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THIRTY-FOUR YEARS OF STABLE ISOTOPIC ANALYSES OF ANCIENT SKELETONS IN CHINA: AN OVERVIEW, PROGRESS AND PROSPECTS*

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Stable isotope analysis of ancient skeletons has become a routine method and widely used to answer diverse archaeological questions related to the human (animal) diets since the initial study in 1977. However, this study in china is underestimated and much less unknown to international circles considering the infrequent publications in English journals. In this review paper, the research history in China was overviewed shortly and then, the research progresses concerning different research themes, such as the chronological trajectory of human diets, the development and spread of agriculture, the mechanism of animal domestication, human social hierarchy, and so on, were introduced in detail, trying to draw a rough framework of human dietary evolution given the unique Chinese geography and cultures. At last, the potential research directions were also suggested for the future studies.

KEYWORDS: STABLE ISOTOPE ANALYSIS, SKELETON, CHINA, ARCHAEOLOGY, DIETARY RECONSTRUCTION

INTRODUCTION

Since the pioneering study published in 1977 (Vogel and van der Merwe 1977), the stable isotope analysis of archaeological skeletal tissues (bones or teeth) has developed quickly and the methodology has greatly improved, from the study of a single element (C) to multiple elements (C, N, H, O, S, Sr and more), from bulk sampling to sequential sampling and from bulk tissues to specific compounds (Makarewicz and Sealy 2015). Furthermore, the research themes have extended as well, from answering the simple questions on palaeodiets to revealing more complex and broader archaeological subjects (see the reviews of Lee-Thorp 2008; Schoeninger 2014; Makarewicz and Sealy 2015).

Compared to Europe and the Americas, the studies of stable isotope analysis in China have been overlooked, probably due to the infrequent publication in English. This situation means that most international scholars have little idea of Chinese isotopic research, which hinders the potential academic exchange and cooperation between China and other countries. However, China has made incredible developments and achievements in this subject, and has become one of the most important and influential places in the world for archaeometrists to work.

First, in this paper, an overview of the history of stable isotope analysis in China will be provided, and then the main research advances will be narrated in detail according to different research themes. Finally, the future areas of research will be discussed.

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AN OVERVIEW OF PAST ISOTOPIC RESEARCH IN CHINA

In contrast to the first study undertaken in North America by geochemists (Vogel and van der Merwe 1977), the initial isotopic research in China was conducted by specialists in radiocarbon dating. The first isotopic study was published in 1984 by Professors Lianzhen Cai and Shihua Qiu in *Archaeology*, in Chinese (Cai and Qiu 1984). However, it was not until the late 1990s that this study was further developed in China.

In 1998, the principle of carbon and nitrogen stable isotope analysis was reintroduced in the review by Professor Changsui Wang (1998). Later, the progress on how to collect nitrogen gas released by collagen combustion for nitrogen stable isotope analysis was presented by Professors Shihua Qiu and Lianzhen Cai, and Dr Xuelian Zhang, at the Fifth National Symposium on Archaeometry held in Hefei, Anhui, in 1998 and at other national conferences in the early 2000s. In 2000, the first review written in Chinese by the author, mainly introducing the methodology of palaeodietary reconstruction, such as the stable isotopes (C, N, Sr) and trace elements (Sr, Ba), was published (Hu *et al*. 2000). In addition, trace elements (Ba, Sr) and stable isotope (C, N) data of human bones were discussed in the Ph.D. dissertation of the author, who was jointly instructed by Professor Changsui Wang and Professor T. Douglas Price, with additional help from Dr James Burton and Professor Stanley Ambrose (Hu 2002). Shortly afterwards, Professor Xuelian Zhang summarized the palaeodietary research through the domestic and international lens (Zhang 2003) and published the first formal isotopic data (C, N) of humans in a Chinese journal (Zhang and Wang 2003). Therefore, the period from the late 1990s to the early 2000s witnesses the revival of isotopic analysis in China.

In the meantime, the isotopic study of Chinese skeletons also caught the attention of international scholars. Dr Pechenkina, in cooperation with isotopic specialists and Chinese archaeologists, published the first human and animal isotopic data (C, N) in North China in the *Journal of Archaeological Science* (Pechenkina *et al*. 2005). This publication and the following paper (Hu *et al*. 2006) demonstrated the value of isotopic studies in China to the international academic community.

