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CHILDHOOD DIET: A CLOSER EXAMINATION OF THE EVIDENCE FROM DENTAL TISSUES USING STABLE ISOTOPE ANALYSIS OF INCREMENTAL HUMAN DENTINE*

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Incremental dentine analysis utilizes tissue that does not remodel and that permits comparison, at the same age, of those who survived infancy with those who did not at high temporal resolution. Here, we present a pilot study of teeth from a 19th-century cemetery in London, comparing the merits of two methods of obtaining dentine increments for subsequent isotope determination. Covariation in $\delta^{13}C$ and $\delta^{15}N$ values suggests that even small variations have a physiological basis. We show that high-resolution intra-dentine isotope profiles can pinpoint short-duration events such as dietary change or nutritional deprivation in the juvenile years of life.

KEYWORDS: FAMINE, LONDON, DENTINE, STABLE ISOTOPES, HIGH RESOLUTION, CARBON, NITROGEN, JUVENILE

INTRODUCTION

The isotope ratios of carbon and nitrogen in archaeological bone collagen have been used extensively to investigate past human diet. Several studies have employed isotope methods to detect variations in the timing of weaning in order to assess biocultural behaviours such as birth spacing (Katzenberg and Herring 1996; Wright and Schwarcz 1999; Dupras and Tocheri 2007; Jay *et al*. 2008; Prowse *et al*. 2008). Earlier isotope studies of weaning age relied on differences in bone collagen values from individuals of different age at death—particularly infants and juveniles (Jay *et al*. 2008; Nitsch *et al*. 2011), but this approach means that non-survivors are targeted. More importantly, it can only provide a very general indication of weaning age, because bone is a dynamic tissue that is constantly remodelled (Hedges *et al*. 2007). In contrast to bone, primary dentine does not remodel (Nanci 2003) and therefore has the potential to provide a more tightly time-bound archive related to food and drink ingested during growth. The timing of the development of human teeth (Hillson 1996; AlQahtani 2009) and the direction and rate of growth of the dentine are well established (Dean and Scandrett 1995; Nanci 2003), so that accurate age

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ranges may be attributed to each sample. Incremental sampling that follows the known pattern of dentine development on faunal samples has produced a high-resolution isotopic record of dietary and metabolic changes, allowing the comparison of seasonal dietary variations (Balasse *et al*. 2001; Kirsanow *et al*. 2008; Wiedemann-Bidlack *et al*. 2008). Since different teeth develop at different times of life, the time scale can be expanded by inter-tooth comparison, where it is possible to measure multiple teeth from an individual. However, the amount of processed collagen required for mass spectrometry has meant that the time resolution available from a single human tooth has been relatively poor, because of their small size. Early attempts produced three or four subsamples of permanent human teeth that represent an average value from three or more years of life (Fuller *et al*. 2003). However, sampling of dentine at much higher resolution is possible, as shown in a recent study by Eerkens *et al*. (2011), where 5–10 increments were obtained for the first permanent maxillary molars (M1s) of individuals in order to examine weaning patterns amongst Californian hunter–gatherer–foragers.

The development of a method for high-resolution dentine sampling offers the opportunity to investigate childhood dietary variation in individuals who survived childhood, thus avoiding the need to consider abnormal physiological factors prior to death (Wood *et al*. 1992). Developing teeth will only be present in individuals who died in childhood. However, a significant advantage of incremental dentine analysis of teeth with incomplete root development is that an isotope profile can be obtained from tissue that was developing at the time of death. This offers the opportunity to compare the dentine profiles of individuals who died with the profiles of similar-aged children who survived into adulthood, and thus investigate dietary factors that may have impacted on the health of the child.

The main challenge for detecting such short-term changes in dentine is the ability to sample it in a manner that is sensitive to the developmental trajectory and reflects time-constrained periods, while also delivering enough material for isotope analysis. Thus, before describing the experimental results, we first discuss dentine structure and development.

Dentine development

Human dentine is secreted and mineralized in a two-phase process, whereby the odontoblasts secrete an initial dentine matrix, or predentine, which is then mineralized by the deposition of short (20–100 nm) crystals of carbonate hydroxyapatite within the collagen fibre matrix. The rate of dentine secretion in a permanent tooth is relatively consistent at 4–6 μm per day throughout the cuspal areas of permanent teeth (Dean and Scandrett 1995). The retreating odontoblasts move away from the enamel/dentine junction (EDJ), producing a layer of newly secreted predentine (Hillson 1996). The mineralizing front follows the same path 10–20 μm behind, suggesting that the dentine is secreted and fully mineralized in approximately 3–8 days.

The formation of root dentine begins from the cement/dentine junction (CDJ) and proceeds at a rate of 1.3–1.5 μm per day. The rate changes, rising to the same rate as that for the cuspal dentine within the bulk of the root, and then reducing again to 1.3 μm per day as the odontoblasts approach the pulp chamber (Dean and Scandrett 1995). As the odontoblasts move towards the pulp chamber, they leave tissue within the dentinal tubules throughout the thickness of the dentine, maintaining a network for nutrients. The tubules follow the path of dentine formation and are S-shaped rather than linear (Dean and Scandrett 1995; Nanci 2003) (Fig. 1). However, sampling that follows the curve of the visible dentinal tubules may not greatly improve the resolution of the signal, because mineralization proceeds from the EDJ or CDJ towards the pulp chamber. Consequently, even if a single layer of dentine between dentinal tubules is sampled, it

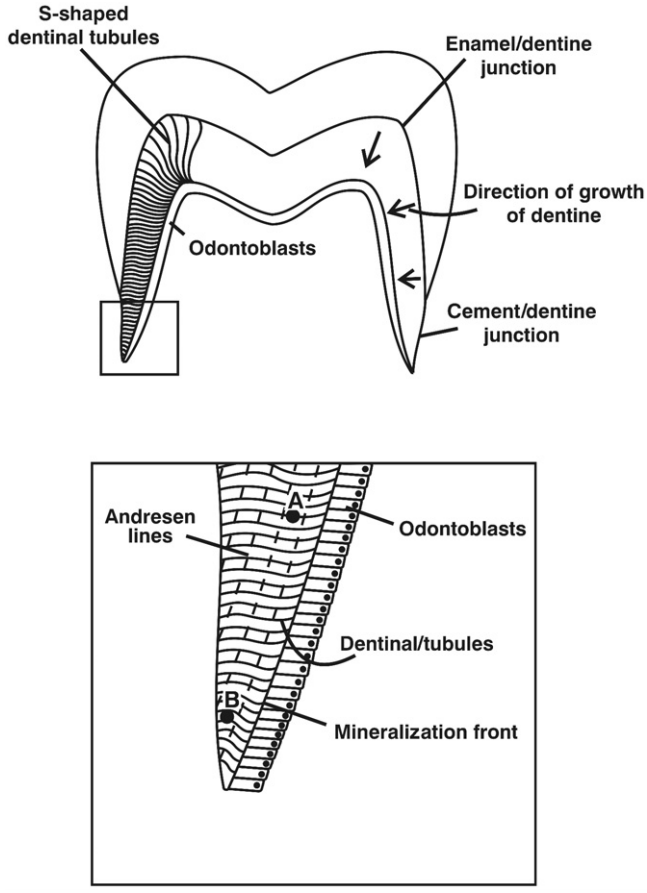

Figure 1 *A diagram showing the direction of dentine development in a human molar tooth, the relationship between Andresen bands and the mineralizing front, and points A and B within the same Andresen band.*

will contain dentine secreted by each odontoblast in the layer over a period of time, which still produces an average value. At a rate of 3–5 μm per day, this could be 100–150 days depending on the thickness of the dentine between EDJ /CDJ and the pulp chamber. The Andresen bands (represented by dashed lines in Fig. 1) record diurnal changes in the position of the secreting front (and therefore the mineralization front) of the odontoblasts. It can be seen from Figure 1 that points A and B are within the same Andresen band, and mineralized at the same time, although separated by a number of dentinal tubules. Sampling along the Andresen bands would represent the ideal, but is technically difficult because these are only visible with microscopy and a longitudinal ground section, and the resulting samples would be too small for isotope analysis.

