WEANING AND CHILDHOOD DIET AT CA-CCO-297

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We reconstruct age of weaning and childhood diet in a population of individuals from CA-CCO-297, using stable isotope carbon and nitrogen analysis of dentinal collagen from permanent first molars. Because dental tissues do not remodel, teeth record a dietary signature of an individual at the time the teeth were forming, between age 0 and 9.5 for first molars. Results for six individuals with intact weaning curves indicate that children were weaned, on average, around 2.7 years of age. Five additional individuals did not display a weaning curve, likely due to heavy occlusal wear that removed the earlier-growing portions of the tooth. We also show that after weaning, children were consuming a diet rich in marine foods, and that isotopically, there is little difference between the diets of toddlers (age 1-3), children (age 4-9), and adults at CCO-297.

CA-CCO-297

CCO-297 is a single-component occupation site in Richmond, California (Figure 1) that was occupied for about 250 years by ancestors of the Ohlone tribe between A.D. 1450 and 1700. Nels Nelson, from the University of California at Berkeley, originally recorded the shell mound in 1905. Between 2011 and 2013, Alta Archaeological Consulting undertook salvage archaeological excavation while Pacific Gas and Electric Company (PG&E) relocated an existing natural gas line through the site (DeGeorgey 2013).

METHODS AND APPROACH

Collagen is a structural protein found in human bones that is synthesized from dietary intake. Stable isotope analysis of bone and dentinal collagen allows researchers to reconstruct certain aspects of an organism's diet (DeNiro and Epstein 1980; Schoeninger and DeNiro 1984). Bone remodeling is a life-long process where adult bone is reabsorbed into the body and new bone tissue is formed to replace the old in a process known as ossification (Price and Burton 2001). Because of this process, isotopic data from bone collagen reflect an individual's diet for the last 10-20 years before death. By contrast, dentinal collagen in human teeth does not remodel, and the stable isotope measures are reflective of diet during the time those teeth were forming. First molars begin growing at birth, the crown is completed at the cementum enamel junction (CEJ) around age 3, and the apical root tip closes around 9 or 10 years of age (Hillson 1996).

The sample size for this study consists of 11 first molars from different individuals at the site (Table 1). Some represent discrete burials, while others represent isolated first molars or first molars embedded in isolated mandible fragments. The samples were collected and analyzed with the permission of the State-appointed Most Likely Descendant (MLD), Ramona Garibay.

To reconstruct age at weaning and early childhood diet, we followed the methods of previous researchers (Eerkens et al. 2011; Fuller et al. 2003). Before destructive analysis began, we photographed the molars and removed and saved enamel, alveolar bone, and dental calculus, if present, for future isotopic studies. The first molars were cut in half using a low-speed saw, and the enamel and cementum were abraded away with a drill bit to remove any possible exogenous contaminants (e.g., soil). Samples were then rinsed and sonicated in deionized water, left to dry, and placed in 0.5M hydrochloric acid (HCl) at 5°C to demineralize. The HCl solution was replaced every day until the molar became spongy in texture and no longer reacted with the HCl solution (about 7-14 days). After demineralization, the sample

Figure 1. Map showing CA-CCO-297 and the surrounding geographic region.

Table 1. Burials and/or catalog numbers from CA-CCO-297 included in this study.

Individual Burial/Catalog	Sex	Age at Death
Burial 3	Male	Adult
Burial 11	Indeterminate	Adult
Burial 15	Male	Mature
Burial 16	Indeterminate	Child
Catalog # 40	Indeterminate	Indeterminate
Catalog # 56	Indeterminate	Indeterminate
Catalog # 129	Indeterminate	Mature
Catalog # 261	Indeterminate	Indeterminate
Catalog # 314	Indeterminate	Indeterminate
Catalog # 533	Indeterminate	Indeterminate
Catalog # 626	Indeterminate	Indeterminate

