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Evaluating sodium hydroxide usage for stable isotope analysis of prehistoric human tooth dentine

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ABSTRACT

Analyzing carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in serial samples of human tooth dentine can aid in reconstructing life history events such as weaning and diet. As dentine does not remodel after formation, it retains the isotopic signatures of the foods ingested during a tooth's development, allowing investigation of the diet consumed during this time. Microsampling human archaeological tissues is becoming increasingly popular but no consensus has been reached on the best method to remove soil humates from such small samples. It is important to remove these humates, as they can alter collagen $\delta^{13}C$ values. This study presents an adjustment to a commonly used method for removing humates from bone collagen samples, the sodium hydroxide soak. Here we compare dentine microsamples from five modern unburied teeth that received the usual 20 hour NaOH soak to microsamples from an archaeological tooth for which NaOH treatment time was reduced to 6 h. The results show that microsamples from modern material tolerate the standard NaOH treatment well despite their tiny size. In the archaeological tooth, the six hour treatment was sufficient to remove humates without damaging the collagen of the small and fragile prehistoric dentine microsamples. Even in these trial samples, the $\delta^{13}C$ and $\delta^{15}N$ values provide some interesting insights into dietary changes during development, underscoring the benefits of analysis at the intra-individual level.

1. Introduction

Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ measured in human tooth dentine are often used to reconstruct past diets and investigate certain life history events such as breastfeeding and weaning (Beaumont et al., 2013a,b; Beaumont and Montgomery, 2016; Eerkens et al., 2011; Fuller et al., 2003; Henderson et al., 2014; King et al., 2018; Sandberg et al., 2014; Van der Sluis et al., 2015). While dentine collagen has not been used as frequently as bone collagen, it has the advantage of providing dietary information at a higher resolution. The chemical signatures from food and water ingested during the development of an individual's dentition are retained in the dentine. These signatures remain unaltered, as dentine is not remodeled once formed (Nanci, 2013). The collagen of a bone permits insight into diet through a dietary signal that has been averaged over the most recent years of an individual's life. Turnover rates vary between different types of bone, with ribs providing information on the last few years prior to death while long bones represent a much longer period (Cox and Sealy, 1997; Hedges et al., 2007; Parfitt, 2002; Tsutaya and Yoneda, 2015). This phenomenon can be used to examine the changing diets of individuals at different points in time (Cheung et al., 2017; Pollard et al., 2012; Xia et al., 2018). For childhood diets, however, dentine collagen provides a higher resolution.

The application of dentine microsampling provides insights into childhood diet, both for individuals who died during childhood and for those who survived past their developmental years into adulthood. By comparing the composition of an individual's primary dentine from teeth formed at different life stages, it is possible to track dietary changes from infancy to early adulthood. The dentine of the three permanent molars (M1, M2, and M3) represents approximately 20 years of developmental time: the M1, from birth to roughly 9–10 years; the M2, between 2 and 16 years; and the M3, between roughly 12 and 20 years (AlQhatani et al., 2010; Beaumont et al., 2013a; Eerkens et al., 2011; Hillson, 1996). The isotopic changes within the dentine can potentially be linked to changes in diet including weaning (Eerkens and Bartelink, 2013; Eerkens et al., 2016; Fuller et al., 2003; Van der Sluis et al., 2015).

This pilot study investigates $\delta^{13}C$ and $\delta^{15}N$ ratios in dentine microsamples from five modern permanent molars and one archaeological permanent first molar. This small study is part of a larger PhD research

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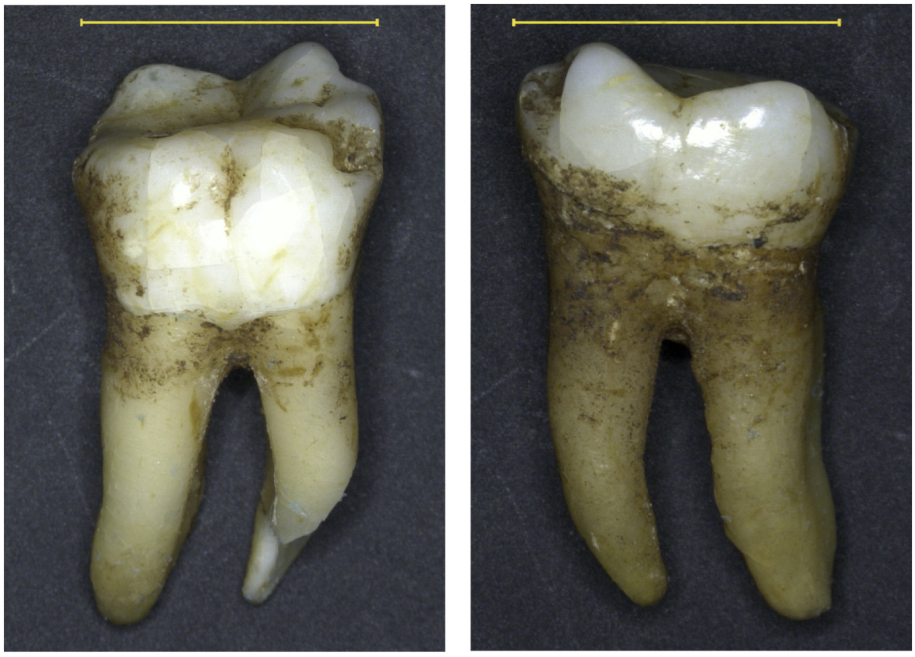

