Isotope analysis of incremental human dentine: towards higher temporal resolution

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Abstract

Here we present a novel method which allows the measurement of the stable isotope ratios of carbon (δ13C) and nitrogen (δ15N) from much smaller samples of dentine than previously possible without affecting the quality parameters. The reconstruction of the diet of past populations using isotopic analysis of bone collagen is a well-established tool. However, because of remodelling of bone throughout life, this gives a blurred picture of the diet. The analysis of δ13C and δ15N from tiny increments of dentine utilizes tissue that does not remodel and permits comparison, at the same age, of those who survived infancy with those who did not at high temporal resolution. This new method has been tested on archaeological teeth from two sites: three molar teeth from the 19th Century Kilkenny Union Workhouse Famine cemetery, Ireland; and three from the Anglian (5-7th centuries AD) cemetery at West Heslerton, Yorkshire, England, selected on the basis of their varied preservation. The methods of incremental dentine sectioning described in Beaumont et al (2013)[1] were carried out and a sub-section removed prior to denaturing and lyophilisation. The two sample sets, dentine and collagen from each section, were measured by isotope ratio mass spectrometry. The profiles produced from each of the six teeth studied show close correlation in isotope ratios indicating that demineralized dentine which has not been denatured and lyophilised produces isotope ratios comparable with dentine collagen. This finding allows analysis of extremely small samples of dentine which could previously not be measured using current instruments and methods.

Keywords: Stable isotopes; Palaeodiet; Lyophilization; Microsampling

Introduction

Dietary reconstruction of the diet by measuring the stable isotopes of carbon (δ13C) and nitrogen (δ15N) in human body tissues is a well-accepted part of both modern and archaeological investigations of human behaviour and health. For example, measurements of δ15N in modern human hair have been used to investigate anorexia and severe morning sickness [2, 3] and from archaeological bone and dentine to show changes in diet within a population which can be related to behaviour [4-7]. Early paleodietary studies used collagen extracted from bulk samples of bone and dentine, which gives an average value for the isotopes incorporated during the growth period of the tissue [8, 9]. In teeth, this will represent the period of years during childhood or adolescence, and from bone will reflect up to the last 10 years of life depending on the age of the individual and the element sampled [10].

Recently, due to improvements in instrumentation, researchers have been able to measure smaller samples, and this has allowed the analysis of incrementally-forming tissues such as primary dentine which form and mineralize at a regular rate and do not remodel [11] thus recording the isotopic values from the diet during the period of formation. Small samples taken at intervals which can be related to the age at which a tooth formed have allowed the examination of dietary changes during growth with high temporal resolution [1, 12-17]. However, there are still limits on the size of the samples. One of the limiting factors is the amount of dentine collagen which can be produced by the methods which were established using bone samples, as some of the yield is lost during the processing. Because of the complex, appositional nature of dentine formation, even these sampling strategies have resulted in each increment representing an average of a period of time [11].

In order to improve the yield, thus reducing the size of sample and improving temporal resolution, new methods have been developed. These include the punching out of discs of dentine which have formed before the neo-natal line in deciduous teeth for measurement [18], although the authors found that the quality parameters suffered as a result of the sample size, and the processing of collagen with centrifugation to move contaminants to the bottom of the microtube rather than filtration [1].

Because the latter method necessarily means that the dentine and any contaminants not removed by the centrifugation remain in the sample, and the collagen sample produced was often large enough to provide several duplicates, it was reasoned that demineralised dentine may be measurable by isotope mass spectrometry without being denatured. This study investigates whether this is feasible and if the measurements can be relied upon, even in cases where the dentine preservation is poor.

Materials and Methods

The samples were taken from three permanent second molar teeth (M2) from individuals from the mid-19th-century Kilkenny Union Workhouse (prefix KUW) Famine cemetery, Ireland, and from three permanent molar teeth, an M1, M2 and M3 from individuals from the Early Anglo-Saxon Period (5-7th centuries AD) cemetery of West Heslerton, Yorkshire, England (prefix HP) [19, 20] (Table 1). The KUW teeth were very well-preserved, macroscopically resembling modern, extracted, un-buried teeth (dentine graded as 1 following Montgomery 2002, 121)[21]. The teeth from West Heslerton were chosen to represent the range of tooth preservation found at the site where burials were made on chalk, in ditches and in free-draining sands. The three teeth selected were graded for dentine preservation as 3 (M1 preservation good), 4 (M2 preservation satisfactory) and 5 (M3 preservation poor) [21](see Table 1 and Figure 1).