was rinsed and measured, taking into account the location of the CEJ and the dentino-enamel junction (DEJ) if present. The sample was then micro-sectioned in serial samples 1-2 mm thick using a cleaned scalpel. Depending on the degree of occlusal surface wear and other preservation limitations (e.g., a broken root tip), we were able to slice 5-12 serial sections per molar (see Figure 2). Demineralized serial sections were then placed in separate glass scintillation vials and treated with 0.125M sodium hydroxide (NaOH) for 24 hours to remove any humic contaminants. After 24 hours, the samples were triple rinsed in deionized water, submerged in pH≈3 solution, and placed in an oven at 80°C for the collagen to solubilize. The solubilized collagen was then freeze-dried. $\delta^{15}N$ and $\delta^{13}C$ were measured from the samples by continuous-flow mass spectrometry (PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer) at the Stable Isotope Facility at UC Davis. Due to the required 1 mg of collagen for each mass spectrometer run, we had to combine some consecutive serial samples to reach the minimum amount. Across the 11 individuals in the study, we averaged between eight and nine measurable serial samples per molar.

Dentinal collagen taken from serial samples in human teeth can be used to study dietary behaviors during the time those teeth formed because dentin incorporates carbon and nitrogen as teeth grow through childhood. The observed carbon and nitrogen isotope measures are reflective of the dietary choices of the individual, especially in terms of the protein sources consumed. $\delta^{15}N$ is the main isotopic signature to estimate age at weaning due to its trophic level effect, where $\delta^{15}N$ increases about 2 to 4‰ with each successive trophic level on a given food web (Minagawa and Wada 1984; Schoeninger 1985). Infants who are predominantly breastfeeding tend to be enriched in $\delta^{15}N$ one trophic level over that of their mothers (Fuller et al. 2006). When a child begins the process of weaning, $\delta^{15}N$ begins to decrease

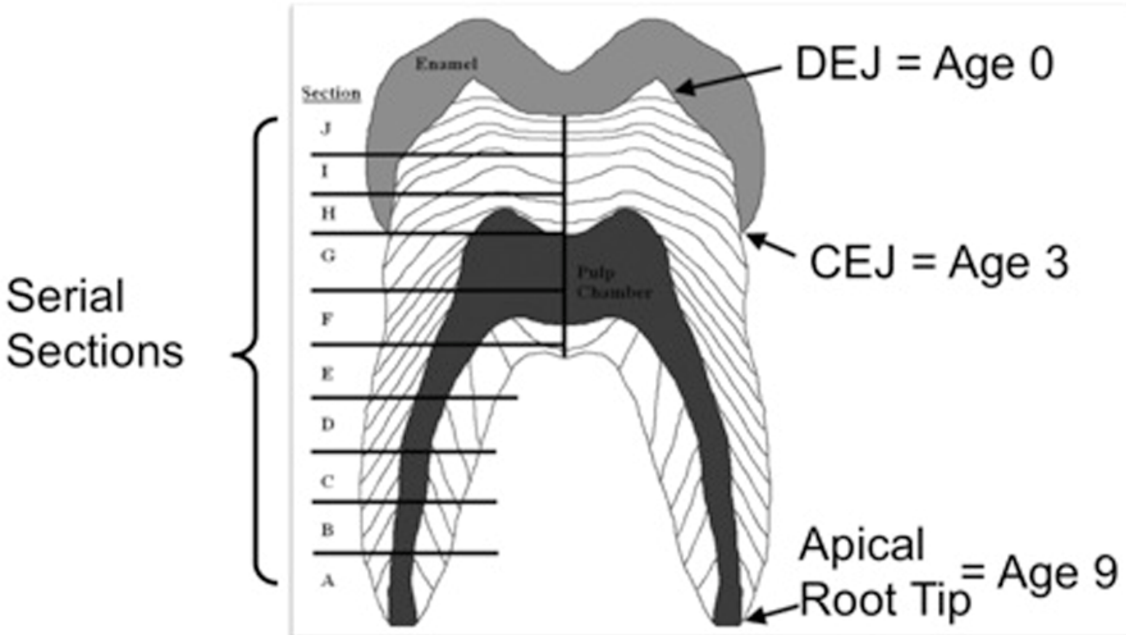

Figure 2. Diagram of a first molar depicting age-related landmarks and serial sections.

