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A new method of dentine microsampling of deciduous teeth for stable isotope ratio analysis

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ABSTRACT

Carbon and nitrogen stable isotope ratio analysis of dentine is a powerful tool for examining early childhood diet in past populations. Serial sampling of the dentine can reconstruct an individual's changing diet. Previous serial studies have used homogenized samples that give broad results for age categories. This study presents a new dentine microsampling technique for use in stable isotope ratio analysis that provides stable isotope signals for three important juvenile life stages: fetal life, breastfeeding, and weaning.

A sample of 35 modern deciduous teeth was collected in collaboration with the Department of Pediatric Dentistry, University of Alberta. One half of each tooth was examined histologically to locate the neonatal line, and the other half was sampled for isotopic analysis. Microsamples of dentine were collected occlusal to the neonatal line, directly apical to the neonatal line, and from the growing edge of the tooth; these should reflect the diet of the mother during pregnancy, followed by the infant's breastfeeding and weaning diets. The results of the isotopic assay show dietary changes in individual children over time that can be reasonably explained in terms of modern infant feeding practices in a diverse modern sample.

While the technique will be useful to many stable isotope researchers, it is particularly suited for studying the changing diet of a single individual. The results indicate that microsamples must be above 0.3 mg to give reliable simultaneous results for carbon and nitrogen, though accurate nitrogen results alone can be gained at much smaller weights. Further research will apply this methodology to archaeological remains.

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1. Introduction

Although a number of stable isotope studies have microsampled non-human dentine (Balasse et al., 2001; Kirsanow et al., 2008), few have attempted to microsample human dentine (Eerkens et al., 2011). Here we propose a novel microsampling method that uses smaller human dentine samples than those described in the literature and attempts to reconstruct diet around known life stages based on patterns of human tooth growth. The method identifies a life event – birth – from the neonatal line of deciduous teeth and uses that demarcation to define specific areas of the tooth to microsample. This study has two goals. The first is to improve the precision and range of dentine-based dietary reconstructions to allow for the study of more specific age related diets. Here we look at two postnatal life stages: the period shortly after birth and older infancy, when the tooth completed formation. The second goal is to examine dentine from above the neonatal line in an attempt to extend analyses to the period before birth. This would allow the study of isotope values in fetal life. Outside of work on archaeological fetal remains, which are rare, and a few modern human studies, very little has been done with this life stage using stable isotope ratio analysis (Dupras and Tocheri, 2007; Fuller et al., 2006a). The ability to recover stable isotope signals from the uterine environment would allow researchers to address novel and interesting questions about past populations including relationships between maternal nutrition and infant mortality.

1.1. Background

1.1.1. Stable isotopes and weaning

Tissue stable carbon and nitrogen isotope ratios reflect dietary values as well as metabolic processes (Katzenberg, 2008; Lee-Thorp, 2008). Trophic level effects in $\delta^{13}C$ and $\delta^{15}N$ have been

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demonstrated in human mothers and their nursing infants using fingernail and hair samples (Fogel et al., 1989; Fuller et al., 2006a). At birth, an infant's $\delta^{15}N$ value is close to that of its mother. Its tissues will maintain this value for a variable period that depends on growth rate and tissue turnover before being replaced by an elevated breastfeeding signal (Fuller et al., 2006a; Katzenberg and Pfeiffer, 1995; Katzenberg et al., 1996; Kinaston et al., 2009; Richards et al., 2002). This trophic level elevation is approximately 2‰–3‰ during peak breastfeeding; it occurs because the infant is consuming its mother's milk, a bodily secretion with $\delta^{15}N$ similar to her tissues (Fogel et al., 1989; Fuller et al., 2006b; Katzenberg and Pfeiffer, 1995; Katzenberg et al., 1996; Katzenberg, 2008; Schurr, 1998). Infants that are exclusively bottle fed show no enrichment (Fuller et al., 2006a). The infant's tissue $\delta^{15}N$ changes again as supplementary foods are introduced. These depress overall dietary $\delta^{15}N$ by introducing new dietary protein sources that tend to have lower $\delta^{15}N$ than breast milk. In response, the child's tissue $\delta^{15}N$ values begin to trend downward and eventually stabilize at a new level after weaning has finished. The shift may be characterized by a steep drop or a slow descent, reflecting the speed of weaning and the length of the mixed feeding period. Fogel et al. (1989) found that the infants in their study had fingernail $\delta^{15}N$ values close to those of their mothers three to five months after weaning.

Carbon isotope values are also affected by breastfeeding and weaning. Fuller et al. (2006a) found that exclusively breastfed infants had a smaller $\delta^{13}C$ trophic enrichment of around 1‰ over their mothers, while bottle fed infants showed no enrichment. As with $\delta^{15}N$, infant $\delta^{13}C$ elevation begins to decline when weaning commences. In some studies this decline is more rapid than that seen for $\delta^{15}N$, leading some authors to suggest that $\delta^{13}C$ is more sensitive than $\delta^{15}N$ to the introduction of weaning foods (Dupras et al., 2001; Fuller et al., 2006a; Richards et al., 2003; White et al., 2001).