A single root was removed from each of the permanent human molar teeth sampled. Five of the teeth were cleaned, demineralized and then sectioned using method 2 in Beaumont et al. (2013)[1] to produce 1mm sections. Because of poor preservation, the third molar from West Heslerton was embedded in plaster and sectioned into 2mm sections using method 1 from Beaumont et al. (2013)[1]. Each root section then had a wedge of demineralized dentine removed using a scalpel, thus including a representative portion of the thickness of the main sample and the same averaging of the time of growth of the section (Figure 2). The larger part of the section was denatured by heating at 70°C for 24 hours, centrifuged, frozen and then freeze-dried. 0.5 mg samples were weighed into tin capsules and measured in duplicate. The dentine wedges were weighed, freeze-dried and re-weighed into tin capsules prior to measurement. For some sections there was insufficient tissue to take a wedge and achieve sufficient collagen: these were denatured, lyophilised and measured in order to see the complete trend in the dentine isotopic profile. All samples were measured by combustion in a Thermo Flash EA 1112 and introduction of separated N2 and CO2 to a Finnigan Delta plus XL via a Conflo III interface at the Stable Isotope Laboratory at the School of Archaeological Sciences, University of Bradford. The instrument was calibrated using both laboratory and international standards: the analytical error at 1 standard deviation was ±0.2‰ or better.

The results have been compared visually using graphs showing changes in the dentine δ13C and δ15N profiles over the approximate age of the individual, and in terms of the range of variation between the values of the collagen and dentine samples.

Results

Table 2 shows the results for each dentine section, with the δ13C and δ15N and the C:N ratio reported for each sample of dentine and collagen. The sectioning process commences with sample number 1 with the earliest forming dentine at the enamel dentine junction in the cusp of the tooth

and is numbered consecutively to the apex of the root. The C:N ratios were within the range accepted by van Klinken [22] as being representative of collagen (reference) and the yield for the collagen was always higher than 1.2% recommended by Ambrose [23] because no filtration was carried out. The demineralised dentine samples lost approximately 50% of their mass during freeze-drying.

It can be seen from the profiles for all 6 teeth (Figure 3) that the δ13C and δ15N values for the collagen and dentine from each section are extremely well-correlated, and most are within the reported 1 SD error for the instrument. The largest difference was found for δ15N (3‰) in section 2 of sample HP2BA897 the M1tooth from West Heslerton for which the dentine was graded as 3.

Discussion

The profiles produced from both sample sets resemble those seen in other studies such as Eerkens at al., Beaumont et al. and Sandberg et al. [1, 13, 17] in terms of the patterns of variation seen in both δ13C and δ15N over the growth period of the tooth, all of which used denatured and lyophilised collagen. The close matching of the values measured in the treated and untreated samples from the same sections in this study across a range of dates and dentine preservation states suggests that it would be possible to achieve reliable results from smaller samples of dentine, thus paving the way for improving temporal resolution. It may now be possible to micro-sample based on the incremental developmental lines visible by microscopy within the dentine and achieve more accurate age estimation for the isotopic information gleaned.

Conclusion

The ability to achieve reliable isotopic values from the measurement of smaller samples of human dentine will allow researchers to achieve a temporal resolution for dietary reconstruction which is comparable to that of incremental hair samples, i.e. 2-3 months, and may pave the way to resolve short-term and seasonal variations which were previously lost due to the averaging of the signal. More research is necessary to improve the method, particularly in terms of sampling in the direction of growth rather than across the incremental layers.