Tissues that are laid down after the primary dentine has formed should be considered when designing a sampling strategy. Cementum is a mineralized tissue that is part of the periodontium. From the middle third of the root to the apex, it has the potential to remodel over time, becoming thicker around the root apex (Nanci 2003). Secondary dentine is laid down very slowly throughout life by the odontoblasts on the wall of the pulp chamber in permanent teeth after the tooth is fully developed. Tertiary dentine is produced at specific sites on the pulpal wall in response to

damage on the dentine surface such as wear or caries (van Rensburg 1987; Nanci 2003). The dentinal tubules of older individuals become occluded over time by the deposition of intratubular dentine, causing dead tracts seen as root translucency in the apical area (Nanci 2003). The impact of these minor tissues on dentine collagen isotope results is expected to be minor relative to the bulk of the tissue and may be avoided altogether by appropriate sample selection and preparation. For instance, the thin cementum layer can be removed during any mechanical cleaning of the tooth root surface and secondary dentine may be avoided through selecting, where possible, caries-free and unworn teeth, where such deposits should be minimal, or by reaming out the pulp cavity before sampling. As the root develops, it will grow in thickness towards the pulp chamber as well as in length (Fig. 1). Comparison of the most recently mineralized edge of a developing tooth with the same section of a fully developed tooth may run the risk of ignoring this thickening, but because each increment represents an average over a period of time, should not affect the general trends seen.

MATERIALS

The samples consisted of teeth from eight 19th-century individuals from the Lukin Street cemetery in London. Results were obtained for four second permanent maxillary molars (M2) and five first permanent maxillary molars (M1) (Table 1). The second molars and two of the first molars were sectioned using method 1; and two of the first molars using method 2. The sex of the individuals and the developmental stage of the tooth, where applicable, are shown in Table 2.

This pilot study uses incremental sampling of dentine from a population for which documentary and archaeological evidence suggests that there will be dietary differences between individuals and also during the growth of teeth within an individual. The individuals were from the Cemetery of the Catholic Mission of St Mary and St Michael (Lukin Street), London, excavated in 2004 by the Museum of London Archaeology (MOLA). The cemetery was in use for 11 years (1843–54) over the period of the Great Irish Famine (1846–50), and documentary and epigraphic evidence strongly suggests that it may contain first-generation Irish migrants (Powers 2008). Given the timing and the Irish surnames traceable to rural districts most affected by the Famine, the population could include rural Irish who had migrated to London during the Famine. In the first half of the 19th century, the rural population of Ireland was almost completely reliant on the potato as the major source of their calories, with very little else other than buttermilk, and oats when the potato was out of season (Clarkson and Crawford 2001, 79). During the Famine, relief food in the form of maize imported from the United States ('Indian Meal') was supplied by the English from March 1847 (O'Gráda 1999, 123) for a period of approximately 2 years. For many, it was the only source of nutrition (Clarkson and Crawford 2001, 121). In a general sense, we would expect that the carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios of collagen from the tissues of an individual eating the restricted Irish potato-based diet should be different from those related to the varied London diet, which often included fish (Tames 2003, 27). Importantly, maize is a C_4 plant with $^{13}C/^{12}C$ ratios distinct from plants using the C_3 pathway of carbon fixation (Smith and Epstein 1971) (i.e., all the most economically important plants in Britain at this time); thus this transient dietary change should be detectable in the developing teeth of affected children. Furthermore, it has been shown that nutritional stress can cause a rise in the nitrogen isotope ratios in an individual's hair (Fuller *et al*. 2004; Mekota *et al*. 2006). Such episodes of dietary change may be detectable in discrete dentine increments—unlike bone collagen, which will change over time. The aim of this study was to identify Famine survivors who were buried in London, and it is possible that some of these individuals are recording nutritional distress in

Table 1 *Isotope data and collagen quality indicators for dentine sections from teeth from Lukin Street, Tower Hamlets*

Sample number	$\delta^{13}C$	$\delta^{15}N$	*%C*	*%N*	*C:N*
Method 1					
Luk 1567 M2 1	−19.1	14	39.5	13.9	3.3
Luk 1567 M2 2	−19.6	13.1	40.8	14.7	3.2
Luk 1567 M2 3	−19.9	12.8	40.9	14.8	3.2
Luk 1567 M2 4	−20.1	12.6	41.9	15.2	3.2
Luk 1567 M2 5	−20.2	12.7	40.9	14.8	3.2
Luk 1567 M2 6	−20.3	12.5	41.4	15	3.2
Luk 1567 M2 7	−20	12.4	41.4	15.1	3.2
Luk 1567 M2 8	−20.2	12.2	35.1	12.8	3.2
Luk 1567 M2 9	−20.6	11.7	41	15	3.2
Luk 1459 M1 1	−19.8	13.1	40.1	14.6	3.2
Luk 1459 M1 2	−20.1	12.5	40.6	13.8	3.4
Luk 1459 M1 3	−19.9	12.3	40.3	14.8	3.2
Luk 1459 M1 4	−20	12.1	41.2	15	3.2
Luk 1459 M1 5	−19.7	12	40.8	15	3.2
Luk 1459 M1 6	−19.5	11.9	39.3	14.4	3.2
Luk 1459 M1 7	−19.4	11.7	43	15.9	3.2
Luk 1459 M1 8	−19.5	11.7	42.3	15.5	3.2
Luk 1459 M1 9	−19.4	11.7	41	15.4	3.1
Luk 1459 M1 10	−19.6	11.6	40.6	14.5	3.3
Luk 1459 M1 11	−19.6	11.4	40	14.7	3.2
Luk 1459 M1 12	−19.7	11.3	40	14.8	3.2
Luk 1459 M1 13	−19.8	11.1	40.8	15	3.2
Luk 1459 M1 14	−19.8	11.1	42.5	15.6	3.2
Luk 1459 M1 15	−19.9	11	40.8	14.8	3.2
Luk 1459 M1 16	−20	11.3	40.7	14.5	3.3
Luk 1459 M1 17	−19.7	11.4	40.7	15	3.2
Luk 1459 M1 18	−19.9	11.3	41.2	15	3.2
Luk 1459 M2 1	−18.8	14	38.3	14	3.2
Luk 1459 M2 2	−18.9	13.2	38.2	14.2	3.1
Luk 1459 M2 3	−19.3	12.9	36.3	13.2	3.2
Luk 1459 M2 4	−19.5	12.7	36.3	13.2	3.2
Luk 1459 M2 5	−19.7	12.4	36.1	13.1	3.2
Luk 1459 M2 6	−19.7	12.2	40.9	14.9	3.2
Luk 1459 M2 7	−19.7	12.1	36.6	13.4	3.2
Luk 1459 M2 8	−19.9	11.7	36.3	13.1	3.2
Luk 1459 M2 9	−20	11.8	37.3	13.6	3.2
Luk 1459 M2 10	−20	11.8	33.9	12.4	3.2
Luk 1459 M2 11	−20	11.7	36	13.2	3.2
Luk 1459 M2 12	−19.8	11.7	34.1	12.3	3.2
Luk 1459 M2 13	−19.8	11.6	35.9	13	3.2
Luk 1459 M2 14	−20.3	11	31.3	11.2	3.3
Luk 1404 M1 1	−19.7	12.5	38.4	13.8	3.2
Luk 1404 M1 2	−19.6	12.6	38.6	13.9	3.2
Luk 1404 M1 3	−19.7	12.6	39.9	14.4	3.2
Luk 1404 M1 4	−19.7	12.4	40.9	14.7	3.2
Luk 1404 M1 5	−19.6	12.4	37.6	13.7	3.2
Luk 1404 M1 6	−19.5	12.1	38.6	14	3.2