and falls in line with that of an adult in the area. The speed at which $\delta^{15}N$ measurements drop depends on how suddenly a child is weaned onto solid foods. Using the serial samples from first molars, we can observe the weaning process and estimate the age at weaning. $\delta^{13}C$ is useful, as it can help distinguish certain aspects of an individual's diet, specifically the importance of dietary proteins derived from C3 vs. C4 photosynthesizing plants or marine sources in an individual's diet (Farquhar et al. 1989). In prehistoric California, C4 plants were not extensively consumed, unlike the extensive consumption of the C4 plant maize in other parts of the Americas (Bartelink 2006, 2009). In the context of paleodiet in prehistoric California, $\delta^{13}C$ is effective at helping distinguish between marine and terrestrial protein sources (Schoeninger and DeNiro 1984).

RESULTS AND DISCUSSION

To calculate the age associated with each serial sample, we used landmarks (DEJ = 0 years, CEJ = 3 years, apical root tip = 9.5 years) and estimated growth rate within the crown and root (Eerkens et al. 2011).

In teeth that had observable weaning curves, the $\delta^{15}N$ values were highest in the serial sections associated with the crown (ca. 0-3 depending on occlusal wear). The $\delta^{15}N$ values were 2 to 4‰ above what is to be expected of the mother's breast milk, and began to decrease with each successive serial sample until all individuals averaged out around adult values in later childhood (see Figure 3). Using both the decreasing $\delta^{15}N$ signature and the age-assigned serial sections, we were able to calculate an age at weaning for six individuals. The average age of weaning for these individuals was 2.7 years of age. CCO-297 shows earlier weaning ages in comparison to other California sites during different time periods (Eerkens and Bartelink 2013). Earlier weaning may reflect lower levels of parental investment due to stressors.

Due to the majority of our samples coming from burials of indeterminate sex, we were not able to detect sex-based differences in weaning and post-weaning diet at CCO-297. Of the burials, only 3 and 15 were from a distinguishable sex, both male, and neither had a detectable weaning curve.

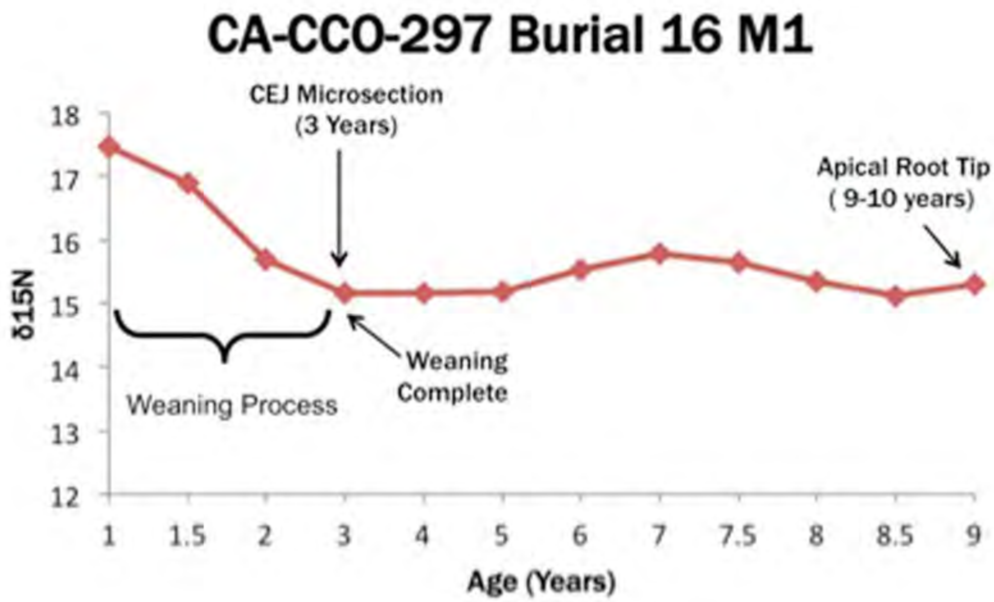

Figure 3. δ^{15}N measures and weaning curve of Burial 16.