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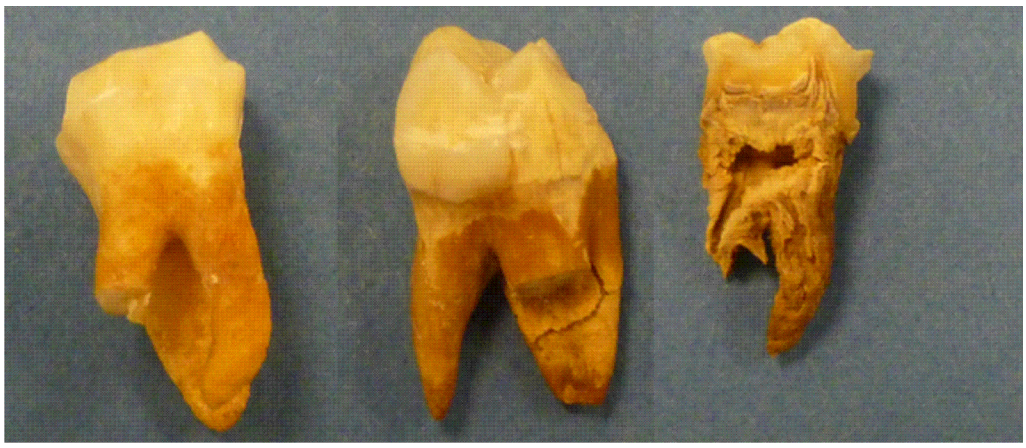

Figure 1. Molar teeth from West Heslerton, from the left HP2BA897 (M1), HP2BA503 (M2), HP2BA903 (M3) showing the range of macroscopic preservation of the dentine. Image: J Beaumont. See Table 1 for details.

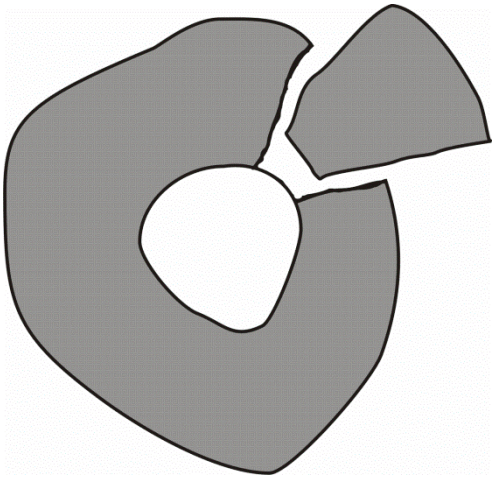

Figure 2. Diagram showing wedge of tissue removed from each demineralized horizontal section of root.