Fig. 1. The archaeological tooth used in the treatment trials. Panel a shows the buccal aspect and panel b shows the lingual aspect. Scale: blue bar = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Photo taken by Victoria M. van der Haas.

project that aims to extend and investigate the life histories of Holocene hunter-gatherers in the Cis-Baikal, Siberia, Russia. For that study, sets of permanent teeth (n = 80) taken from 49 hunter-gatherers from the Cis-Baikal region are being analyzed as part of a long-term ongoing project known as the Baikal Archaeology Project (Weber and Bettinger, 2010; Weber et al., 2002, 2010, 2016). Before applying the microsampling method to the additional 79 prehistoric teeth, we wished to confirm the reliability and appropriateness of our method for removing soil humates from the microsamples. Soil humates are common organic contaminants of archaeological collagen; if not removed, they can alter the $\delta^{13}C$ values of collagen samples (Katzenberg, 1989; Katzenberg et al., 1995; Liden et al., 1995). In current research on bulk collagen samples, two techniques are commonly used to remove humates from collagen samples. The older technique consists of soaking the sample in 0.125 M NaOH for 20 h (Ambrose, 1990; DeNiro and Epstein, 1978; Lee-Throp et al., 1989; Tuross et al., 1988; for recent examples see Katzenberg et al., 2012; Ren et al., 2017; Waters-Rist and Katzenberg, 2010). It has commonly been assumed that removal of humates will also be achieved during ultrafiltration, which removes soil contaminants and degraded collagen fragments by separating molecules based on their weight (Blatt et al., 1965; Brown et al., 1988; Cheung et al., 2017; Craig et al., 2013; Müldner and Richards, 2005). However, a recent test of humate removal techniques on archaeological bone samples suggests that ultrafiltration is not in fact effective for this purpose, and that NaOH treatment should be used (Szpak et al., 2017).

Although some dentine microsampling studies include one of these steps (e.g. Burt, 2015; Eerkens and Bartelink, 2013; Greenwald et al., 2016), a number of others do not report using any method for the soil humate removal, perhaps due to concerns about loss of mass in the tiny microsamples (Beaumont et al., 2013a,b; Beaumont and Montgomery, 2016; Fuller et al., 2003; Henderson et al., 2014; Sandberg et al., 2014; Van der Sluis et al., 2015). As discussed below, such concerns would be valid. It is also possible NaOH was not used in any of these studies because the teeth did not exhibit obvious signs of humic acid contamination and were relatively recent in age. The Baikal samples are older, dating to the middle Holocene, and a NaOH soak is considered part of the necessary protocol in our stable isotope laboratory for such samples.

While ultrafiltration is an effective means of sample purification, it carries risks as it increases the risk of losing even more collagen from already very small samples. Mass yields and amino acid profiles of NaOH treated samples indicate that some collagen is also lost to this treatment, which was the original reason for restricting treatment duration to 20 h (Boutton et al., 1984; Katzenberg, 1989; for more recent confirmation of this effect see Szpak et al., 2017). Anecdotally, our laboratory has noted that 20 hour NaOH treatments can produce excessive degradation in poorly preserved collagen samples and that shorter treatment times are more prudent, a precaution that is common in the published literature (e.g. Rick et al., 2011). Concerns that a 20 hour treatment might damage the far older Baikal teeth provided the impetus to experiment with a time change in the sodium hydroxide soak, using the abbreviated 6 hour treatment time that has been usual in our laboratory for fragile samples.