Table 1 (*Continued*)

Sample number	$\delta^{13}C$	$\delta^{15}N$	*%C*	*%N*	*C:N*
Luk 1404 M1 7	–19.6	11.7	39.8	14.6	3.2
Luk 1404 M1 8	–19.5	11.2	40.4	14.8	3.2
Luk 1404 M1 9	–19.6	10.9	38.7	14.2	3.2
Luk 1404 M1 10	–19.6	10.8	37.9	13.9	3.2
Luk 1404 M1 11	–19.7	10.8	36.1	13.3	3.2
Luk 1404 M1 12	–19.6	11.3	38	13.9	3.2
Luk 1404 M1 13	–19.7	11.3	38.8	14.1	3.2
Luk 1404 M2 1	–19.4	12.1	40	14.6	3.2
Luk 1404 M2 2	–19.3	12.2	39.9	14.6	3.2
Luk 1404 M2 3	–19.5	12.4	42.6	15.6	3.2
Luk 1404 M2 4	–19.6	12.6	38.5	14.2	3.2
Luk 1404 M2 5	–19.7	12.6	40.4	14.8	3.2
Luk 47 M1 1	–20	12.3	39.3	12.9	3.5
Luk 47 M1 2	–20.4	12.5	40.6	14.8	3.2
Luk 47 M1 3	–20.1	11.8	40.5	14.5	3.3
Luk 47 M1 4	–20	11.7	41.8	15.2	3.2
Luk 47 M1 5	–20	11.7	36	13	3.2
Luk 47 M1 6	–20	11.8	62.5	22.7	3.2
Luk 47 M1 7	–20	11.9	40.3	14.7	3.2
Luk 47 M1 8	–19.9	11.9	35.9	13	3.2
Luk 47 M1 9	–19.9	11.9	33.5	11	3.5
Luk 47 M1 10	–19.9	11.9	37.2	13.5	3.2
Luk 47 M1 11	–19.8	12	36.9	13.4	3.2
Luk 47 M1 12	–19.8	12	40	14.5	3.2
Luk 47 M1 13	–19.9	12.2	39.7	14.1	3.3
Luk 47 M1 14	–19.8	12	39.2	14.1	3.2
Luk 47 M1 15	–19.9	12.1	40.6	14.8	3.2
Luk 47 M1 16	–20	12.1	41.1	15	3.2
Luk 47 M2 1	–20	12.3	40.6	14.5	3.3
Luk 47 M2 2	–20.4	12.5	41.7	14.9	3.3
Luk 47 M2 3	–20.1	11.8	42	15	3.3
Luk 47 M2 4	–20	11.7	41	14.6	3.3
Luk 47 M2 5	–20	11.7	41.5	14.9	3.3
Luk 47 M2 6	–19.4	12.3	41.3	14.9	3.2
Luk 47 M2 7	–20	11.9	42.5	15.3	3.3
Luk 47 M2 8	–19.9	11.9	39.5	14.2	3.3
Luk 47 M2 9	–20.7	11.9	40.7	14.6	3.3
Luk 47 M210	–19.9	11.9	41.2	14.8	3.3
Luk 47 M2 11	–19.8	12	41.2	14.7	3.3
Luk 47 M2 12	–19.8	12	40	14.4	3.2
Luk 47 M2 13	–19.9	12.2	40.1	14.2	3.3
Luk 47 M2 14	–19.8	12	38.7	13.8	3.3
Luk 47 M2 15	–19.9	12.1	35.7	13.2	3.2
Luk 47 M2 16	–20	12.1	40.5	14.6	3.2

Table 1 (*Continued*)

Sample number	$\delta^{13}C$	$\delta^{15}N$	*%C*	*%N*	*C:N*
Method 2					
Luk 1212 M1 1	–19.7	15.4	59.6	21.8	3.2
Luk 1212 M1 2	–20.4	13.1	66.4	24.4	3.2
Luk 1212 M1 3	–20.9	11.2	66.4	24.6	3.2
Luk 1212 M1 4	–21.5	10.4	69.6	25.7	3.2
Luk 1212 M1 5	–21.3	10	73	26.9	3.2
Luk 1212 M1 6	–21.2	9.7	56.7	20.9	3.2
Luk 1212 M1 7	–20.9	9.4	59.2	22	3.1
Luk 1212 M1 8	–20.1	9.6	61.1	22.4	3.2
Luk 1212 M1 9	–19.5	10	58.5	21.7	3.2
Luk 1212 M1 10	–19.1	10.7	76.5	28.3	3.2
Luk 259 M1 1	–19	13.4	42	15.8	3.1
Luk 259 M1 2	–18.8	15.8	41	15.4	3.1
Luk 259 M1 3	–18.9	15.6	40.7	15.3	3.1
Luk 259 M1 4	–18.9	15.2	42.1	16	3.1
Luk 259 M1 5	–19.1	13.9	41.5	15.8	3.1
Luk 259 M1 6	–19.3	13.5	43.7	16.7	3.1

The collagen yield for all samples was in the range of 15–20% by weight.