From 2005, and especially from 2010 to the present, isotopic research in China has flourished. In addition to C and N, other stable isotopes, such as H, O, S and Sr, are now being applied to reconstruct human diets, human mobility, the palaeoenvironment and the palaeoclimate. Further, the installation and setting up of stable isotope ratio spectrometers by Peking University in 2012 and the University of Chinese Academy of Sciences in 2014, specifically for archaeological studies, has dramatically improved the research progress and makes it possible to collect large isotopic data sets.

RESEARCH PROGRESS

To date, more than 120 papers on stable isotope analysis of ancient skeletons in China have been published in Chinese and English journals. Although two isotopic reviews have previously been reported (Chen 2014; Zhang 2016), the research progress in China has not been summarized in a systematic way. Here, the research advances are introduced on the basis of different subjects in a general chronological order. Unfortunately, given the focus of the research themes presented here and the space limits, not all isotopic studies in China are included in this review.

Adaptations of modern humans in the Late Pleistocene

In recent years, the discovery of modern human fossils in China has greatly expanded the views on the origin and dispersal of modern humans in East Asia during the Late Pleistocene. However, human dietary diversities and adaptations are less clear, mainly due to the lack of direct evidence, although the broad spectrum of modern human diets in the Late Pleistocene has been widely suggested (Gao 2014).

To date, there has only been one case study using stable isotope analysis to directly investigate human diets in the Late Pleistocene. The stable isotopic data (C, N, S) of the Tianyuan human from Beijing, dated to approximately 40 ka BP, indicate that this individual consumed large quantities of freshwater resources, which might be related to the pressures of increased human population during this time and provides solid evidence of a broad-spectrum dietary revolution in eastern Eurasia (Hu *et al*. 2009a). Unfortunately, no similar studies on human fossils have yet been undertaken, making human dietary adaptations in the Late Pleistocene still less understood.

Agricultural developments and human subsistence patterns during the Neolithic

It is well known that China is the agricultural origin of two crops in the world; that is, rice (*Oryza sativa*) (C_3 plants) and millets (C_4 plants), including foxtail millet (*Setaria italica*) and broomcorn millet (*Panicum miliaceum*). The archaeobotanical studies show that both of these plants might have been domesticated about 10 000 years ago in the Yangtze River Valley (Zuo *et al*. 2017) and the Yellow River Valley (Yang *et al*. 2012), respectively. However, both of these agricultural systems developed along different pathways during the Neolithic (Zhao 2011).

Supplementary to the archaeobotanical evidence, the stable isotope (C, N) analysis of human bone collagen provides direct evidence of human diets and the extent to which humans relied on agricultural products. Carbon isotope ratios can differentiate C_3- or C_4-based foods, while nitrogen isotope ratios can be used to determine the trophic level (Lee-Thorp 2008). Furthermore, the carbon isotope values of collagen mainly reflect the contribution of carbon in food protein (Ambrose and Krigbaum 2003). Since rice and millets belong to C_3 and C_4 plants respectively, the C and N isotope values of human collagen can be used to infer the different trajectories of agricultural developments in China, which include the crops themselves and/or animals fed by the crops and their products.

Due to the good preservation of human skeletons in North China, most previous isotopic studies have concentrated on this region, and these large isotopic data sets are used to decipher details about the development of millet agriculture during the Neolithic.

Currently, there have been no published isotopic data of humans from the early Neolithic (*c*.11 000–9000 BP) in North China. During the Middle Neolithic (*c*.9000–7000 BP), the human isotopic data (C, N) in the Upper, Middle and Lower Yellow River Valley (Hu *et al*. 2008; Barton *et al*. 2009; Atahan *et al*. 2011b) indicate that millet agriculture contributed a little to human subsistence and that humans still relied on hunting and gathering, except in the Liao River Valley in Inner Mongolia, where more positive carbon isotope values were observed in human collagen (Liu *et al*. 2012). However, in the Late Neolithic (7000–5000 BP), during the Yangshao Culture period, quite positive carbon stable isotope values are observed in humans and animals (pig, dog) bones at numerous archaeological sites in North China, covering a large area of the central Yellow River Valley and some regions of the Liao River Valley (X. Zhang 2003, 2006; Pechenkina *et al*. 2005; Cui *et al*. 2006; Lin *et al*. 2010, 2013; X. Zhang *et al*. 2010, 2012a, 2015b, 2016b; Atahan *et al*. 2011a,b, 2014; Guo *et al*. 2011, 2016; Chen *et al*. 2017). This large isotopic shift from the Pre-Yangshao Culture period to the Yangshao Culture period strongly suggests that millet agriculture was highly developed and played an important part in cultural expansion and human migrations. Even in the later periods such as the upper Neolithic (5000–4000 BP) and the earliest dynasty in China, the legendary Xia dynasty (*c*.2070–1600 BC) in the Central Plains, positive carbon isotope values of human and animal bones were found in North China (Chen *et al*. 2016a,b; Dai *et al*. 2016), although complex agricultural system, including millets, rice, wheat, beans and hemp, were also developed at that time (Zhao 2011, 2014).