2. Stable isotopes, collagen and sodium hydroxide

2.1. Stable isotope analysis

Stable isotope analysis is an analytical technique that provides information on past diets, with the most commonly used isotopes being carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). The technique was first used on archaeological specimens by Vogel and Van Der Merwe (1977) to distinguish between the consumption of C_3 and C_4 plants. Since then, stable isotope analysis has revolutionized the way archaeologists study human and faunal remains (Lee-Thorp, 2008; Price, 2015). The stable isotope composition of the sample is determined through mass spectrometry and expressed in per mil (‰) relative to an international standard using the following equation:

$$\delta R‰ = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where R_{sample} is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample, and $R_{standard}$ represents the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ content of the international standards, which are AIR and Vienna Pee Dee Belemite (VPDB), respectively (Price, 2015).

Table 1
Summary of carbon and nitrogen data for all tooth samples.

VPS 1–5 are the modern specimens; H2000.224 is the archaeological specimen. S-# refers to the dentine section with S-01 representing the earliest forming dentine (top part of the crown).

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C content (%)	N content (%)	C:N
VPS 1 S-01	−18.1	12.1	44.18	15.64	3.3
VPS 1 S-02	−17.7	11.6	44.89	15.45	3.4
VPS 1 S-03	−17.5	11.4	45.00	15.92	3.3
VPS 1 S-04	−17.6	11.4	44.21	15.81	3.3
VPS 1 S-05	−17.7	11.6	44.47	15.78	3.3
VPS 1 S-06	−17.8	11.6	44.32	15.81	3.3
VPS 1 S-07	−17.7	11.8	43.96	15.70	3.3
VPS 1 S-08	−17.9	11.8	43.63	15.29	3.1
VPS 1 S-09	−17.8	11.9	44.73	15.82	3.3
VPS 1 S-10	−17.7	11.9	44.17	15.64	3.3
VPS 1 S-11	−17.8	11.9	44.53	15.67	3.3
VPS 1 S-12	−17.8	12.0	44.46	15.78	3.3
VPS 1 S-13	−17.9	12.0	44.45	15.81	3.3
VPS 1 S-14	−17.9	12.1	44.73	15.99	3.3
VPS 1 S-15	−17.9	12.0	44.54	15.77	3.3
VPS 1 S-16	−17.9	12.0	44.54	15.77	3.3
VPS 1 S-17	−17.9	12.1	44.55	15.85	3.3
VPS 1 S-18	−17.8	11.9	42.39	15.07	3.3
VPS 1 S-01	−18.1	12.1	44.18	15.64	3.3
VPS 1 S-02	−17.7	11.6	44.89	15.45	3.4
VPS 1 S-03	−17.5	11.4	45.00	15.92	3.3
VPS 1 S-04	−17.6	11.4	44.21	15.81	3.3
VPS 1 S-05	−17.7	11.6	44.47	15.78	3.3
VPS 1 S-06	−17.8	11.6	44.32	15.81	3.3
VPS 1 S-07	−17.7	11.8	43.96	15.70	3.3
VPS 1 S-08	−17.9	11.8	43.63	15.29	3.1
VPS 1 S-09	−17.8	11.9	44.73	15.82	3.3
VPS 1 S-10	−17.7	11.9	44.17	15.64	3.3
VPS 1 S-11	−17.8	11.9	44.53	15.67	3.3
VPS 1 S-12	−17.8	12.0	44.46	15.78	3.3
VPS 1 S-13	−17.9	12.0	44.45	15.81	3.3
VPS 1 S-14	−17.9	12.1	44.73	15.99	3.3
VPS 1 S-15	−17.9	12.0	44.54	15.77	3.3
VPS 1 S-16	−17.9	12.0	44.54	15.77	3.3
VPS 1 S-17	−17.9	12.1	44.55	15.85	3.3
VPS 1 S-18	−17.8	11.9	42.39	15.07	3.3
VPS 2 S-01	−18.3	12.3	46.61	16.00	3.4