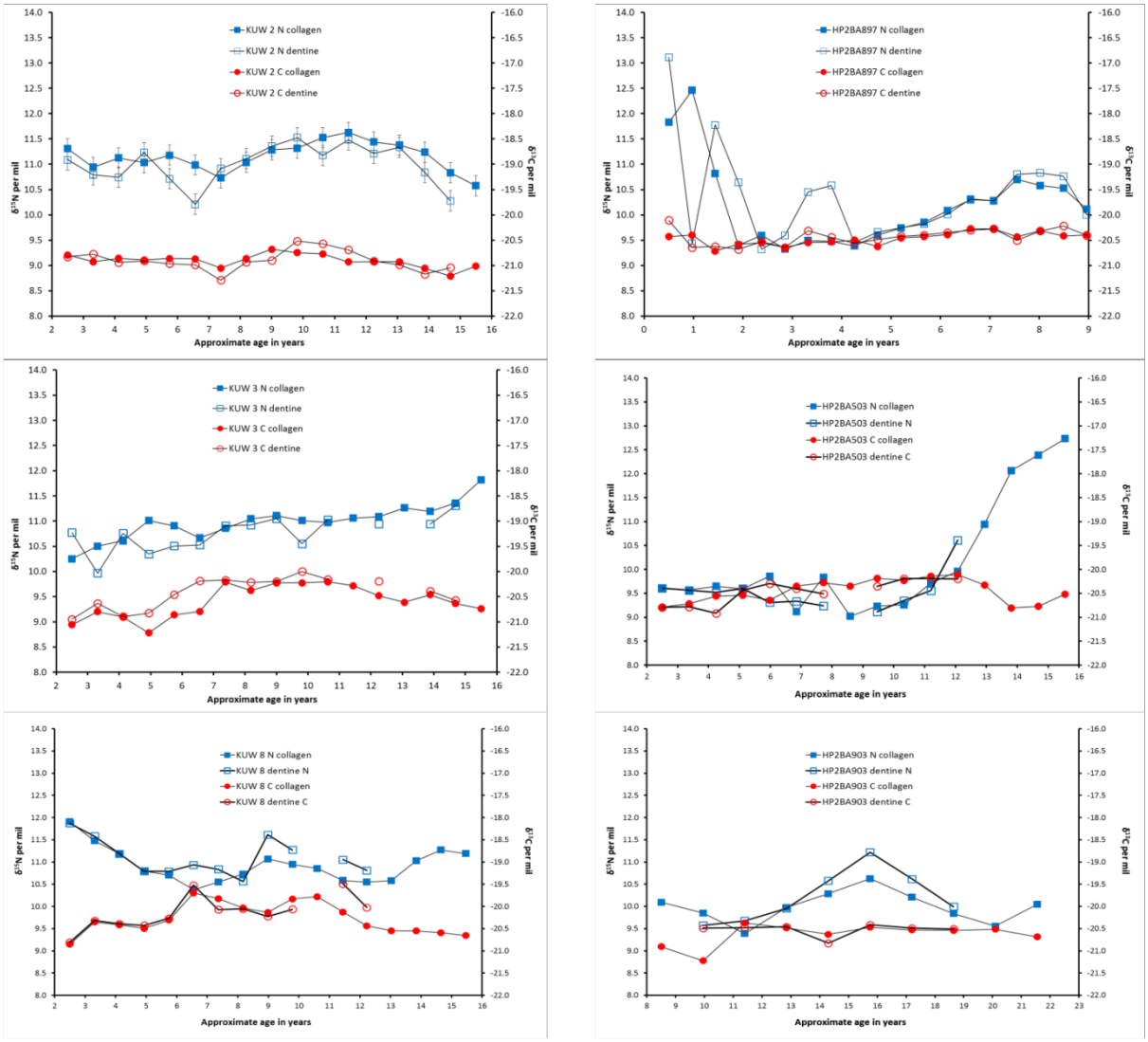

Figure 3. Plots of δ13C and δ15N profiles against approximate age for molar teeth from Kilkenny Union Workhouse and West Heslerton.

Sample number	Site	Tooth type	Dentine preservation score	Description
KUW 2	Kilkenny Union Workhouse	M2	1	Visually indistinguishable from modern
KUW 3	Kilkenny Union Workhouse	M2	1	Visually indistinguishable from modern
KUW 8	Kilkenny Union Workhouse	M2	1	Visually indistinguishable from modern
HP2BA897	West Heslerton	M1	3	Preservation good -
HP2BA503	West Heslerton	M2	4	Preservation satisfactory -
HP2BA903	West Heslerton	M3	5	Preservation poor -

Table 1 Tooth samples and dentine preservation scores (after Montgomery 2002, 121[21])

