal bone of individual M6:B; (c) chop wounds on the distal side of the right supraorbital margin of individual M6:C].

According to the results of the Heigouliang population studied here (Figures 2 and 4), the majority of the individuals shared similar dietary patterns regardless of age, sex, status and burial treatment. Given the social and ethnic diversity of this population, this finding is somewhat surprising but could reflect the fact that while variations did exist in the Heigouliang community, these differences were not manifested in the individual subsistence patterns that can be investigated through stable carbon and nitrogen isotope ratio analysis. This could be a result of the fact that Chinese historical sources describe the pastoralists as having life ways largely

revolved around the seasonal migratory management of domestic herding animals (Sima, 1959; Ban, 1962; Fan, 1965). Even in modern times, nomadic pastoralism has been seen as having 'low productivity' and a simple form of organisation compared with the complexity in social structure enabled by agriculture (Shelach, 1999). Thus, the pastoral systems are always relatively fragile and highly variable, operating at a community level and being determined 'according to the risks presented by environmental, social, political and economic interaction among pastoralists and their neighbours' (Koryakova & Epimakhov, 2006; Frachetti, 2008). For our study on the Heigouliang cemetery, different groups of the community were possibly loosely organised, rather than being distinctively stratified; thus they displayed relatively uniform dietary patterns among the majority of the population. Considering that the outlier values of the whole Heigouliang database are from individuals of tomb M6 and individual M5:F, it is possible that the diversity of burial treatment and grave goods are consequences of the interactions of diverse cultural and ethnic groups, rather than social status. Again, future studies on larger sample size looking at the stable isotope ratios of sulfur or strontium could help resolve this question in more detail by looking for migrants in the population, and this is an area of active research (Richards *et al.*, 2003; 2008; Nehlich, 2015).

## Conclusions

Located along the key crossroad connecting east and west, the Iron Age communities of the Tianshan area of Xinjiang are generally referred to as nomadic pastoralists with a high degree of social, economic and ethnic complexity. An isotopic investigation of the humans from the early Iron Age cemetery site of Heigouliang was conducted along with microfossil analysis of dental calculus to reconstruct dietary patterns. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results revealed that the overall diet was based on  $\text{C}_3$  terrestrial protein sources, including sheep, but that some individuals were consuming a mixed  $\text{C}_3/\text{C}_4$  diet. Identifiable starch grains preserved by the dental calculus of the tooth samples show that the  $\text{C}_3$  plant foods included wheat and/or barely, while foxtail millet and/or common millet were the sources of the  $\text{C}_4$  plants. The archaeological remains point to diversity and hierarchy in a turbulent society that had different burial features, grave goods and ethnicities buried in the Heigouliang cemetery. However, at the population level, no dietary differences were detected between the burial owners and the sacrificial victims. Variable isotopic results were found associated with individuals displaying signs of bone trauma, possibly suggesting that some of the sacrificial victims could have been captured warriors that were sacrificed for the burial owners, as described in the historical literature. Finally, it is important to recognise that this study was unfortunately limited in size for the humans and especially for the animals, and it is possible that an examination of a larger and better characterised set of skeletal specimens might reveal the possibility of a more direct link between social diversity and diet in the future, and this is an area of active research.

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