Table 2 *Lukin Street tooth samples and the sectioning method used, age and sex (as determined by osteological and epigraphic data) and stage of tooth development (after Moorees* et al. *1963*)

Sample number	*Tooth type*	*Sectioning method*	*Sex*	*Age (years)*	*Developmental stage*
Luk 1567	M2	1	F	36–45	AC
Luk 1404	M1	1	F	18–25	AC
Luk 1404	M2	1	F	18–25	AC
Luk 47	M1	1	U	12–17	AC
Luk 47	M2	1	U	12–17	Rt3/4
Luk 1459	M1	1	F	12–17	AC
Luk 1459	M2	1	F	12–17	Rt3/4
Luk 259	M1	2	M	1–5	Rt1/2
Luk 1212	M1	2	M	6–11	Rt1/2

the dentine shown by a rise in the nitrogen isotope ratios within the early-forming dentine, and by a rise in the carbon isotope ratios if maize had been introduced into the diet. The restricted potato-based diet should be distinguishable from that of individuals who had grown up in London through the use of a combination of both carbon and nitrogen isotope ratios.

METHODS

Two approaches for sampling human tooth dentine for high-resolution stable light isotope analysis were undertaken for comparison. The two methods used explore the practical issues involved in obtaining an accurately measured sample with minimal loss of tissue and maximum yield.

Each method utilized incremental sampling along the tooth axis, following the direction of growth of the root towards the apex.

Method 1 was developed to allow the sampling of accurately measured increments based on size to establish what yield of collagen could be obtained, and is a refinement of the work by Fuller *et al*. (2003). Initially, the increments were 1.5 mm for the first tooth sampled; section widths were reduced on subsequent teeth to establish the smallest feasible increment for sufficient yield of collagen for measurement. Sections were taken on successive teeth at 1.25 mm, 1.0 mm and finally 0.75 mm. It was found that some of the 0.75 mm increments from near the base/apex of the roots had collagen yields that were too low to allow duplicate or even single analyses. For all subsequent teeth sampled, a 1 mm increment was used and this produced sufficient collagen to allow duplicate analyses in all cases.

Method 2 was based on the work of Kirsanow *et al*. (2008), demineralizing the dentine before sectioning, but in this study using a 1 mm increment to ensure sufficient collagen for duplicate measurement. Collagen produced from each sample was measured by isotope ratio mass spectrometry (IR–MS) to establish the nitrogen and carbon stable isotope ratios.

Method 1: embedding prior to sectioning

Surface debris was removed from each tooth by air abrasion. The palatal root and the corresponding portion of coronal dentine were sectioned using a diamond saw in a straight, motor-driven dental handpiece, and the overlying enamel removed using dental burs and saws. Instruments were cleaned between samples with 4M nitric acid. The dentine was embedded in a block of a 50:50 mixture of dental plaster and dental stone measuring 1 cm × 1 cm × 10 cm, with the root apex to the end of the block. The block was then sectioned transversely using a Buehler Isomet slow-speed saw fitted with a micrometer gauge, an abrasive wafering blade and a cooling water bath. Accurately measured sections were produced throughout the length of the dentine, starting from the root apex. The dentine slices were then removed from the plaster, and placed in labelled microtubes. In order to achieve sufficient collagen for duplicate analyses, a minimum dentine weight of 10 mg was required before demineralization.

Method 2: sectioning after demineralization

Each tooth was cleaned by air abrasion before being sectioned. Using a diamond saw in a dental handpiece as above, the palatal root of the tooth and the corresponding crown was removed. As much as possible of the enamel was carefully removed, leaving the EDJ intact. The complete dentine section was demineralized (see method below). The demineralized dentine section retained its original shape, and was divided into transverse samples at 1 mm intervals by hand using a sterile scalpel and optical loupes, measured by metal ruler along its length. In these teeth, the sectioning commenced from the coronal dentine horn (i.e., the opposite direction from method 1). Each sample was labelled for identification before processing. In order for each dentine section to produce sufficient collagen for duplicate measurements, a minimum weight after demineralization of 2.5 mg was required.

Collagen extraction and isotope analysis

We applied the same method for collagen extraction and isotopic analysis to all samples. Collagen was prepared from the dentine sections using a protocol based on the modified Longin

method (Brown *et al*. 1988; O'Connell and Hedges 1999). The samples, after sectioning in method 1, and prior to sectioning in method 2, were demineralized in 0.5M hydrochloric acid at about 4°C. The pre-cut sections demineralized quickly, within 7–10 days, while the whole roots took 21–28 days. The demineralized sections were rinsed with deionized water and placed in sealed microtubes with a pH 3 hydrochloric acid solution at 70°C for 24 h to denature the collagen. No filtration was carried out, although any debris at the base of the microtubes was removed after centrifugation. The samples were frozen and then freeze dried.

Samples were measured in duplicate by combustion in a Thermo Flash EA 1112 and introduction of separated N_2 and CO_2 to a Delta plus XL via a Conflo III interface. Laboratory and international standards were interspersed throughout each analytical run. The results are expressed using the delta notation in parts per thousand (per mil or ‰) relative to the international marine limestone PDB standard for carbon and Ambient Inhalable Reservoir (AIR) for the nitrogen as follows:

$$\delta^{15}\mathrm{N} = R_{\mathrm{sample}} / R_{\mathrm{standard}} - 1,$$

where R is the isotope ratio $^{15}\mathrm{N}/^{14}\mathrm{N}$ in this case (Coplen 2011). The error for the carbon and nitrogen isotope ratio measurements determined from repeated measurement of international and laboratory standards is ±0.2‰, 1 s.d.

Determining the time of life represented by each section

For all permanent teeth, crown initiation occurs when the ameloblasts and odontoblasts begin to move away from the EDJ secreting enamel and dentine matrix, respectively. If the dentine within a permanent tooth is forming at about 3–5 μm per day (Dean and Scandrett 1995), then it will take at least 200 days for the dentine to reach a thickness of 1 mm. This means that the first 1 mm sample of crown dentine represents at least the first 9 months of dentine formation. Thus the isotope values will be an average from the date of crown initiation over approximately 9–12 months. For this reason, each sample is shown as representing the mid-point of the age during which the section would form; for example, the first section for the M1s is shown as representing 0.5 years. The length of the roots of the same tooth varies between individuals (Hillson 1996). In this pilot study, sampling widths were used regardless of the actual length of the root. This has required calculating an approximate age for each section based on the developmental ages at commencement and completion of the dentine, the actual length of the root and the number of sections. For example, using the AlQahtani Atlas (2009), the age at which an M2 begins to form is approximately 2.5 ± 0.5 years, and the age at apex closure is 15.5 ± 0.5 years. Thus the tooth takes approximately 13 years to fully form. Where a fully formed tooth measures 15 mm from the tip of the coronal dentine to the apex, and 1 mm increments were cut, each increment represents 1/15th of 13 years. The midpoint for each age range was used as the value for the *x*-axes. Where 0.75 mm increments were cut, and with a longer tooth with more sections, the temporal resolution is higher. For samples where the tooth was still developing, the stage of development at death was assessed using the descriptions of Moorees *et al*. (1963) and the average age at that stage established from AlQahtani (2009). The number of years taken to reach that stage (i.e., average age at death – average age at initial tooth formation) was then divided by the number of increments obtained to achieve an estimate of the age for each increment. Where two teeth were sampled from the same individual, it was assumed that any variation in developmental age would be the same for both teeth.

