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Technical Note: Light stable isotope analysis of human remains from Nemrik 9, Iraq

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Abstract: *Human remains from the Pre-Pottery Neolithic A are rare and therefore present a precious source for elucidating human lifestyle during a crucial period of subsistence transition. Human bone samples from Nemrik 9, Iraq, were analysed to test their suitability for light stable isotope analysis from collagen. None of the samples yielded signatures that conform to the agreed international standards of quality control applied to the analysis of carbon and nitrogen isotopic ratios. The results are discussed in the context of comparable studies from the Near and Middle East considering the thermal and depositional history at the Nemrik site.*

Key words: stable isotopes; human remains; Nemrik; Iraq; PPNA

Nemrik, a site associated with a Pre-Pottery Neolithic A (PPNA) habitation horizon dated to c. 9500–8500 BC (Kozłowski 2002), is located in the Tigris Valley at the southern rim of the Kurdish Plateau, Iraq. Nemrik 9 has yielded human remains from the PPNA occupation phase and they have been subject to a number of anthropological studies pertaining to skeletal and trace element evidence of subsistence patterns (Molleson 2006; Szostek et al. 2006) as well as to dental characteristics (Szlachetko & Zadurska 2006). Human remains from this time period are still a rarity and maximisation of anthropological information is paramount to understanding the socio-ecology and cultural history of human communities during this important formative phase of sedentary agricultural subsistence. To test whether skeletal remains of this age and provenance are suitable for more sophisticated analytical reconstruction of dietary habits, five specimens of compact bone from human femora were analysed for stable isotope ratios of carbon and nitrogen from bone collagen.

Principles of stable light isotope analysis

Chemical analysis of human remains provides the most immediate access to information about the diet of past individuals and populations. A commonly used method

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employs the analysis of stable light isotope ratios of carbon and nitrogen measured from bone collagen. Isotopes are species of the same chemical element. While their number of protons is identical, they differ in atomic weight due to varying numbers of neutrons in their nucleus and the variants considered here are stable, i.e. not subject to radioactive decay. The standard notations $\delta^{13}C$ or $\delta^{15}N$ refer to the ratios of the heavier to the lighter stable isotope, ^{13}C to ^{12}C and ^{15}N to ^{14}N, respectively. Stable isotope ratios from collagen lend themselves to palaeodietary analysis because they largely reflect protein intake from different foodstuffs (Ambrose 1993). Depending on trophic level, for example plant or animal protein, the isotopic ratio undergoes systematic alterations, a process known as fractionation (Schwarcz & Schoeninger 1991). The mass differences of isotopes, even though small and measured in parts per thousand ('per mil'), influence the reaction kinetics during metabolic processes, which results in the step-wise discrimination against the slightly heavier isotope. Therefore, during the passage from one trophic level to the other or, following the same principle, from one tissue to the other, the end-product gets isotopically lighter, i.e. depleted in the heavier of the two isotopes. Therefore, the value of an isotopic ratio indicates trophic position relative to other individuals and those organisms that supply the food web. Trophic level spacing for carbon typically amounts to 1-3‰, and for nitrogen 3-5‰. Carbon isotope ratios are measured against the internationally agreed baseline ratio of bicarbonate in the PeeDee Belemnite formation (now the Vienna PeeDee Belemnite standard, vPDB), while nitrogen ratios vary against nitrogen in air (Ambient Inhalable Reservoir, AIR). Stable light isotope ratios measured from bone collagen reflect the regular and average diet of the last five to ten years before death (Tieszen & Fagre 1993). In addition to fractionation, origin of foodstuffs and biochemical properties allow further differentiation of dietary intake.

Plants generally follow two major different photosynthetic pathways, depending on whether carbon dioxide is incorporated into a three-carbon (Hatch-Slack cycle) or four-carbon compound (Calvin-Benson cycle) during absorption. Accordingly, there is more ^{13}C carbon in C_4 plants, resulting in less negative values when compared against the reference standard. Isotope ratios therefore permit distinction between these groups of plants, which constitute the vast majority of vegetable matter humans use for cultivation and consumption. C_3 plants comprise cultivars from temperate climates, including common staples such as wheat, rye or barley and most vegetables, while C_4 plants originate from more arid and/or tropical zones, including maize, sugar cane, millet and sorghum, but also native grasses and chenopods. A smaller group of plants, so-called C_3-C_4 intermediates, have developed the ability to switch between the C_3 and C_4 pathways in response to fluctuating environmental conditions, for example water stress or salinized soils (cf. Katzenberg 2008).