Considering the acidic soils, which are not suitable for skeletal preservation and the extraction of collagen, the isotopic work in the Yangtze River Valley is not as popular as that in North China and is fragmentary. However, the limited isotopic data available offer a rough framework for understanding the development of rice agriculture and human subsistence strategies in this region.

There is one case study on stable isotope analysis of human bones from the Jiahu site, Henan Province during the Middle Neolithic (9000–7000 BP), which is located within the Huai River Valley (Hu *et al*. 2006). Although the negative carbon isotope values do not directly reflect rice consumption, some of the humans had relatively low nitrogen isotope values, which might be related to the occurrence of rice agriculture (Hu *et al*. 2006). More isotopic studies were conducted during the Late Neolithic (7000–5500 BP) in the Lower Yangtze River Valley. Negative carbon isotope values and high nitrogen isotope values were observed in human bones at several sites in this region (X. Zhang & Wang, 2003; Hu *et al*. 2007; G. Zhang *et al*. 2015a), indicating that humans relied mainly on hunting terrestrial animals and fishing for marine or freshwater resources, which is in agreement with the zooarchaeological results in this region (Yuan 2012). Therefore, rice agriculture in the Yangtze River Valley seemed to not be well developed until at least *c*.6 ka BP, despite the earlier presence of carbonized rice grains and phytoliths (Zhao 2014).

In South China, there have been few isotopic studies relating to the Neolithic, due to the extremely bad preservation of human skeletons. However, the much higher nitrogen isotope values and intermediate carbon isotope values of humans from the sites of Liyundun, Guangdong Province (Hu *et al*. 2010) and Shitanshan, Fujian Province (Wu *et al*. 2016) strongly suggest that humans consumed large quantities of marine resources around 8000–5000 BP.

In summary, the isotopic data of humans during the Neolithic provide direct evidence of agricultural developments and human subsistence strategies in China. Three patterns of human subsistence strategy had existed around 6000 years ago (Hu *et al*. 2010). Among them, only millet agriculture in North China was highly developed at that time, which could account for why the first Chinese dynasty (the Xia dynasty) was later found in the Yellow River Valley region.

The exchange of millets and wheat across Eurasia

During the Bronze Age or even earlier, the whole Eurasian continent witnessed large-scale human migrations, which facilitated the occurrence of food globalization (Jones *et al*. 2016). During this process, millets native to the East were found outside China and in Central Asia and Europe (Miller *et al*. 2016), while the barley and wheat from the Middle East were discovered in China in *c*.4000 BP (Zhao 2015). The Western radiation of millets and the Eastern spread of wheat and barley compose the prehistoric Silk Road connecting the East and the West, almost 2000 years before the formal Silk Road of the Han dynasty (202 BC – AD 220).

The Xinjiang Autonomous Region is located in the heart of the Eurasian continent and is the region that bridges the East and the West. Archaeological evidence from physical anthropological studies, ancient DNA analyses and archaeobotanical studies shows that humans were an admixture of European and Mongolian traits (Gao *et al*. 2015; Wei 2017) and that multiple crops, such as wheat, barley and millets, have been grown together (Yang *et al*. 2014; Jiang *et al*. 2015) since the Bronze Age. Recent stable isotope analyses (C, N) of human bones at many archaeological sites dated to the Bronze Age and Iron Age, covering the eastern, western and central Xinjiang area, indicate that the millets dispersed from the Yellow River Valley and were an important and necessary component of human diets, although humans consumed a large amount of animal protein and relied on animal husbandry (Zhang and Zhu 2011; Lin *et al*. 2013; Qu *et al*. 2013, 2017; Si *et al*. 2013; Wang *et al*. 2016, 2017; Zhang *et al*. 2016a). This isotopic pattern suggests that millets had a positive impact on this human admixture and cultural fusion in Xinjiang. Further, in view of the human stable isotopic data from central Asia and Europe, the concept of an ‘Isotopic Millet Road’ has been put forward to describe the processes of millet radiation from the East to the West (Wang *et al*. 2017).