RESULTS

While many dentine increments were small, they nonetheless produced sufficient collagen for duplicate measurements of optimal-weight samples normally required for the instrument; that is, 0.5 mg. All the C:N ratios were within the range of 3.1–3.5, indicating that collagen of acceptable quality had been recovered (van Klinken 1999). Any covariation of $\delta^{13}C$ and $\delta^{15}N$ is therefore not caused by the measurement of less than optimal collagen in the IR–MS. The collagen yield from the dentine samples from both methods and both molars was in the range of 10–19% by weight before demineralization. There was no correlation between the method used and the overall collagen yield.

Luk 1567 M2: covarying $\delta^{13}C$ and $\delta^{15}N$

The carbon and nitrogen isotope ratios of the incremental dentine samples from Luk 1567 M2 are presented in Figure 2. This second molar contains dentine that formed between 2.5 and 15.5 years of age and demonstrates differences in isotope ratios throughout the period of growth of the tooth. Although the absolute $\delta^{13}C$ range is less than 2‰ (per mil) and $\delta^{15}N$ up to 3‰, both profiles vary sequentially and increase gradually over time, showing a strong positive linear correlation ($r^2 = 0.9$).

Figures 3–7 display the results from all the other teeth sampled. Isotope ratios are plotted on the *y*-axis and the age at formation on the *x*-axis, starting with the earliest-forming dentine on the

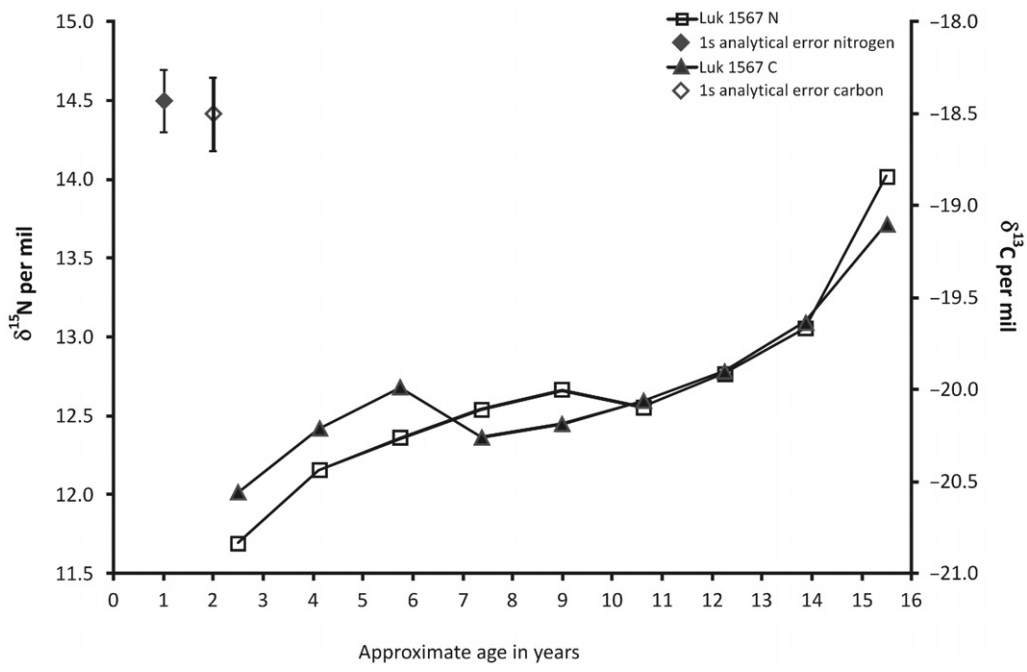

Figure 2 *$\delta^{13}C$ and $\delta^{15}N$ values of dentine sections against age for Luk 1567, second maxillary permanent molar, from Lukin Street, Tower Hamlets.*

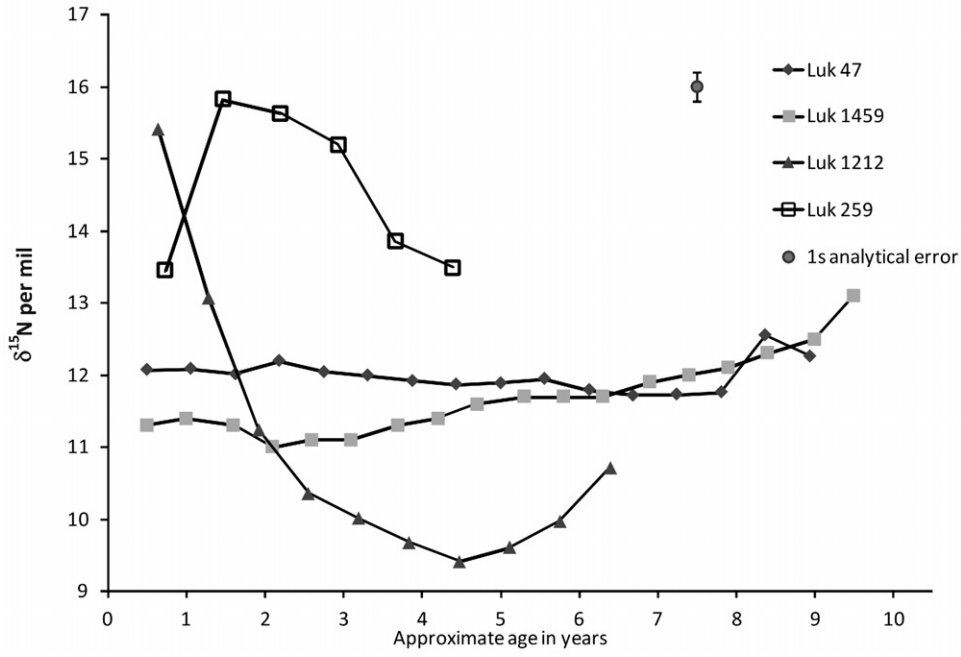

Figure 3 *$\delta^{15}N$ values against age for dentine sections of first permanent molars for four individuals from Lukin Street, Tower Hamlets.*

left-hand side of the plot. Temporal profiles for $\delta^{13}C$ and $\delta^{15}N$ were produced from all teeth, spanning a minimum of 7 years for Luk 259 up to a maximum of 13 years for Luk 47.

Luk 1459, Luk 47, Luk 1212 and Luk 259 M1s

Figures 3 and 4 represent the variations in $\delta^{15}N$ and $\delta^{13}C$ for first molars from four individuals from Lukin Street. Two were produced by using sectioning method 1 (Luk 47 and 1459) and two by using method 2 (Luk 1212 and 259). Luk 1212 and 259 were still developing at the time of death. The data from both methods produced profiles that show variations in the isotope ratios throughout the life of each individual. Luk 1212 and Luk 259 demonstrate greater changes over time, especially for $\delta^{15}N$ (Fig. 3). For Luk 259, $\delta^{15}N$ increases by 2.5‰ in the first year, followed by a gradual drop of 2.5‰ over the next 3 years, which continues until the death of the individual. For Luk 1212, $\delta^{15}N$ falls by 6‰ over the first 4 years, and then rises by 1‰ in the 2 years before death. Luk 259, Luk 1459 and Luk 47 have profiles that show $\delta^{13}C$, while varying by up to 0.5‰, becoming gradually lower with age. For Luk 1212, the $\delta^{13}C$ values decrease initially from –19.7 to –21.5‰, but then rise over time, reaching their highest level at the age of 6.5 years, at –19.1‰ (Fig. 4).