Sample number	$\delta^{15}N‰$	$\delta^{13}C‰$	C:N		differences $\delta^{15}N‰$	differences $\delta^{13}C‰$
KUW 2 1	11.3	-20.8	3.2		0.2	0.0
KUW 2 2	10.9	-20.9	3.3		0.2	-0.2
KUW 2 3	10.9	-21.0	3.2		0.2	0.0
KUW 2 4	11.0	-20.9	3.2		-0.2	0.0
KUW 2 5	11.2	-20.9	3.3		0.5	0.1
KUW 2 6	11.0	-20.9	3.3		0.8	0.1
KUW 2 7	10.7	-21.1	3.3		-0.2	0.2
KUW 2 8	11.0	-20.9	3.3		-0.1	0.1
KUW 2 9	11.3	-20.7	3.3		-0.1	0.2
KUW 2 10	11.3	-20.7	3.3		-0.2	-0.2
KUW 2 11	11.6	-20.9	3.3		0.5	-0.4
KUW 2 12	11.6	-20.9	3.3		0.1	-0.2
KUW 2 13	11.4	-20.9	3.3		0.2	0.0
KUW 2 14	11.4	-20.9	3.3		0.0	0.1
KUW 2 15	11.2	-21.1	3.3		0.4	0.1
KUW 2 16	10.8	-21.2	3.3		0.6	-0.2
KUW 2 17	10.6	-21.0	3.3			
KUW 2 1 dentine	11.1	-20.8	3.2			
KUW 2 2 dentine	10.8	-20.8	3.2			
KUW 2 3 dentine	10.7	-20.9	3.2			
KUW 2 4 dentine	11.2	-20.9	3.3			
KUW 2 5 dentine	10.7	-21.0	3.3			
KUW 2 6 dentine	10.2	-21.0	3.3			
KUW 2 7 dentine	10.9	-21.3	3.4			
KUW 2 8 dentine	11.1	-20.9	3.2			
KUW 2 9 dentine	11.4	-20.9	3.3			
KUW 2 10 dentine	11.5	-20.5	3.3			
KUW 2 11 dentine	11.2	-20.6	3.4			
KUW 2 12 dentine	11.5	-20.7	3.3			
KUW 2 13 dentine	11.2	-20.9	3.3			
KUW 2 14 dentine	11.3	-21.0	3.3			
KUW 2 15 dentine	10.8	-21.2	3.4			
KUW 2 16 dentine	10.3	-21.0	3.4			
KUW 3 1	10.2	-21.1	3.3		-0.5	-0.1
KUW 3 2	10.5	-20.8	3.2		0.5	-0.2
KUW 3 3	10.6	-20.9	3.2		-0.2	0.0
KUW 3 4	11.0	-21.2	3.2		0.7	-0.4
KUW 3 5	10.9	-20.9	3.2		0.4	-0.4
KUW 3 6	10.7	-20.8	3.2		0.1	-0.6
KUW 3 7	10.9	-20.2	3.2		0.0	0.0

Sample number	$\delta^{15}N‰$	$\delta^{13}C‰$	C:N		differences $\delta^{15}N‰$	differences $\delta^{13}C‰$
KUW 3 8	11.0	-20.4	3.3		0.1	-0.2
KUW 3 9	11.1	-20.2	3.2		0.1	0.0
KUW 3 10	11.0	-20.2	3.3		0.5	-0.2
KUW 3 11	11.0	-20.2	3.2		-0.1	0.0
KUW 3 12	12.7	-17.9	3.1			
KUW 3 13	11.1	-20.5	3.3		0.1	-0.3
KUW 3 14	11.3	-20.6	3.3			
KUW 3 15	11.2	-20.5	3.3		0.3	-0.1
KUW 3 16	11.4	-20.6	3.3		0.1	-0.1
KUW 3 17	11.8	-20.7	3.4			
KUW 3 1 dentine	10.8	-21.0	3.3			
KUW 3 2 dentine	10.0	-20.6	3.3			
KUW 3 3 dentine	10.8	-20.9	3.3			
KUW 3 4 dentine	10.3	-20.8	3.3			
KUW 3 5 dentine	10.5	-20.5	3.3			
KUW 3 6 dentine	10.5	-20.2	3.3			
KUW 3 7 dentine	10.9	-20.2	3.3			
KUW 3 8 dentine	10.9	-20.2	3.3			
KUW 3 9 dentine	11.0	-20.2	3.3			
KUW 3 10 dentine	10.6	-20.0	3.3			
KUW 3 11 dentine	11.0	-20.2	3.3			
KUW 3 12 dentine						
KUW 3 13 dentine	10.9	-20.2	3.4			
KUW 3 14 dentine						
KUW 3 15 dentine	10.9	-20.4	3.4			
KUW 2 16 dentine	11.3	-20.6	3.4			
KUW 8 1	11.9	-20.8	3.3		0.0	0.0
KUW 8 2	11.5	-20.4	3.2		-0.1	0.0
KUW 8 3	11.2	-20.4	3.2		0.0	0.0
KUW 8 4	10.8	-20.5	3.2		0.0	-0.1
KUW 8 5	10.7	-20.3	3.2		-0.1	0.0
KUW 8 6	10.4	-19.7	3.2		-0.6	-0.2
KUW 8 7	10.5	-19.8	3.2		-0.3	0.2
KUW 8 8	10.7	-20.0	3.2		0.2	0.0
KUW 8 9	11.1	-20.1	3.2		-0.5	0.1
KUW 8 10	10.9	-19.8	3.2		-0.3	0.2
KUW 8 11	10.9	-19.8	3.2			
KUW 8 12	10.6	-20.1	3.2		-0.5	-0.6
KUW 8 13	10.6	-20.4	3.2		-0.3	-0.4
KUW 8 14	10.6	-20.5	3.2			
KUW 8 15	11.0	-20.6	3.2			