VPS 2 S-02	−17.7	11.7	44.43	15.64	3.3
VPS 2 S-03	−17.5	11.5	43.71	15.38	3.3
VPS 2 S-04	−17.4	11.5	44.20	15.43	3.4
VPS 2 S-05	−17.6	11.5	44.14	15.52	3.3
VPS 2 S-06	−17.7	11.7	38.55	13.32	3.4
VPS 2 S-07	−17.8	11.7	44.16	15.48	3.4
VPS 2 S-08	−17.8	11.8	44.13	15.48	3.4
VPS 2 S-09	−17.9	11.8	45.51	15.88	3.4
VPS 2 S-10	−17.9	11.9	44.56	15.73	3.3
VPS 2 S-11	−17.9	12.1	44.98	15.82	3.3
VPS 2 S-13	−17.8	11.9	44.61	15.74	3.3
VPS 2 S-14	−17.8	11.9	44.10	15.41	3.4
VPS 2 S-15	−17.8	12.1	43.62	15.34	3.3
VPS 2 S-16	−17.8	12.0	44.43	15.63	3.3
VPS 2 S-17	−17.8	12.0	44.44	15.60	3.3
VPS 3 S-01	−17.8	12.1	44.39	14.16	3.6
VPS 3 S-02	−17.5	11.6	44.83	15.53	3.4
VPS 3 S-03	−17.3	11.2	43.35	15.14	3.4
VPS 3 S-04	−17.7	11.4	45.69	15.70	3.4
VPS 3 S-05	−17.8	11.7	44.85	15.70	3.4
VPS 3 S-06	−17.7	11.8	44.40	15.60	3.3
VPS 3 S-07	−17.4	11.8	44.39	15.58	3.3
VPS 3 S-08	−17.4	11.9	44.74	15.69	3.4
VPS 3 S-09	−17.4	11.9	44.02	15.35	3.4
VPS 3 S-10	−17.3	11.8	44.08	15.28	3.4
VPS 3 S-11	−17.2	11.8	44.13	15.43	3.4
VPS 3 S-12	−17.5	12.1	39.80	15.56	3.0
VPS 3 S-13	−17.8	12.1	44.49	15.51	3.4
VPS 3 S-14	−17.9	12.1	44.64	15.65	3.4
VPS 3 S-15	−17.8	12.3	44.42	15.65	3.3
VPS 4 S-01	−18.0	11.9	44.54	15.42	3.4
VPS 4 S-02	−18.1	11.5	42.35	14.38	3.4
VPS 4 S-03	−17.9	11.6	44.40	15.52	3.4
VPS 4 S-04	−17.8	11.4	43.75	15.38	3.3
VPS 4 S-05	−17.7	11.6	44.18	15.68	3.3
VPS 4 S-06	−17.6	11.5	44.41	15.67	3.3
VPS 4 S-07	−17.5	11.6	44.15	15.72	3.3
VPS 4 S-08	−17.4	11.7	45.08	15.85	3.3
VPS 4 S-09	−17.3	11.6	44.49	15.67	3.3
VPS 4 S-10	−17.5	11.6	45.55	15.80	3.4
VPS 4 S-11	−17.4	11.6	45.03	15.94	3.3
VPS 4 S-12	−17.4	11.8	44.28	15.61	3.3
VPS 4 S-13	−17.4	11.9	45.20	15.89	3.3
VPS 4 S-14	−17.5	12.0	44.36	15.66	3.3
VPS 4 S-15	−17.5	12.3	44.16	15.71	3.3
VPS 4 S-16	−17.5	12.5	44.47	15.61	3.3
VPS 4 S-17	−17.3	12.7	43.87	15.60	3.3
VPS 4 S-18	−17.4	12.7	43.59	15.21	3.4
VPS 5 S-01	−14.3	11.8	39.20	15.30	3.0
VPS 5 S-02	−15.2	10.3	43.42	15.23	3.4
VPS 5 S-03	−16.0	8.9	43.75	15.39	3.3
VPS 5 S-04	−16.4	8.3	43.78	15.40	3.3
VPS 5 S-05	−16.0	7.9	43.79	15.35	3.4
VPS 5 S-06	−16.6	7.9	44.29	15.47	3.4
VPS 5 S-07	−16.3	8.3	43.26	15.15	3.4
VPS 5 S-08	−15.4	8.8	43.87	15.33	3.4
H2000.224 S-01	−19.9	13.2	43.97	15.64	3.3
H2000.224 S-02	−20.0	11.6	43.55	15.61	3.3
H2000.224 S-03	−19.7	11.2	43.90	15.56	3.3
H2000.224 S-04	−19.6	11.0	44.06	15.50	3.3
H2000.224 S-05	−19.5	10.9	44.30	15.59	3.3
H2000.224 S-06	−19.4	11.0	44.42	15.56	3.4
H2000.224 S-07	−19.5	10.8	45.11	15.54	3.4
H2000.224 S-08	−19.7	10.8	49.06	17.21	3.4
H2000.224 S-09	−19.7	11.0	42.87	14.74	3.4
H2000.224 S-10	−19.6	11.3	43.41	15.38	3.3
H2000.224 S-11	−19.4	11.4	43.53	15.47	3.3
H2000.224 S-12	−19.5	11.4	44.20	15.67	3.3
H2000.224 S-13	−19.4	11.3	44.14	15.59	3.3
H2000.224 S-14	−19.2	11.2	44.33	15.73	3.3
H2000.224 S-15	−19.1	11.3	44.10	15.64	3.3
H2000.224 S-16	−19.2	11.5	43.79	15.50	3.3
H2000.224 S-17	−19.3	11.6	42.77	15.12	3.3