M1 and M2 pairs: extending the temporal range

The results for three individuals (Luk 1404, Luk 1459 and Luk 47) are presented in Figures 5, 6 and 7, respectively. These plots show that $\delta^{13}C$ and $\delta^{15}N$ vary throughout the dentine increments

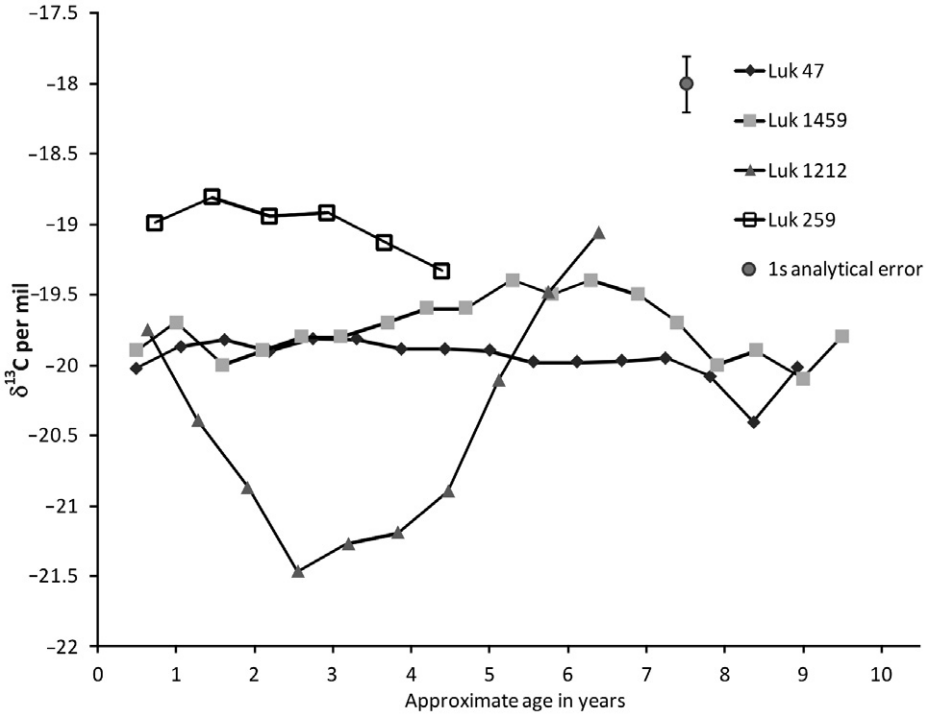

Figure 4 *$\delta^{13}C$ values against age for dentine sections of first permanent molars for four individuals from Lukin Street, Tower Hamlets.*

of these teeth, and the temporal range can be extended by sampling two teeth from the same individual with overlapping developmental ages. Where the dentine sections overlap in date, the isotope ratios are very close, in most cases within analytical error.

DISCUSSION

Both methods showed that it was possible to produce sufficient good-quality collagen to obtain duplicate $\delta^{13}C$ and $\delta^{15}N$ measurements from very small (approximately 10 mg) samples of dentine. Samples of this size permit high-resolution intra-dentine profiles to be obtained over a range of up to 13 years of childhood from a single tooth, and more when sequentially forming teeth are combined.

Eerkens *et al*. (2011) recently used a similar technique to investigate weaning in a group of Californian hunter–gatherers. Their M1 profiles are remarkably similar to those in this study. In their study, the sampling widths (and hence the period reflected) varied from crown to root: this appears to have been to maximize the amount of collagen produced for measurement. We used the same width of increment throughout each tooth to maintain clarity of the temporal resolution. A 1 mm section appears to be a reasonable choice for producing sufficient collagen from well-preserved teeth. Another difference is in the wear patterns: we used 19th-century samples with very little wear, while the samples used by Eerkens *et al*. (2011) have lost the earliest-forming tissues due to heavy occlusal wear: where tissue is missing this will need to be taken into

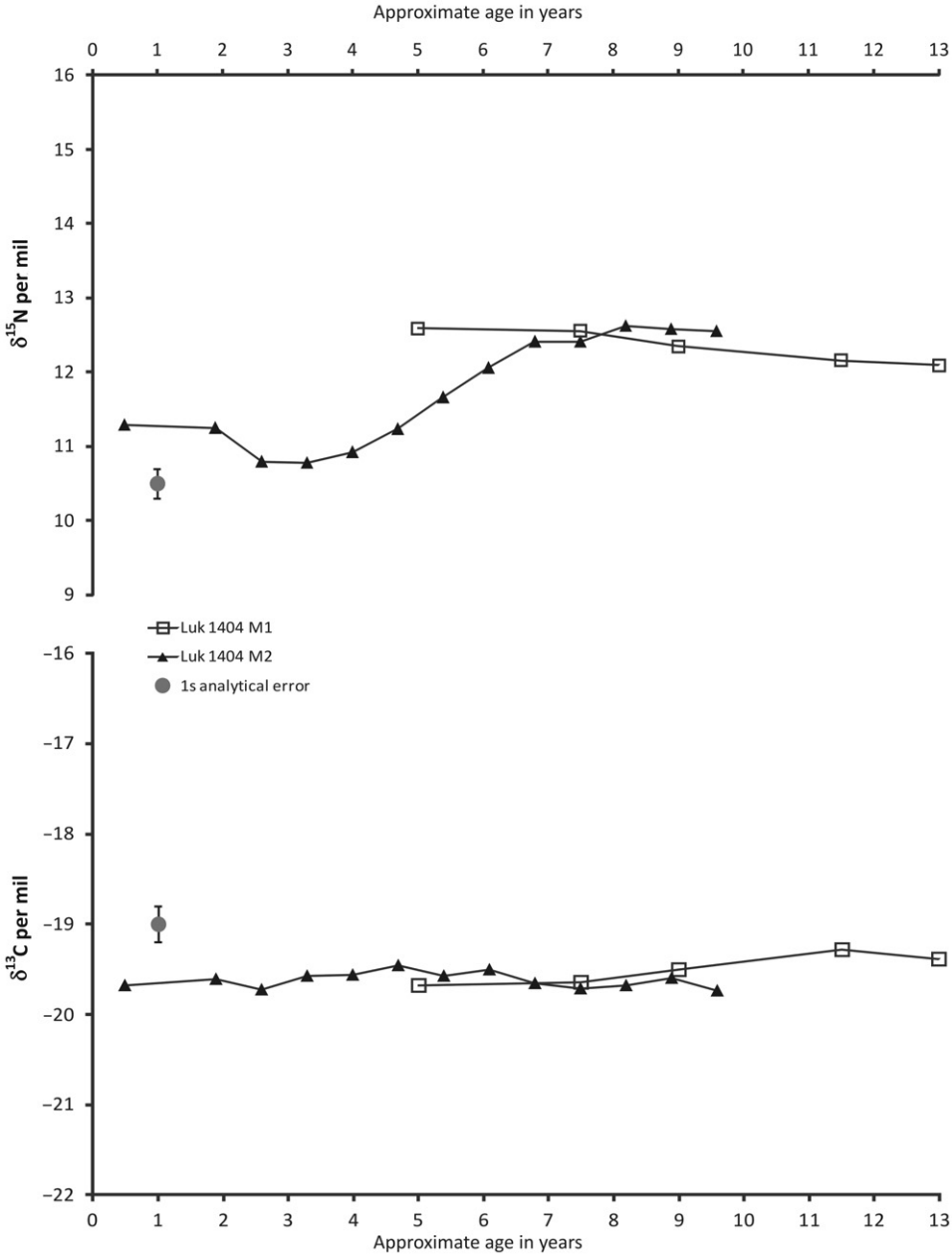

Figure 5 *$\delta^{13}C$ and $\delta^{15}N$ values against age for dentine sections for Luk 1404, first and second permanent maxillary molars, from Lukin Street, Tower Hamlets.*

account in the interpretation of the results in relation to the average age assigned to each dentine increment, especially when addressing weaning.