In addition, the influx of barley and wheat also had important effects on human populations and cultures in China. To date, the early records of barley and wheat are mainly found in the north-western region of China, in *c*.4000 BP (Zhao 2015). Stable isotope ratio analyses (C, N) of human bones in the area indicate that there was a dietary transition for humans from mainly C_4-based foods (Ma *et al*. 2014, 2015; X. Zhang *et al*. 2015b) to C_3-/C_4-based foods (Liu *et al*. 2014, 2016; Ma *et al*. 2016), suggestive of the increased cultivation and consumption of wheat and barley (C_3 plants) by humans (Liu *et al*. 2014, 2016; Ma *et al*. 2016). This dietary shift might be related to the cooler and drier climatic event that occurred around 3600 BP (Ma *et al*. 2016).

However, in the Central Plains, the barley and wheat did not develop as successfully as in the north-western area, although they were present just a little later. Archaeobotanical evidence shows that the agricultural system of multiple crops, including millets, rice, wheat, bean and hemp, had been established as early as during the Longshan Culture period and developed further during the Xia–Shang–Zhou dynasties (2070–221 BC) (Zhao 2014). Nevertheless, the highly positive carbon isotope values of humans suggest that millets still contributed significantly to the diets of humans and domestic animals (Hou *et al*. 2013b,b; Zhang and Zhao 2015; Chen *et al*. 2016a,b; Dai *et al*. 2016; Zhou and Garvie-Lok 2015; Zhou *et al*. 2017). Yet, during the Han dynasty, humans with more negative carbon isotope values have been found, which is indicative of increased consumption of wheat-based foods by humans (Hou *et al*. 2012; Atahan *et al*. 2014; Zhou and Garvie-Lok 2015; Zhou *et al*. 2017). The popularization of wheat agriculture in North China was caused by the government policy to encourage wheat cultivation and the appearance of stone grinding mills to make the crop grains into powder (Hou *et al*. 2012; Zhou *et al*. 2017). Therefore, since the Han dynasty, wheat has become increasingly important in human daily lives and amongst the main resources for making noodles in North China.

Conflicts with and integration of the nomads and the farmers in Chinese history

Geographically speaking, China is located in a relatively closed environment and has mainly been based on an agricultural economy, for self-maintenance, throughout the various historical periods. On the other hand, there had been continuous occupations by nomads living on the Mongolian Plateau since the Zhou dynasty (1046–221 BC). The conflicts with, and integration between, the nomads and the farmers compose the whole of Chinese history and form the basis of the Chinese population as far as broad concepts are concerned.

The middle southern region of Inner Mongolia is the main passageway for the nomads to travel to the Central Plains. There was a long-term coexistence of human populations with different physical characteristics during the Pre-Qin period (Zhang and Zhu 2010). The stable isotope analyses (C, N) of these human populations in the early periods show that millet agriculture substantially contributed to human diets, although their diets were diverse and included much animal protein (Q. Zhang *et al*. 2006; Zhang and Zhu 2012a), while in later periods, human diets became more homogenous and relied more on millet-based foods (Q. Zhang *et al*., 2012a,b; X. Zhang *et al*. in press). This transition of human diets is highly related to the admixture of the nomads and local farmers, and to the continuous northward movements of humans from different kingdoms in the Central Plains during the Eastern Zhou dynasty (Zhang *et al*. in press).

