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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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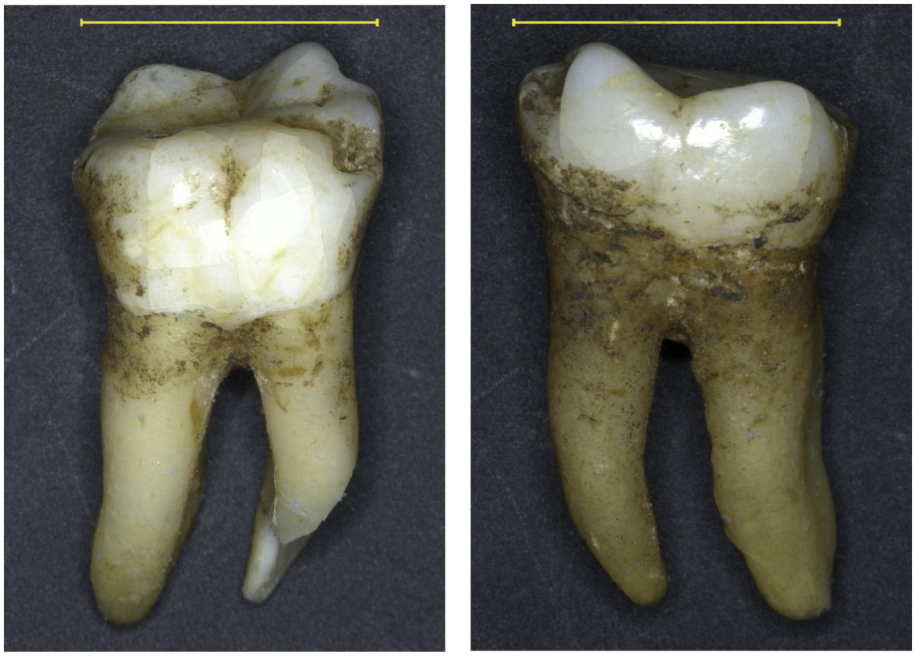

Fig. 1. The archaeological tooth used in the treatment trials. Panel a shows the buccal aspect and panel b shows the lingual aspect. Scale: blue bar = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Photo taken by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