Carbon isotopic signatures thus allow the identification of relative amounts of major plant groups contributing to the diet. Carbon derived from animal protein is isotopically different from that derived from plants of the same habitat and as a result, human $\delta^{13}C$ values will be less negative when domestic or wild animals are contributing to the diet in measurable quantities. In the marine biotope, carbon isotopic ratios are generally more enriched and therefore enable the detection of seawater sources, especially when the terrestrial food components are C_3-based. The isotopic ratios of nitrogen in bone collagen best describe protein intake from animal sources due to their much higher protein content compared with plants, reflecting, for example, trophic level effects caused by meat consumption. As with carbon, fractionation occurs in both the terrestrial and marine biotope.

Methods

Sample preparation and measurement of stable isotope ratios

Duplicate samples of c. 500mg weight were taken from cortical bone in human and terrestrial animal specimens, while vertebrae were sampled from fishes. Bone surfaces were cleaned using air abrasion with an aluminium oxide powder to remove adhering soil particles, and then subjected to a modified Longin method (Brown et al. 1988) for collagen extraction: samples are demineralised in 0.5M HCl at 2-5°C and then gelatinised at 72°C for 48h in deionised water adjusted to pH 3, with 0.5M HCl. This process typically took as long as 14 days, but occasionally, extraction times were extended depending on the actual size of the specimen. The extraction mix was filtered using Ezee filter separators (Elkay Laboratory Products, Basingstoke) to remove insoluble materials and then was purified again using Amicon Ultra-4 centrifugal filters (Millipore) to remove contaminants lower than 30,000 nominal molecular weight limit (Brown et al. 1988). The resulting solutions were lyophilised, a sub-sample of 0.4 ± 0.1mg combusted and analysed by Isotope Ratio Mass Spectrometry (Finnigan Delta Plus XL).

Quality control

Methionine standard reference material, with known both $\delta^{13}C$ (-26.6‰) and $\delta^{15}N$ (-3.0‰) values (Elemental Microanalysis, Devon, UK) was measured at regular intervals in tandem with samples of bone collagen to examine the accuracy and precision of analytical methods, together with internal and external certified laboratory standards (e.g. IAEA standards, bovine liver, fish gel etc.; see **Table 1**). Collagen yield, the percentages of carbon and nitrogen, and the C:N ratio were recorded to control for possible effects of diagenetic processes (Ambrose 1993). As a sector standard in conjunction with the sample preparation method employed here, collagen yields

as low as 0.5% were deemed acceptable (van Klinken 1999), however, usually only yields of 1% and higher are considered sufficient to indicate preservation of authentic collagen. For this study, samples that yielded between 0.5 and 1% collagen were considered suspect and any samples with collagen yields below 0.5% were discarded from the analysis to take account of local sediment conditions that could have potentially impaired collagen preservation. Samples not having a C:N ratio between 2.9 and 3.6 (the range known for native bone collagen) (Ambrose 1993) were omitted.

Results and discussion

Isotopic and quality control data are presented in **Table 1**. A comparison with internationally agreed protocols (see above) reveals that, whilst all standards to control for precision and accuracy of the measurements confirm fully satisfactory procedures, none of the human bone collagen samples met the quality control criteria to yield results, which can be used as meaningful indicators of dietary patterns. The weight percent figures for both carbon and nitrogen are below acceptable values and none of the C:N ratios fall within the range of figures known for native bone collagen. It has to be concluded, therefore, that the skeletal remains, at least those that were sampled, have undergone substantial degradation and loss of the organic component of the bone tissue.