In this study, all the teeth where development was complete (i.e., the apex closed) show similar-shaped profiles, regardless of the absolute isotope ratios. This would suggest that there

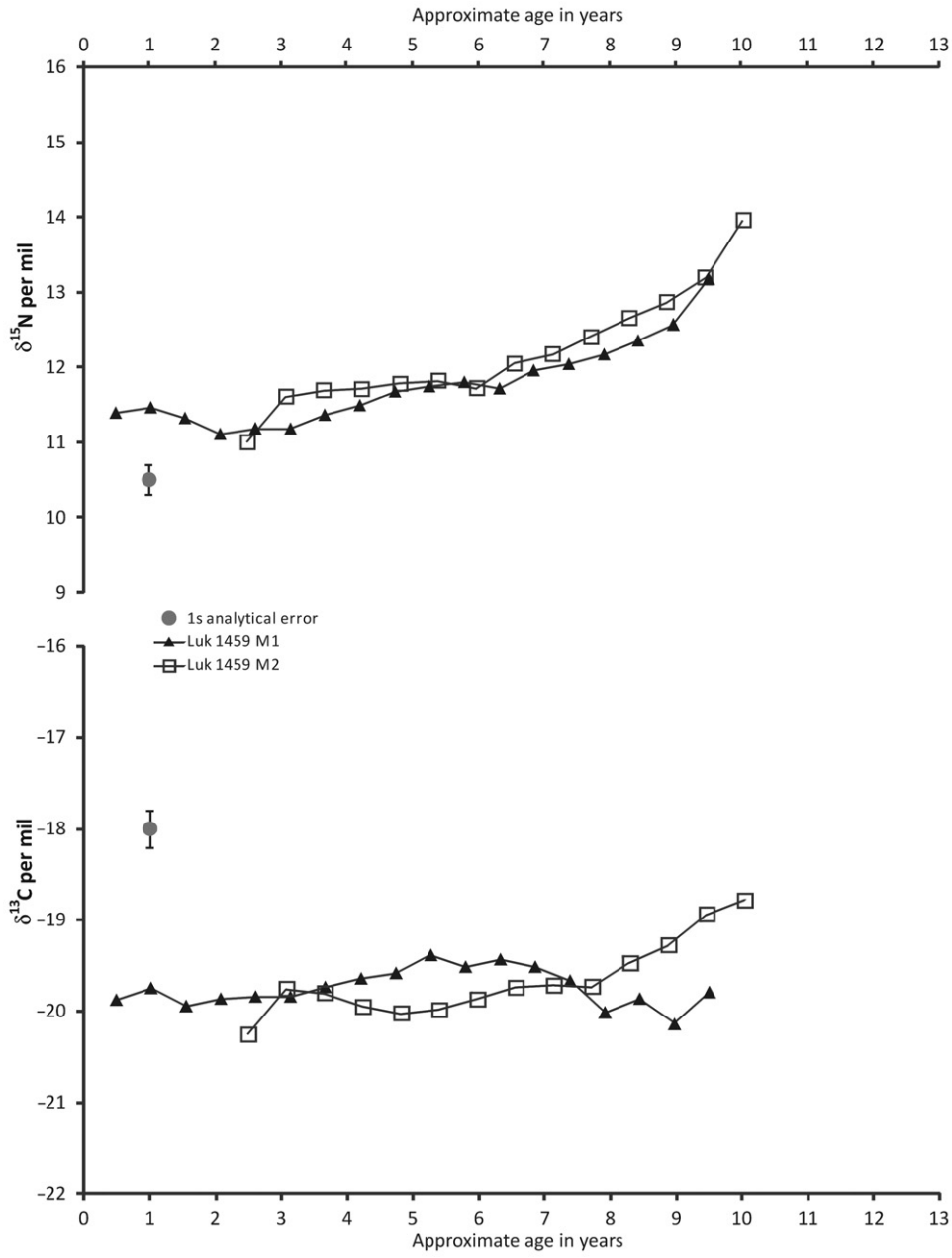

Figure 6 *$\delta^{13}C$ and $\delta^{15}N$ values against age for dentine sections for Luk 1459, first and second permanent maxillary molars, from Lukin Street, Tower Hamlets.*