The most representative example of the integration of the nomads and the farmers comes from the Xianbei ethnic group in Chinese history. The Xianbei originally lived in the northern steppe and had a nomadic lifestyle (Zhu 2002). The Tuoba Xianbei, one branch of the Xianbei, rose initially in the northern steppe and founded the Northern Wei dynasty (AD 386–557) in Luoyang, Henan. A comparison of the isotopic data of human bones during different periods indicates a sharp increase in the carbon isotope values and a dramatic decrease in the nitrogen isotope values (G. Zhang *et al*. 2015c). This dietary transition was caused by the wide adoption of millet agriculture and the popularization of the local agricultural cultures in the Central Plains (G. Zhang *et al*. 2015c). Thus, the millet agriculture in the Central Plains seems to play an active role as a catalyst, to stimulate the integration of two ethnic groups and make the Xianbei one of the important contributors to modern Chinese populations.

Domestication and feeding strategies of animals

The domestication of animals offers humans not only meat resources but also close companions in their daily lives. By means of the stable isotope analysis of human and animal bones, the relationship between them can be identified, which sheds further light on the clues with regard to animal domestication and feeding strategies by humans (Hu *et al*. 2009b, 2014).

Stable isotope analysis of dogs at the Dadiwan site, Gansu, dated to the middle Neolithic, indicates that they consumed substantial amounts of C_4-based foods (millets) (Barton *et al*. 2009). The isotopic inconsistency between dogs and wild animals strongly suggests that the dogs were domesticated (Barton *et al*. 2009). Thus, the isotopic record supplies direct evidence of domestication of the dog by at least 8000 years ago in China. Subsequently, numerous studies on humans and dogs dated to different periods spanning from the Late Neolithic to the Three Dynasties have indicated that their isotopic data are quite similar (Chen *et al*. 2012, 2016b, 2017; Hou *et al*., 2013a,b; Zhang and Zhao 2015; Dai *et al*. 2016). This dietary similarity strongly suggests that humans had a close relationship with dogs and that dogs played an important part in human society, such as work and hunting animals, pets and so on.

Although wild boars and domestic pigs are considered omnivores, the isotopic data of domestic pigs can be different from those of wild boars due to the intentional feeding practices by humans during the process of domestication in North China (Guan *et al*. 2007, 2008). The dietary heterogeneity is observed in the pig population at the Yuezhuang site, Shandong (Hu *et al*., 2009b), providing solid support for the domestication of pigs at *c*.8000 BP in North China. In later periods, spanning from the Late Neolithic to the Three Dynasties, most of the pigs in North China had isotopic data similar to those of humans and dogs (Chen *et al*. 2012, 2015a, 2016b,b, 2017; Hou *et al*., 2013a, b; Zhang and Zhao 2015; Dai *et al*. 2016), which confirms the importance of pigs in human society as meat, as well as their close relationship with humans.

It has been modelled that cats also follow the commensal pathways towards domestication (Zeder 2012). Stable isotope analysis of humans and animals at the Quanhucun site, Shaanxi Province, dated to the Late Neolithic, indicated that humans, cats and rodents consumed substantial amounts of millet-based foods (Hu *et al*. 2014). Thus, the mutual relationship between humans and cats had been formed by 5300 BP in China, providing the earliest evidence of a commensal pathway of cat domestication in the archaeological record (Hu *et al*. 2014).

It has been suggested that domestic cattle were imported from the Near East and arrived in north-west China around 4500 BP (Lv *et al*. 2017). Nonetheless, some cattle at archaeological sites in North China, dated to the Yangshao Culture and the Longshan Culture, had relatively positive carbon isotope values, indicative of millet by-products as fodder (Chen *et al*. 2012, 2016b). Therefore, the issue of whether there was an independent origin for the domestication of cattle in China still needs to be tested in the near future.

Human social status and hierarchy

As early as in 1984, Cai and Qiu (1984) first proposed the possibility of discerning dietary differences among human social classes by means of carbon stable isotope analysis, and this research has continued to be conducted until the early 2000s. Wei (2004) discussed on the relationship of elevated nitrogen isotope values of human collagen and their social status. Cui *et al*. (2006) made the first study focusing on distinguishing the dietary disparity among humans with different tomb sizes and burial styles at the Zongri site, Qinghai Province, dated to *c*.5000 BP. Additional systematic studies were carried out in the 2010s: at the Qinglongquan site, Hubei Province, dated to the Shijiahe Culture period (*c*.4600–4000 BP), both wild boars and domestic pigs buried in the same tomb (M148) had different isotopic values, suggesting that they might have been contributed by various families and that the tomb owner could possibly have been a leader or a member of an elite class in human society (Chen *et al*. 2015b). A thorough investigation of dietary diversities among humans with unequal tomb sizes and tomb accessory artefacts was found at the Yinxu site, Henan Province, the former capital of the Shang dynasty (1600–1046 BC), which was directly relevant to human social stratification and different localities (Cheung *et al*. 2017a,b; X. Zhang *et al*. 2017). Recently, a dietary and physical comparison between the males and females in central China during the Eastern Zhou dynasty (770–221 BC) has suggested the gradual decrease of female social status and health in human society (Dong *et al*. 2017).