Such a negative result, as disappointing as it may be, is not unusual (Weiner & Bar-Yosef 2004). Similar observations were made in comparable circumstances on the Arabian Peninsula (Grupe & Schutkowski 1989), where diagenetic alteration of the bone tissue occurred as the result of the specific depositional environment in desert arid soil, which led to quantitative loss of the organic portion. Analyses of bone samples from several arid sites in ancient Egypt also produced failure of sufficient and stable collagen in a number of cases (Thompson et al. 2005). The mechanism to suggest here is linked to high levels of evapo-transpiration in the microenvironment surrounding the burials. Substantial temperature differences between night and day cause oscillating capillary water transport in the soils and sediments and facilitate hydrolysis of collagen and cleaving of the polypeptide chains (Piepenbrink & Schutkowski 1987), thus allowing for gradual leaching of fragmented proteins from the interred bones. Even though these examples reported on skeletal remains of much younger age (2nd millennium BC) than those from Nemrik, a situation analogous to the concept of thermal age (Smith et al. 2003), by which molecular and structural preservation of a specimen is governed by its thermal history rather than actual age, is likely to apply here. Conditions unfavourable to the preservation of complex biomolecules produce a higher rate of degradation than would be expected from the age of the sample alone. In the case of Nemrik it is the combination of historic age and specific burial conditions,

which result in deterioration at the molecular level despite satisfactory macroscopic preservation.

In contrast, human remains recovered from more favourable depositional conditions in the Near and Middle East have produced valid and meaningful light stable isotope results from bone collagen, as analyses of PPNA material from Nevalı Çori, Turkey (Lösch et al. 2006) or of Middle Bronze Age remains from Sidon, Lebanon

Table 1. Human remains from Nemrik 9 – Results of light stable isotope analysis, including quality controls.

Identifier	Weight (mg)	%N	δ^{15}N	%C	δ^{13}C	C/N	Yield %
			Nemrik				
NK2545a	1.15	0.3	5.23	2.9	-24.05	13.07	0.6
NK2545b	1.02	0.2	7.10	2.5	-23.71	12.81	
NK2549a	1.07	0.1	12.88	1.4	-27.14	13.35	0.3
NK2549b	0.60	0.2	-4.33	1.9	-27.41	13.11	
NK2549a[1]	1.65	0.2	7.80	1.2	-26.05	9.62	0.5
NK2549b[1]	1.07	0.1	-3.81	1.4	-24.81	12.98	
NK3352a	1.07	0.1	-10.96	0.8	-22.79	8.99	0.9
NK3352b	1.21	0.1	4.48	0.8	-17.51	14.30	
NK3357a	1.33	0.1	-20.08	0.3	-18.75	5.65	2.1
NK3357b	1.29	0.0	6.77	0.3	-13.59	9.85	
NK3372a	1.18	0.1	23.53	0.6	-21.71	9.48	0.8
NK3372b	1.34	0.1	16.77	0.7	-25.71	14.32	
			Standards				
Bovine liver	1.25	10.1	7.64	48.6	-21.66	5.64	measured
			7.65±0.25		-21.59±0.25		certified
Fish gel	1.00	22.0	14.13	56.9	-15.63	3.02	measured
	1.00	20.1	14.51	52.0	-15.50	3.02	measured
	1.47	16.9	14.51	43.2	-15.51	2.98	measured
	1.65	16.4	14.44	41.7	-15.37	2.98	measured
	1.34	16.1	14.47	41.2	-15.43	2.99	measured
			14.45		-15.52		certified
IAEA600	0.33	28.3	1.05	49.4	-28.30	2.04	measured
	0.49	28.1	1.30	48.3	-27.72	2.00	measured
	1.03	27.6	1.08	46.8	-27.59	1.98	measured
			1.0±0.2		-27.77±0.04		certified
N2	0.47	19.9	20.70				measured
	0.57	20.2	20.48				measured
			20.41±0.2				certified
IAEA CH7	0.28			83.5	-32.11		measured
					-32.15±0.05		certified

[1] Sample NK2549 was measured twice in duplicate to check for reproducibility.

(Schutkowski & Ogden 2011) demonstrate. Analysis of other tissues, e.g. tooth enamel, from samples with a thermal history likely similar to that at Nemrik have been more successful (e.g. Gregoricka 2013, samples from the 3rd millennium BC) because of the virtual lack of organic matter and the densely packed mineral, resulting in much less diagenetic susceptibility of enamel in comparison with bone.

