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A mini me? Exploring early childhood diet with stable isotope ratio analysis using primary teeth dentin

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ABSTRACT

Objectives: Reconstruct childhood diet using teeth collected in Edmonton in a collaborative effort between the Departments of Dentistry and Anthropology at the University of Alberta. **Methods:** Deciduous teeth needing extraction were collected from 33 children for stable isotope ratio analysis of diet. Tooth dentin was microsampled in three locations using a newly developed technique to reconstruct the changing pattern of participants' diet through early childhood including breastfeeding practices.

Results: The microsampling method can reconstruct diet with tiny samples (0.3 mg). The results reconstruct fetal isotope ratios, which showed significant variation. $\delta^{15}\text{N}$ values indicate some children were being breastfed (7/17), while others were likely bottle fed (10/17). Surprisingly, the early childhood results do not show the range of diets expected in adults based on known eating habits. Toddler diets form a tight cluster implying diets of similar isotopic composition in almost all of the households despite potential cultural and class distinctions ($\delta^{15}\text{N}$ values 11–11.5‰, $\delta^{13}\text{C}$ values around –18‰). The $\delta^{13}\text{C}$ values show a strong C_3 dependence for most children, a two outliers show C_4 (–12‰) dependence indicating a possible corn based diet.

Conclusions: Microsampling can potentially track each child's diet through early childhood. For this group of children, both breastfeeding and bottle feeding was practiced. However, the percent of breastfed infants was less than reported Canadian rates. Surprisingly, the choice to breastfeed or to bottle feed was not linked to the choice of toddler diet. All toddler diets were narrower in scope than adult diets.

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1. Introduction

There is a huge range of modern adult diets that makes dietary analysis using stable isotope ratio analysis difficult. What we eat is also important to our health, particularly our dental

health, where heavy carbohydrate diets can contribute to high caries rates. This paper outlines some of the uses of stable isotope ratio analysis for understanding the diet of modern children on both a population and individual basis. By independently analyzing breastfeeding versus bottle feeding, the method may also provide a way to determine

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over-reporting of breastfeeding by mothers trying to give researchers the “correct” answer. The research presented here is an excellent example of the innovative research that can stem from the collaboration of physical anthropologists and dentists who are interested in studying childhood nutrition, diet, and health.

Stable isotope ratio analysis is an accepted way to reconstruct diet in past and present populations.^{1,2} Omnivores, carnivores, and herbivores have different stable isotope ratios because the isotopic composition of their diets are different in a patterned way. Nitrogen and carbon analysis of animals and other foods in a food web allows unknown diets to be studied. When an animal eats another its $\delta^{15}\text{N}$ ratios are enriched due to preferential excretion of ^{14}N causing enrichment in ^{15}N relative to dietary protein.^{1,2} The trophic level effect in nitrogen is the key to studying weaning in children. A trophic elevation of $\sim+3\%$ is found in breastfed human infants because they are consuming their mother’s secretions.^{1,2} Bottle fed infants will not show this trophic effect, as they have a diet with similar isotopic composition to that of their mothers. Nitrogen can be used to determine the timing of breastfeeding and weaning by looking at the rise and fall of $\delta^{15}\text{N}$ values. By tracking this pattern, it is possible to determine what kinds of proteins the child is weaned onto. $\delta^{13}\text{C}$ ratios reflect C_3 or C_4 plant consumption. C_3 plants (more negative, -26% global average) are grains such as wheat or rye, while C_4 plants (less negative, -12% global average) include corn, sugarcane, and millet.^{1,2} $\delta^{13}\text{C}$ ratios of C_3 plants vary wildly (-24% to -36%) depending on environmental conditions like temperature and moisture. Human collagen is offset by $\sim 3\text{--}5\%$ from the diet.² Marine diets also result in less negative $\delta^{13}\text{C}$ ratios, as the marine source of carbon, dissolved carbonate (0%), is less negative than atmospheric CO_2 (-7%). This can lead to difficulty in interpretation in areas with C_4 plants and marine resources are present. However, $\delta^{15}\text{N}$ ratios are also affected by marine resource use (higher trophic elevation). By analyzing both carbon and nitrogen isotopes it is possible to determine if C_4 or marine resources are being utilized. Modern Western diets usually contain a mix of C_3 and C_4 plants, and therefore have intermediate carbon values.^{1,2} White and Schwarcz³ found in their study of ancient Maya that diets dominated by C_4 foods had enriched $\delta^{13}\text{C}$ ratios (-9 to -12%), while $\delta^{13}\text{C}$ ratios were depleted (-15%) during periods of mixed C_3 and C_4 plant use. Extreme C_4 diets are often still found in Mesoamerican descendent populations. These differences can be used to identify different food utilization practices of cultural groups within the larger population and could give insight into the retention specific food preferences and practices of specific groups. $\delta^{13}\text{C}$ also exhibits a small $\sim 1\%$ enrichment with breastfeeding.^{1,2}