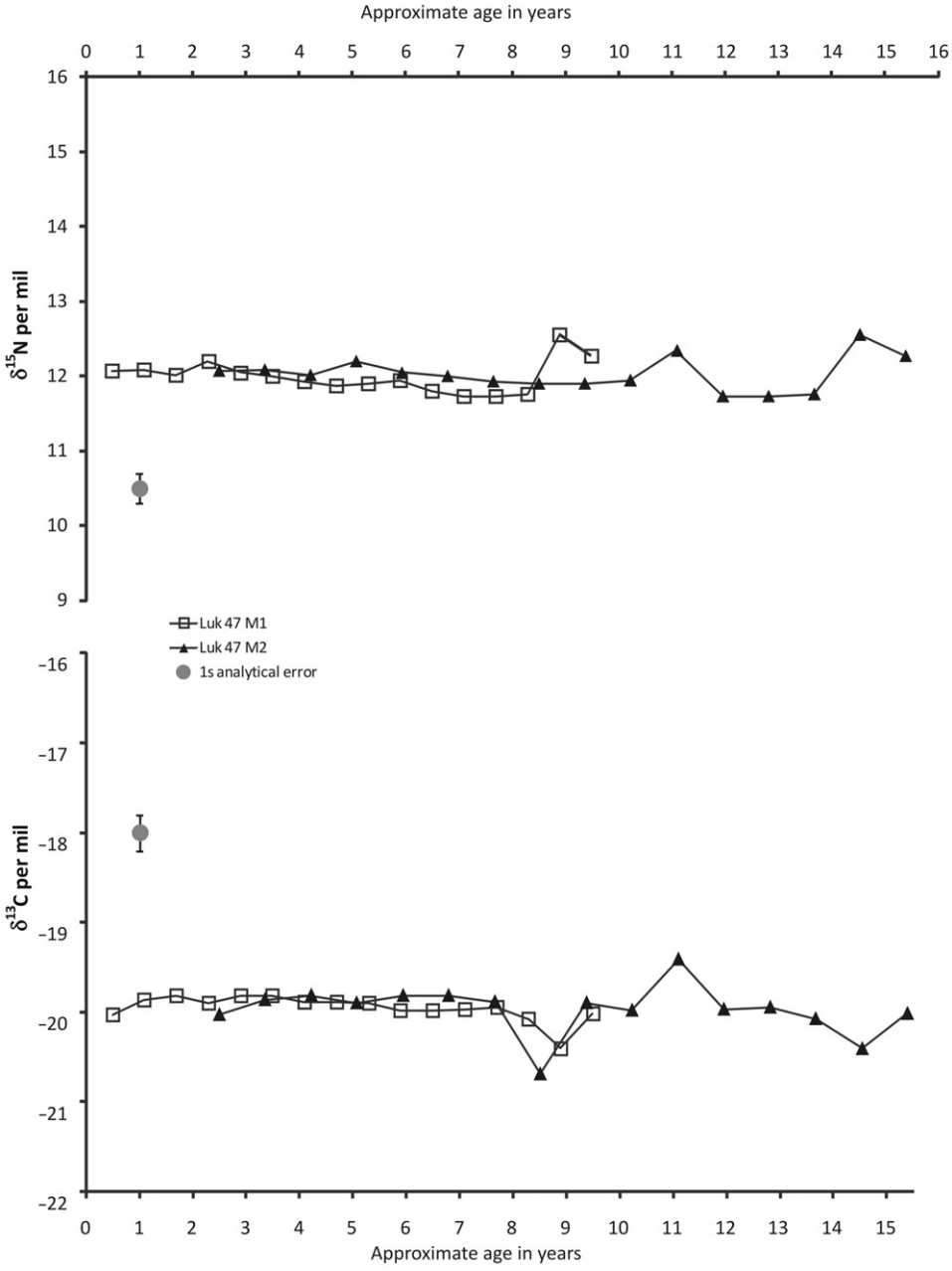

Figure 7 *$\delta^{13}C$ and $\delta^{15}N$ values against age for dentine sections from Luk 47, first and second permanent molars, from Lukin Street, Tower Hamlets.*

may be an underlying physiological trend. Where there is a more marked rate of change in $\delta^{15}N$ or $\delta^{13}C$ (e.g., Fig. 2, Luk 1567; Figs 3 and 4, Luk 1212) and where the absolute values change by more than 2‰, these could represent a change in the diet or possibly a physiological response to a major life event. A time lag may also be possible before changes in the isotope ratios are seen due to the turnover of amino acids in the tissues, and the averaging discussed above. Luk 1567 appears to have a gradual change in both $\delta^{15}N$ and $\delta^{13}C$ from the age of 2.5 years until 15.5 years. These changes are consistent with a change from a terrestrial, plant-based diet to a higher-trophic-level diet with a marine component. Similar dietary differences were seen between low-status and high-status individuals in medieval England (Müldner and Richards 2005). Within the tissues of one individual, this could represent a change in diet after the age of three, when the first dentine increment would have formed.

The population in the Lukin Street cemetery is thought to include Famine survivors, and it is possible that some of these individuals are recording nutritional distress in the dentine shown by a rise in $\delta^{15}N$ within the early-forming dentine increments (Fig. 3, Luk 1212 and Luk 259). An alternative explanation could be that this is a weaning pattern, with the initial rise in $\delta^{15}N$ being due to the consumption of breast milk, followed by weaning and a corresponding drop as seen in bone collagen samples from infants of different ages within cemetery populations rather than individuals (Jay *et al*. 2008).

The $\delta^{13}C$ profile for Luk 1212 appears to be very different from the other three M1s shown (Fig. 4). This could be an indication of a dietary change that includes consumption of a C_4 plant such as maize. In the case of Luk 1212, there is the potential to produce a range of dietary interpretations if a bulk sample was taken from different areas of the crown or the root tip.

The $\delta^{15}N$ and $\delta^{13}C$ profiles for the M2 from Luk 1404 (Fig. 5) suggest that, because only five sections were taken, the values are averaged over the time of growth of the tooth when compared with the larger variations seen in the first molar. With both Luk 1459 (Fig. 6) and Luk 47 (Fig. 7), the profiles suggest that the variations seen in the first and second molars are related to age: the overlapping $\delta^{15}N$ and $\delta^{13}C$ profiles show that the small variations within the dentine are generally the same in both teeth, mostly within analytical error. Where there are larger variations, these can be seen in the profiles of both M1 and M2 at the same age; for example, Figure 6, Luk 1459, $\delta^{15}N$ at age 6–9 years, and Figure 7, Luk 47, $\delta^{13}C$ at age 7–9 years. This confirms that the profiles reflect changes in the diet over time in teeth with overlapping developmental ages. This finding extends the age range that can be investigated using this technique.

Comparison of the methods

Method 1, where the dentine is still hard and embedded in plaster, permitted accurately measured sections to be produced. However, dentine is lost as the rotating saw grinds away tissue during the process. Using method 2, the softened dentine is cut into sections with a scalpel: it can be difficult to achieve as high a level of accuracy as with method 1, but less tissue is lost.

Since each incremental sample contains tissue from a developmental period of several months, the precision of the measurement of the increment width probably does not greatly affect the result (Zazzo *et al*. 2006).

We would recommend method 2 for well-preserved teeth: less dentine is lost in the cutting process, giving better time resolution. Cutting by hand also offers the potential to sample in a way that is more sensitive to the tooth morphology; for example, for curved roots, or the thinner tip of a developing or resorbing root. While the process of demineralization takes longer, there is no

requirement for embedding or access to a cutting blade. We would recommend method 1 where collagen preservation is poor, allowing the tooth to be cut while still hard, permitting the production of accurately measured increments.

CONCLUSIONS

The results of this pilot study demonstrate that it is possible to produce collagen of acceptable quality using either method from archaeological teeth, and both have advantages and disadvantages that could be applied to different samples.

Each tooth sampled demonstrates the presence of markers for dietary and metabolic features that may result from more transient influences than have previously been observed archaeologically. The time scale within which these features are observed is exceptionally detailed, and represents an opportunity to shift away from comparing dietary information within a population, to within each individual. The nearest parallels are studies on hair and fingernails in living subjects (Fuller *et al*. 2004; Mekota *et al*. 2006; Huelsemann *et al*. 2009). The use of incremental dentine allows the comparison of those who have died during infancy and childhood with those who have survived, and could be used to test the validity of using isotope ratios obtained from the bone collagen of individuals who died in childhood to reconstruct variations in diet with age within a cemetery population. The use of deciduous teeth, where available, could give information about the perinatal nutritional status of children, and combining time sequences from teeth with different developmental times from the same individuals could allow the reconstruction of up to 20 years of diet and nutrition. In particular, the results presented here demonstrate that dietary and physiological changes other than weaning may also be recorded by the $\delta^{13}C$ and $\delta^{15}N$ profiles found in incremental dentine collagen.

This study therefore illustrates the need for new research using incremental analysis to investigate the high temporal resolution of isotopic information recorded in human dentine.

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