Conclusion

The general state of preservation of human skeletal remains of extended time depth and in latitudes characterised by unfavourable conditions of aridity and evapo-transpiration renders the survival of biomolecules unlikely. Under less harsh conditions the extraction of collagen and analysis of light stable isotopes from bone even of Pre-Pottery Neolithic date has been successful, but the relatively small corpus of such data from the Middle East testifies to the volatile nature of these endeavours, which are subject to the specific circumstances of the burial environment and the general climatic conditions. More targeted and tissue-specific analyses will have to be employed in future studies to unlock dietary and other lifestyle information from remains of compromised depositional provenance.

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References

Ambrose S.H. (1993), *Isotopic analysis of paleodiets: methodological and interpretive considerations* [in:] "Investigations of ancient human tissues: Chemical analyses in anthropology", K. Sandford (ed.), Langhorne: Gordon & Breach, pp. 1-37.

Brown T.A., Nelson D.E., Southon J.R. (1988), *Improved collagen extraction by a modified Longin method*, Radiocarbon 30:171-177.

Gregoricka L. (2013), *Residential mobility and social identity in the periphery: Strontium isotope analyses of archaeological tooth enamel from southeastern Arabia*, Journal of Archaeological Science 40:452-464.

Grupe G., Schutkowski H. (1989), *Dietary shift during the 2nd millennium BC at prehistoric Shimal*, Oman peninsula, Paléorient 15:77-84.

Katzenberg M.A. (2008), *Stable isotope analysis: a tool for studying past diet, demography and life history* [in:] "The biological anthropology of human skeletons", 2nd edition, M.A. Katzenberg, S.R. Saunders (eds.), New York: Wiley-Liss, pp. 413-441.

Kozłowski, S.K. (2002), *Nemrik: An aceramic village in northern Iraq*, Warsaw: Institute of Archaeology, University of Warsaw.

Lösch S., Grupe G., Peters J. (2006), *Stable isotopes and dietary adaptations in humans and animals at pre-pottery Neolithic Nevalı Çori, southeast Anatolia*, American Journal of Physical Anthropology 131:181-193.

Molleson T. (2006), *Hunters of Nemrik*, Studies in Historical Anthropology 3:5-18.

Piepenbrink H., Schutkowski H. (1987), *Decomposition of skeletal remains in desert dry soil. A roentgenological study*, Human Evolution 2:481-491.

Schutkowski H., Ogden A.R. (2011), *Sidon of the Plain, Sidon of the Sea – Reflections on Middle Bronze Age diet in the Eastern Mediterranean*, Archaeology and History in the Lebanon 34/35:213-225.

Schwarcz H.P., Schoeninger M.J. (1991), *Stable isotope analysis in human nutritional ecology*, Yearbook of Physical Anthropology 43:283-321.

Smith C.I., Chamberlain A.T., Riley M.S., Stringer C., Collins M.J. (2003), *The thermal history of human fossils and the likelihood of successful DNA amplification*, Journal of Human Evolution 45:203-217.

Szlachetko K., Zadurska M. (2006), *Human teeth from Nemrik*, Studies in Historical Anthropology 3:31-91.

Szostek K., Głąb H., Kaczanowski K. (2006), *An analysis of the content of macro- and microelements in the teeth of an Early Neolithic population from Nemrik (Iraq)*, Studies in Historical Anthropology 3:19-29.

Thompson A.H., Richards M.P., Shortland A., Zakrzewski S.R. (2005), *Isotopic palaeodiet studies of ancient Egyptian fauna and humans*, Journal of Archaeological Science 32:451-463.

Tieszen L., Fagre T. (1993), *Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite and soft tissues* [in:] "Prehistoric human bone. Archaeology at the molecular level", J. Lambert, G. Grupe (eds.), Berlin: Springer, pp. 121-155.

Weiner S., Bar-Yosef O. (2004), *States of preservation of bones from prehistoric sites in the Near East: A survey*, Journal of Archaeological Science 17:187-196.

van Klinken G.J. (1999), *Bone collagen quality indicators for palaeodietary and radiocarbon measurement*, Journal of Archaeological Science 26:687-695.