2.2. Collagen

Bone and teeth are composed of organic (collagen) and inorganic (mineral) compounds. While the latter compounds can also be used for stable isotope analysis, only the former will be used for this particular study. Collagen, the main structural protein in human and animal tissues, primarily represents the protein portion of the diet. Collagen $\delta^{13}C$ is elevated ~5‰ above dietary protein, though carbon from dietary lipids and carbohydrates also participates in collagen synthesis, causing substantial departure from this value under some conditions (Ambrose and Norr, 1993; Krueger and Sullivan, 1984; Tieszen and Fagre, 1993). Isotopic differences between diet and consumer are also seen in nitrogen. Tissue $\delta^{15}N$ values of heterotrophs are elevated by about +3‰ to +6‰ over dietary $\delta^{15}N$; the magnitude of this trophic level effect varies in response to factors such as health and growth status, but still generally reflects predator-prey relationships and is useful in foodweb reconstruction (Bocherens and Drucker, 2003; DeNiro and Epstein, 1981; Fuller et al., 2005; O'Connell et al., 2012; Schoeninger et al., 1983).

2.3. Collagen preparation and sodium hydroxide (NaOH)

NaOH has been used to remove humic contaminants from collagen from the earliest days of archaeological stable isotope analysis (DeNiro and Epstein, 1981). Today, NaOH is frequently used for purifying collagen prior to stable isotope analysis (Katzenberg, 2008). While it has been proven that NaOH pre-treatment somewhat lowers collagen yields, it does not affect the collagen stable isotope values when