Other isotopic studies besides C and N

It is well known that bone hydroxyapatite is susceptible to contamination by the burial environment compared to bone collagen (Lee-Thorp 2008). In international circles, the stable isotope analysis (C, O) of bone hydroxyapatite is highly debated, given the uncertainty of retaining the original isotopic signal. This situation is also true of China. To date, only a small number of studies focusing on carbon and oxygen stable isotopes of bone and teeth hydroxyapatite have been reported (Hu *et al*. 2006; Tian *et al*. 2008, 2013; Lanehart *et al*. 2011). Nevertheless, the isotope analysis (C, O) of teeth hydroxyapatite in Chinese animal fossils is widely used to reveal the feeding ecology of these animals.

The first paper on Sr isotope analysis of human bones in China was published in 2008, aiming to understand the human movements at the Jiahu Site, Henan Province (Yin *et al*. 2008). However, it was not until the 2010s that Sr isotope analysis in China expanded. Several Sr isotope analyses of human and animal teeth were undertaken in central and south-western China in order to determine the presence of immigrants in the human or animal populations (Zhao *et al*. 2011b,b, 2012, 2016a, b; Zhang *et al*. 2014). However, in general, this research is not popular in China, possibly due to the complex procedure for extracting Sr and the high cost of measuring Sr isotopes.

In recent years, our team has undertaken continuous studies on the hydrogen and oxygen stable isotope analysis of bone collagen in China, to compare the difference in diets and drinking water of domestic animals (Si *et al*. 2014) and to identify the Dongyi immigrants originating from Shandong at the Xiaoshuangqiao site, Henan Province (Wang *et al*. 2015). In particular, the unique isotopic data (H, O) of bone collagen of the tomb owner at the Shuzhuanglou site, Hebei Province, dated to Yuan dynasty (AD 1206–1368), strongly indicates that he had lived elsewhere, providing evidence to help discover his identity (Cui *et al*. 2015).

Large numbers of sulphur isotopic analyses of bone collagen have only become available after technical improvements in isotopic measurement techniques during the late 2000s (Nehlich 2015). Since the pioneering work on the 'Tianyuan Man', to reveal the consumption of freshwater resources (Hu *et al*. 2009a), only a few studies have been reported on sulphur analysis to identify possible migration of humans in the area, mixed with rice and millet agriculture (Guo *et al*. 2015), and immigrants at the Yinxu site, Henan Province (Cheung *et al*. 2017a). The application of sulphur stable isotope analysis in China is still in the early stages of development.

CONCLUSION AND PROSPECTS

Since the initial study in 1984, stable isotope analysis of ancient skeletons in China has continued over 34 years and great achievements have been made on different research topics. However, we also recognize that China still lags behind the international level in both the methodology and the breadth and depth of research. From the author's viewpoint, some important research directions can be recommended for future studies:

(1) Isotopic fractionation principles specific to Chinese populations. So far, all the isotopic studies carried out in China can be attributed to the application of isotopic fractionation principles that are mainly constructed on the basis of Western dietary habits to Chinese skeletons. Whether isotope fractionation from diets to tissues based on Chinese foods containing large quantities of carbohydrates is distinct from that based on Western diets is still unknown. Thus, controlled experiments on animals fed with specifically Chinese foods are needed to solve this problem.

(2) Human health in the past and up to modern times. Unfortunately, there have been no studies concerning this topic in China using stable isotope analysis. Therefore, more isotopic studies should be carried out in the near future to understand ancient (and modern) human health.

(3) The development of new analytical techniques. As stated just above, the methodology adopted so far has mainly been concentrated on C and N stable isotopes. Unfortunately, this situation is not satisfactory to meet the growing demand for high-resolution isotopic signals. Thus, the development of new sampling procedures and the integration of analytical techniques to the maximum possible extent are urgently needed for future studies.

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