Recently, research has focused on trying to develop serial sampling methods that are better able to track changing diets of individuals and access new areas of human diet, such as fetal diet.^{4–7} For this study, a microsampling methodology for stable isotope ratio analysis developed by the first author was used to look at changing diet, as well as breast feeding and weaning patterns of a population.⁷ This method focuses on single rooted teeth and uses very small microsamples (≥ 0.3 mg) allowing more control in sampling location. Modern teeth were used to create and test the method, before applying

it to archaeological materials. This data is presented here and in Burt and Garvie-Lok.⁷ Although the original goal was archaeological application, it became clear that this method was also valuable for looking at modern childhood diet, as a proxy for mobility, and looking at rates of breastfeeding. A modern child’s diet is complicated as young infants may be breastfed or bottle fed for a variable length of time before being weaned to solid foods. Mixed feeding of infants (i.e. bottle and breastfeeding) is also quite common for modern mothers. Breastfeeding causes an elevation in $\delta^{15}\text{N}$ ratios of infants over their mothers, but this does not occur when infants are eating prepared food such as formula.^{1,8} The prepared food or formulas used are usually isotopically similar to the mothers and the $\delta^{15}\text{N}$ ratios reflect this and there is no peak. The relationship of fetal and maternal pregnancy $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios are being increasingly studied as evidence for a possible offset between these tissues is found.^{8–10} Fetal tissues were thought to form directly from the maternal isotope pools with no fractionation, meaning fetal and maternal tissues formed during pregnancy should match.^{1,2,8–10} Fuller et al.⁹ found that maternal tissue $\delta^{15}\text{N}$ ratios did not hold steady during pregnancy, but were depleted relative to the woman’s diet. They speculated that positive nitrogen balance during pregnancy is causing this effect. Fuller et al.⁸ analyzed hair from mother infant pairs and found that while some infant $\delta^{15}\text{N}$ ratios seemed to match the mother’s ratios this was not uniform. De Luca et al.¹⁰ analyzed hair from a cohort of 239 mother infant pairs. The infant hairs were collected 3 days post birth. Hair like dentin does not remodel once formed and retains the fetal isotope information. Fetal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were closely correlated to that of the mother, but the fetal ratios were statistically significantly elevated. This indicates there may be an offset between maternal isotope composition during pregnancy and fetal ratios. This offset cannot currently be explained by the literature. Our data is interpreted in light of these complicated isotopic relationships.

2. Materials and methods

Thirty-five deciduous teeth were collected from twenty-four children aged 5–12 years during the paediatric dentistry clinics at the University of Alberta. All teeth needed to be extracted for dental reasons. The parents were asked to give informed consent for the donation of extracted teeth and where age permitted, assent of the child was also obtained (University of Alberta ASL REB Ethics Approval #2120). No personal data were collected or analyzed from the children. The dentists collecting the teeth report diverse ethnic and socioeconomic status of participants, though specific demographics are not known. To maximize sample size, all tooth types were collected. Of the 35 teeth donated, two were rendered unusable because of caries or prior dental work and were excluded from the analysis. This left a sample set of 33 teeth, some of which provided limited results due to extreme wear, caries, or root resorption. Teeth were cleaned and analyzed using a new method of microsampling for carbon and nitrogen stable isotope ratio analysis, which was developed on this collection.⁷ Only individuals with both carbon and nitrogen results are discussed in this paper.

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