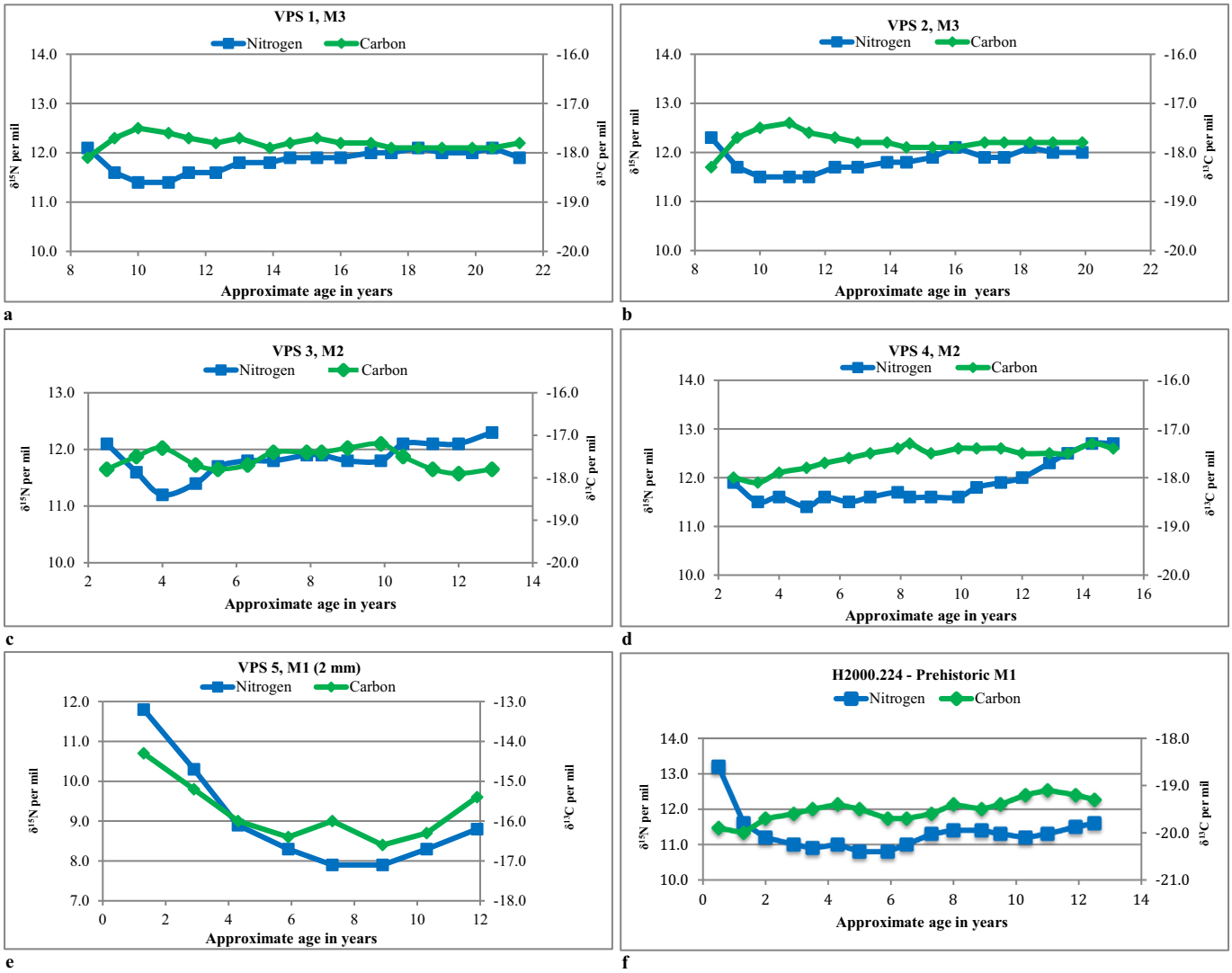

Fig. 2. Dentine microsection stable isotope values for modern teeth.

treatment time is restricted to 20 h or less, and it removes humates that can significantly alter the apparent stable isotope values of the sample (Katzenberg, 1989; Liden et al., 1995; Szpak et al., 2017). The NaOH technique only appears to have been used in a few archaeological microsampling studies, that of Burt (2015), who used a 20 hour soak on medieval English samples, and those of Eerkens (Eerkens and Bartelink, 2013; Eerkens et al., 2011, 2016) and Greenwald et al. (2016), who soaked their samples in NaOH for 24 h. Because work on larger bone collagen samples has clearly demonstrated that NaOH treatment can decrease sample yield and that treatments departing from the common 20 hour standard can be effective in removing humate contamination (Szpak et al., 2017), it is worthwhile to consider whether briefer NaOH treatments may be a good choice for dentine microsample preparation. As a 6 hour soak has been applied to fragile bone collagen in our laboratory in the past, we decided to use this shortened time for dentine microsamples as well.

3. Dentinogenesis

There are three types of dentine in human teeth: primary dentine, which is formed during tooth formation; secondary dentine, which is a continued secretion of dentine later in life; and sometimes tertiary dentine, which is laid down as a response to tooth trauma (Zilberman and Smith, 2001). Dentine formation - dentinogenesis - begins prior to enamel formation and is secreted and mineralized in a two-phase process. Odontoblasts will secret a dentine matrix also known as pre-dentine. The matrix is then mineralized by the deposition of short (20–100 μm) crystals of carbonate hydroxyapatite within the collagen fiber matrix (Beaumont et al., 2013a). The rate in which dentine is laid down in a permanent tooth is 4–6 μm a day throughout the cuspal areas while the formation of root dentine starts from the cement-dentine junction and continues to grow 1.3–1.5 μm per day (Dean and Scandrett, 1995). Dentine is not laid down in straight increments, but rather as concentric cones, giving the dentine layers a more S-shaped profile (Dean and Scandrett, 1995; Nanci, 2013; Smith, 2005). Microsamples will therefore always contain some dentine of the adjacent increments. Dentine does not remodel over time (Nanci, 2013) and will provide insight into dietary change during specific time intervals. Bones, in contrast, continuously remodel, making it more difficult to detect change in diet at different stages of an individual's development. If teeth are not available, using multiple bones from the same individual can provide a broader stable isotope profile of changing diet, and this approach has successfully been used in a number of studies (Cheung et al., 2017; Cox and Sealy, 1997; Pollard et al., 2012; Sealy et al., 1995; Xia et al., 2018).

The development time of life represented by each 1 mm section was determined by Beaumont et al. (2013a) who calculated it that will take roughly 200 days - or nine months - for the dentine to reach a 1 mm

thickness based on the mineralization rate determined by Dean and Scandrett (1995). Alongside tooth developmental calculations using the AlQhatani et al. (2010) Atlas, Beaumont et al. (2013a) created a developmental age estimation time scale in which to plot each dentine increment. It is important to note that as variation exists between and within individuals, each slice represents a developmental period, rather than a fixed point in an individual's life.