1.1.2. Sampling for stable isotope ratio analysis in archaeological weaning studies

Many studies have examined breastfeeding and weaning in past populations using the distinctive stable isotope signatures of collagen laid down during those periods (e.g. Fuller et al., 2006b; Katzenberg and Pfeiffer, 1995; Katzenberg et al., 1996; Katzenberg, 2008; Nitsch et al., 2011; Prowse et al., 2008; Tuross et al., 1988). Most studies rely primarily on nitrogen, with age-related shifts in $\delta^{15}N$ values of juvenile skeletal remains used to estimate the length of breastfeeding in past populations and to suggest when weaning foods were introduced and when weaning was normally completed. Carbon has also been used, with shifts in $\delta^{13}C$ values compared to shifts in $\delta^{15}N$ values to track the introduction of particular solid foods in the diet and $\delta^{15}N$ levels used to estimate the duration of breast milk consumption.

In young juveniles, collagen $\delta^{15}N$ and $\delta^{13}C$ from bones such as ribs likely reflect diet in the few months prior to death due to rapid bone remodeling (Fuller et al., 2006b; Jay et al., 2008; Nitsch et al., 2011). Dentine, which does not remodel, can be used to recover dietary signals from earlier periods in life. Archaeological weaning studies can reconstruct diet by analyzing bone samples from a large number of juveniles and seriating the values by estimated age at death. This method was the earliest to be developed in the literature and is still often used (e.g. Jay et al., 2008; Mays et al., 2002; Nitsch et al., 2011; Richards et al., 2002). Another potential approach is to analyze the dentine of whole teeth, using the $\delta^{13}C$ and $\delta^{15}N$ values for each tooth position as indicators of diet in the age range at which that tooth typically forms. Dupras and Tocheri (2007) analyzed teeth from Roman period Kellis, Egypt. For each tooth, all dentine was processed as a single sample. Mean $\delta^{13}C$ and $\delta^{15}N$ values were then generated for each tooth position. By sampling several individuals in this way, diet can be reconstructed for roughly the first 20 years of life. However, a large sample of teeth is needed and by homogenizing so much dentine, potential information is lost.

Serial dentine sampling within a single tooth is a way to get a more detailed look at diet. Previous serial sampling studies have used relatively large dentine samples. Clayton et al. studied weaning at Matjes River Rock Shelter in South Africa (2006). This was primarily a bone study, but due to underrepresentation of 2–4 year olds in the Matjes sample, the root tips of deciduous canines and deciduous molars were also used. The roots of these teeth complete their formation in the 2–4 year period. By using them as proxies for bone values in the 2–4 year range, Clayton et al. were able to make the series complete enough to interpret. Fuller et al. (2003) investigated weaning times at medieval Wharram Percy. Deciduous second molars, permanent canines and permanent third molars were cut horizontally into three large sections: crown, cervical root half, and apical root half. The results clearly showed changing diet through the breastfeeding and weaning period. Because the crown was analyzed as a single sample, this strategy was not able to reconstruct a fetal life value. It also generalized diet over large periods of time because of the way the teeth were sectioned, and Fuller et al. (2003) remark that sectioning along the lines of growth would improve resolution. While these sampling methods do provide a look at an individual's changing diet, it should be possible to get results that are even more specific through microsampling.

Eerkens et al. (2011) recently published a human dentine microsampling method that utilizes smaller horizontal sections than those analyzed in earlier work. The sample consisted of permanent first, second and third molars. Enamel and cementum were removed from the teeth, which were then fully demineralized using a dilute HCl soak. The demineralized collagen models were then sliced horizontally into 5 to 10 1–2 mm sections. Eerkens et al. report that they could detect isotopic changes attributable to weaning, but that some clarity was lost due to horizontal sectioning of the teeth, which form in parabolic increments.

Dentine microsampling has also been performed on faunal teeth (Balasse et al., 2001; Kirsanow et al., 2008). Balasse et al. (2001) looked at $\delta^{13}C$ and $\delta^{15}N$ in the dentine of cattle. Molars were demineralized and sliced into 4 mm sections. Using this method, the authors were able to detect isotopic shifts corresponding to the known pattern of weaning. Kirsanow et al. (2008) microsampled at an even more precise level. Their study focused on determining seasonal variation in dentine collagen $\delta^{18}O$ and δD values of sheep and goats. Teeth were demineralized in EDTA solution and 1 mm diameter microsamples were taken in a longitudinal series moving down the molars using a punch. Although Kirsanow et al. worked with animals whose teeth are larger than humans' and form in horizontal layers, the punch they used also provides the key to microsampling human teeth.

1.1.3. Human tooth formation

The method presented here is based on the layered, parabolic pattern of human dentine formation and utilizes the neonatal line as a marker of birth. It also takes advantage of the fact that a portion of human deciduous tooth dentine is laid down prior to birth (Scheuer and Black, 2004; Van der Linden, 1983; Zaslansky, 2008), allowing in utero values to be studied in addition to values reflecting breastfeeding and weaning. A consideration of how teeth develop is an important aspect of this method.

Human teeth begin forming with the development of the dental lamina approximately six weeks after conception. The appearance and early development of the tooth germs that form along this

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