Sample number	$\delta^{15}N‰$	$\delta^{13}C‰$	C:N		differences $\delta^{15}N‰$	differences $\delta^{13}C‰$
KUW 8 16	11.3	-20.6	3.2			
KUW 8 17	11.2	-20.7	3.2			
KUW 8 1 dentine	11.9	-20.8	3.2			
KUW 8 2 dentine	11.6	-20.3	3.2			
KUW 8 3 dentine	11.2	-20.4	3.2			
KUW 8 4 dentine	10.8	-20.4	3.2			
KUW 8 5 dentine	10.8	-20.3	3.2			
KUW 8 6 dentine	10.9	-19.5	3.2			
KUW 8 7 dentine	10.8	-20.1	3.2			
KUW 8 8 dentine	10.6	-20.1	3.2			
KUW 8 9 dentine	11.6	-20.2	3.2			
KUW 8 10 dentine	11.3	-20.1	3.2			
KUW 8 11 dentine						
KUW 8 12 dentine	11.1	-19.5	3.2			
KUW 8 13 dentine	10.8	-20.0	3.2			
HP2BA897-1	11.8	-20.4	3.2		-1.3	-0.3
HP2BA897-2	12.5	-20.4	3.2		3.0	0.2
HP2BA897-3	10.8	-20.7	3.2		-1.0	-0.1
HP2BA897-4	9.4	-20.6	3.2		-1.2	0.1
HP2BA897-5	9.6	-20.6	3.2		0.3	0.0
HP2BA897-6	9.3	-20.6	3.2		-0.3	0.0
HP2BA897-7	9.5	-20.5	3.2		-1.0	-0.2
HP2BA897-8	9.5	-20.5	3.2		-1.1	-0.1
HP2BA897-9	9.4	-20.5	3.2		-0.1	0.1
HP2BA897-10	9.6	-20.6	3.3		-0.1	-0.1
HP2BA897-11	9.7	-20.5	3.2		0.0	0.0
HP2BA897-12	9.9	-20.4	3.2		0.0	0.0
HP2BA897-13	10.1	-20.4	3.2		0.1	0.0
HP2BA897-14	10.3	-20.3	3.2		0.0	0.0
HP2BA897-15	10.3	-20.3	3.2		0.0	0.0
HP2BA897-16	10.7	-20.4	3.2		-0.1	0.1
HP2BA897-17	10.6	-20.3	3.2		-0.3	0.0
HP2BA897-18	10.5	-20.4	3.3		-0.2	-0.2
HP2BA897-19	10.1	-20.4	3.2		0.1	0.0
HP2BA897-20	9.6	-20.4	3.3		-0.3	-0.1
HP2BA897-1 dentine	13.1	-20.1	3.2			
HP2BA897-2 dentine	9.4	-20.6	3.2			
HP2BA897-3 dentine	11.8	-20.6	3.2			
HP2BA897-4 dentine	10.6	-20.7	3.2			
HP2BA897-5 dentine	9.3	-20.5	3.2			