4. Materials and methods

4.1. Sample collection

Five modern permanent molars from four anonymous donors from Alberta, Canada and one Early Neolithic (8000–7200 cal BP) individual from the Cis-Baikal region in Siberia, Russia were used for this study. The modern specimens (VPS 1, 2, 3, 4, 5) were extracted for medical purposes and serve as a control as their intact, unburied dentine is not at risk of damage from a 20 hour soak. Samples VPS 1 and VPS 2 are an upper and a lower M3 from the same individual. VPS 3 (lower M2) and VPS 4 (upper M2) come from two additional individuals, as does VPS 5, a badly damaged M1 fragment. Further information on the five modern teeth (e.g., donor sex and dietary history) cannot be provided because donors were assured full anonymity at the time of donation and could not be recontacted. The prehistoric lower M1 (Fig. 1a, b) comes from an Early Neolithic individual who was buried at the Manzurka cemetery (Konopatskii, 1977) in the Cis-Baikal region of Siberia, Russia. All teeth were sectioned longitudinally with a diamond hand held saw. For the modern teeth, the longest root was selected for sampling and the other half was used for different research purposes. For the archaeological tooth the distal root was used for microsampling because the mesial root was broken post-mortem at its apex; the other half of the tooth was kept for future BAP research.

Initial cleaning of the sectioned teeth was done by sonicating the samples in double distilled water. This was followed by demineralization in HCl solution at room temperature. The archaeological tooth was demineralized in 0.5 M HCl solution; modern samples were demineralized in 1.2 M HCl to encourage dissolution of the dense, well-preserved enamel. Demineralization took between 14 and 21 days for all samples, regardless of the difference in HCl solution concentration.

4.2. Microsampling and analysis

The method used for sectioning was similar to Method 2 of Beaumont et al. (2013a,b), where the demineralized dentine is sectioned into 1 mm strips from crown to root using a hand held scalpel. 1 mm sections were used for all teeth but the fragmentary VPS 5, which was sliced into 2 mm sections. Post-slicing, the modern dentine sections received a 20 h 0.125 M NaOH soak, while the archaeological dentine sections received a 6 hour soak in 0.125 M NaOH. After this, all samples were rinsed three times in double distilled water. The collagen was gelatinized by placing the sections in acidulated H_2O (pH 3) and heating in a water bath (80 °C, 48 h). Samples were centrifuged, and the supernatant was drawn off and lyophilized. The resulting purified collagen was weighed on a microbalance and transferred into tin capsules for analysis. Samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$ at the University of Alberta Biogeochemical Analytical Service Laboratory using a EuroVector EuroEA3028-HT elemental analyzer coupled to a GV Instruments IsoPrime continuous-flow isotope ratio mass spectrometer. NIST 8415 whole egg powder SRM was used as an in-house $\delta^{15}N$ and $\delta^{13}C$ QA/QC check throughout analyses, with standard deviations of 0.2‰ and 0.1‰ respectively. The carbon and nitrogen stable isotopic data, C%, N%, and C:N are provided in Table 1.

4.3. Collagen assessment

Several criteria can be used to assess the integrity of collagen; the most commonly recognized are collagen yield, carbon (C%) and nitrogen (N%) content, and C:N (Ambrose, 1990; Sealy et al., 2014; Van Klinken, 1999). Collagen yield is expressed as a weight percentage of the weight of the original bone or dentine sample. However, as teeth were demineralized prior to slicing the dentine, no original weight values were available and it was not possible to calculate the collagen yield. C%, N% and C:N report the composition of the combusted collagen extract. For this study, collagen samples with C% values of 40% or higher, N% values of 14% or higher, and C:N values of 2.9–3.6 were accepted (DeNiro, 1985; Bocherens and Drucker, 2003).

5. Results and dietary interpretation

All microsamples' C:N ratios fall within the acceptable range of 2.9 to 3.6. The modern collagen samples which received the standard 20 hour NaOH soak mainly exhibit a C:N ratio around 3.3. The same can be said for the archaeological sections. The highest C:N value of the sample set is seen in S-01 of VPS 3; this showed a value of 3.6, high but still within the generally accepted range. The C% and N% values for all microsamples also fall above the acceptable minimum values, indicating the collagen is of good quality.

While the main point of this report is the success of the treatment protocol, it is still interesting to consider the dentine section $\delta^{13}C$ and $\delta^{15}N$ values. To allow this, all tooth sections have been plotted against an approximate timeline following Beaumont et al. (2013a) (Fig. 2a–f). The upper and lower M3's belonging to the same individual, VPS-1 and VPS-2 (Fig. 2a, b), are closely similar in terms of their overall $\delta^{13}C$ and $\delta^{15}N$ values and the patterns of change in these values over time. This is an interesting confirmation of a common assumption of the dentine microsampling method – that the information a tooth will provide on an individual's dietary history will vary based on its position (M1, M2 or M3) but not on whether it is an upper or lower tooth. In contrast, VPS-3 (Fig. 2c) and VPS-4 (Fig. 2d), second molars from two different individuals, show two clearly different histories of dietary change in these individuals between the ages of about 2 and 16 years. VPS-5 (Fig. 2e) shows isotopic changes between about 18 months and twelve years of age. This tooth was sliced into 2 mm sections in an attempt to document the utility of 2 mm sections in poorly preserved or fragmentary teeth; while it is evident that 1 mm slices provide better insight into dietary shifts, the 2 mm sections still do trace broad patterns in diet. If the individual backgrounds of these modern donors were known, it would be possible to make some interesting comparisons between their $\delta^{13}C$ and $\delta^{15}N$ values and their life history. This is beyond the scope of this paper, but it is still interesting to note that the values of VPS-1, VPS-2, VPS-3 and VPS-4 fall in the same general $\delta^{13}C$ and $\delta^{15}N$ ranges as post-weaning values measured by Burt (Burt and Amin, 2014; Burt and Garvie-Lok, 2013) on teeth from anonymous modern donors from the same region of Canada. In contrast, VPS-5 shows apparent breastfeeding elevation before 4 years of age, coupled with post-weaning values that are higher than the other modern teeth for $\delta^{13}C$ and lower for $\delta^{15}N$ (Fuller et al., 2006). Given the diverse geographic and cultural origins of modern urban Canadian populations, isotopic variation of this sort can be expected in an anonymous donor sample.

H2000.224 (Fig. 2f) displays a clear breastfeeding and weaning signal with $\delta^{15}N$ values in the first five sections showing a decrease from 13.2‰ to 10.9‰ before going slightly back up and fluctuating between 10.8‰ and 11.6‰. These results suggest that this prehistoric individual was fully weaned around the age of 3.5 years, a finding consistent with previous findings by Waters-Rist et al. (2011) for bone collagen of sub-adults and adults from individuals in the Cis-Baikal. The $\delta^{13}C$ values show the opposite pattern in the first five sections, starting at −19.9‰ and then dropping slightly before rising to −19.5‰. This is inconsistent with the expected pattern for weaning (Fuller et al., 2006), but is consistent with the variable $\delta^{13}C$ seen throughout this individual's later childhood; prior studies of the Cis-Baikal (Katzenberg and Weber, 1999; Katzenberg et al., 2010; Lam, 1994) show that human

$\delta^{13}C$ values in these mobile populations varied in response to factors including locale and resource choice. These results will be discussed in-depth in future publications profiling multiple individuals from the same region and time period.

6. Discussion and conclusion

The results presented in this paper confirm that NaOH treatment of dentine microsamples should be feasible in most situations. In all cases, NaOH treatment produced samples of sufficient weight for analysis with C:N, C% values and N% values characteristic of well-preserved collagen. The results on the modern material support results by Burt (2015), indicating that the standard 20 hour treatment can be applied to modern or well-preserved recent archaeological microsamples without damaging them. The data for the archaeological tooth show that even older and more fragile archaeological microsamples will withstand a 6 hour soak in 0.125 M NaOH. The results suggest that this reduced-duration treatment can remove humates while avoiding damage to the ancient collagen. Had the treatment been too long or not long enough, it should have been apparent from altered C:N or excessive loss of collagen providing insufficient material for analysis. At this time the 6 hour NaOH soak has also been applied to the larger Baikal sample set described in the introduction. While the data are still being interpreted, it can be said at this point that all treated microsamples produced enough collagen for analysis and all C:N fall within the acceptable range of 2.9–3.6. Although teeth are less susceptible to diagenetic alteration than bone, teeth can still be subject to humic contamination depending on the burial conditions. When contamination is suspected and the condition of the teeth makes the standard 20 hour treatment a concern, a reduced 6 hour treatment can safely be applied.

Although dietary interpretation was not the main goal of this preliminary study, it is clear that dietary histories are being captured in the $\delta^{13}C$ and $\delta^{15}N$ values. The values of most modern samples are consistent with post-weaning dentine values measured for the same region of Canada by Burt (Burt and Amin, 2014; Burt and Garvie-Lok, 2013), and results for the single archaeological tooth are consistent with the results of prior studies of material of the same region and era (Waters-Rist et al., 2011).

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Conflicts of interest

None.

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