Sample number	$\delta^{15}N$‰	$\delta^{13}C$‰	C:N		differences $\delta^{15}N$‰	differences $\delta^{13}C$‰
HP2BA897-6 dentine	9.6	-20.6	3.2			
HP2BA897-7 dentine	10.5	-20.3	3.2			
HP2BA897-8 dentine	10.6	-20.4	3.3			
HP2BA897-9 dentine	9.5	-20.6	3.3			
HP2BA897-10 dentine	9.7	-20.5	3.2			
HP2BA897-11 dentine	9.7	-20.4	3.2			
HP2BA897-12 dentine	9.8	-20.4	3.2			
HP2BA897-13 dentine	10.0	-20.3	3.2			
HP2BA897-14 dentine	10.3	-20.3	3.2			
HP2BA897-15 dentine	10.3	-20.3	3.2			
HP2BA897-16 dentine	10.8	-20.5	3.2			
HP2BA897-17 dentine	10.8	-20.3	3.2			
HP2BA897-18 dentine	10.8	-20.2	3.2			
HP2BA897-19 dentine	10.0	-20.4	3.3			
HP2BA897-20 dentine	9.9	-20.3	3.3			
HP2BA503-1	9.6	-20.8	3.2		0.0	0.0
HP2BA503-2	9.6	-20.7	3.2		0.0	0.1
HP2BA503-3	9.6	-20.6	3.2		0.1	0.4
HP2BA503-4	9.6	-20.5	3.2		0.0	-0.1
HP2BA503-5	9.9	-20.6	3.3		0.6	-0.3
HP2BA503-6	9.1	-20.3	3.3		-0.2	0.1
HP2BA503-7	9.8	-20.3	3.2		0.6	0.2
HP2BA503-8	9.0	-20.3	3.2		-0.1	0.0
HP2BA503-9	9.2	-20.2	3.2		-0.1	0.0
HP2BA503-10	9.3	-20.2	3.2		-0.3	0.0
HP2BA503-11	9.7	-20.1	3.2		-0.9	0.1
HP2BA503-12	10.0	-20.1	3.2		-1.5	0.4
HP2BA503-13	10.9	-20.3	3.2			
HP2BA503-14	12.1	-20.8	3.2			
HP2BA503-15	12.4	-20.8	3.3			
HP2BA503-16	12.7	-20.5	3.3			
HP2BA503-1 dentine	9.6	-20.8	3.2			
HP2BA503-2 dentine	9.6	-20.8	3.2			
HP2BA503-3 dentine	9.5	-20.9	3.2			
HP2BA503-4 dentine	9.6	-20.4	3.2			
HP2BA503-5 dentine	9.3	-20.3	3.2			
HP2BA503-6 dentine	9.3	-20.4	3.3			
HP2BA503-7 dentine	9.2	-20.5	3.3			
HP2BA503-8 dentine	9.1	-20.4	3.2			
HP2BA503-9 dentine	9.4	-20.2	3.2			
HP2BA503-10 dentine	9.6	-20.2	3.2			

Sample number	$\delta^{15}N$‰	$\delta^{13}C$‰	C:N		differences $\delta^{15}N$‰	differences $\delta^{13}C$‰
HP2BA503-11 dentine	10.6	-20.2	3.3			
HP2BA503-12 dentine	11.5	-20.5	3.2			
HP2BA903-1	10.1	-20.9	3.4			
HP2BA903-2	9.9	-21.2	3.4		0.3	-0.7
HP2BA903-3	9.4	-20.4	3.2		-0.3	0.1
HP2BA903-4	10.0	-20.5	3.2		0.0	0.0
HP2BA903-5	10.3	-20.6	3.4		-0.3	0.2
HP2BA903-6	10.6	-20.5	3.3		-0.6	-0.1
HP2BA903-7	10.2	-20.5	3.2		-0.4	0.0
HP2BA903-8	9.8	-20.5	3.2		-0.2	0.0
HP2BA903-9	9.6	-20.5	3.3			
HP2BA903-10	10.0	-20.7	3.3			
HP2BA903-2 dentine	9.6	-20.5	3.2			
HP2BA903-3 dentine	9.7	-20.5	3.2			
HP2BA903-4 dentine	10.0	-20.5	3.2			
HP2BA903-5 dentine	10.6	-20.8	3.5			
HP2BA903-6 dentine	11.2	-20.4	3.3			
HP2BA903-7 dentine	10.6	-20.5	3.3			
HP2BA903-8 dentine	10.0	-20.5	3.3			

Table 2. δ13C and δ15N values and C:N ratios for incremental dentine and collagen samples from Kilkenny Union Workhouse and West Heslerton, and differences between the samples. Analytical error is shown as vertical bars on the δ15N profile of KUW 2.