Of the 11 individuals, five had no detectable weaning curve. These individuals showed no significant drop in δ^{15}N, and had relatively stable measures through time. The most plausible explanation for this is the heavy occlusal wear. Because teeth form from the DEJ downward, the earliest-forming dentin is in the upper portions of the crown. Thus, occlusal wear removes the early-forming dentin and can erase the weaning signal. For example, in Figure 4, occlusal wear has removed dentin associated with approximately the first three years of life, when weaning is likely to have occurred. The loss of early-forming dentin, combined with a relatively early weaning age, is the most likely reason why there are no observable weaning curves in these five individuals.

δ^{13}C distinguishes between marine and terrestrial protein sources. The high levels of δ^{13}C across all burials at CCO-297 indicate a stable diet of marine resources. For comparison, a δ^{13}C value near 20.0‰ is typically reflective of a terrestrial-focused diet. Because of the high levels of carbon enrichment, we were interested to see if weanling and older children had comparable isotope signatures, suggesting similar importance of marine-derived foods. To do this, we selected the δ^{13}C and δ^{15}N measures of each individual within the serial sections that correspond to an age of 3 years. We compared this measure to that of the δ^{13}C and δ^{15}N in the lowest apical root sections to provide an estimate of diet at age of 8-9. The benefit of observing differences between the serial sections of first molars is that these samples can account for seasonality and fluctuations in the natural resource availability through the time as the teeth were forming.

Results show that there were no major differences in diet at these different stages in life. In other words, while there might be minor fluctuations in diet through time, on average there was no isotopic difference between a toddler's and an older child's diet at CCO-297. We also have δ^{13}C and δ^{15}N isotopic data on bone collagen from 20 adult burials at CCO-297 provided by Eric Bartelink. This allows us to compare the childhood diet at different ages to that to the adult diet at CCO-297 (see Figure 5). While the average bone collagen δ^{13}C and δ^{15}N is slightly lower than the dentine collagen on average, the difference is not great, suggesting children's diets were isotopically similar to those of adults.

Regardless of differences between childhood and adult values, the overall ^{13}C enrichment seen at the site indicates a consistent inclusion of marine resources throughout childhood and into adulthood. This finding is supported by the site's bay shore location and the presence of shellfish (especially clam),

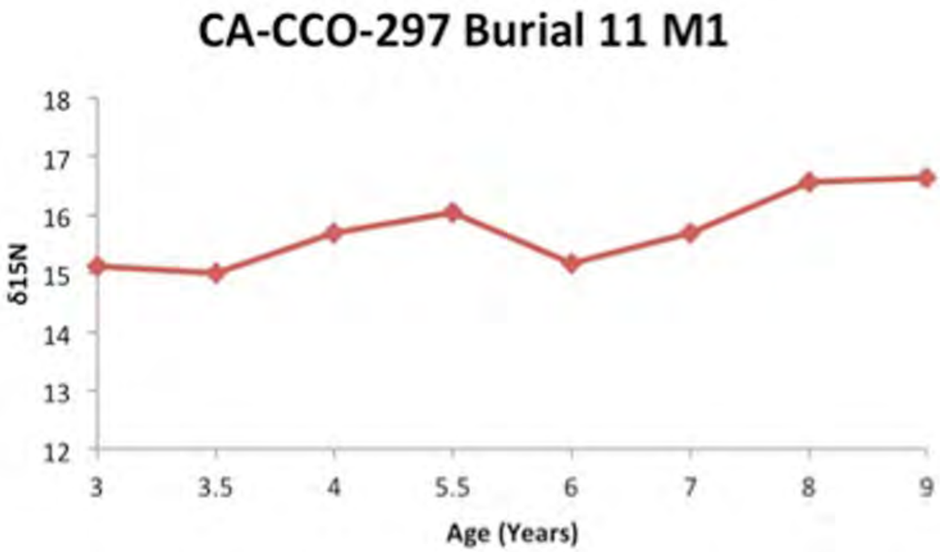

Figure 4. δ^{15}*N measures of Burial 11 showing the absence of a weaning curve.*

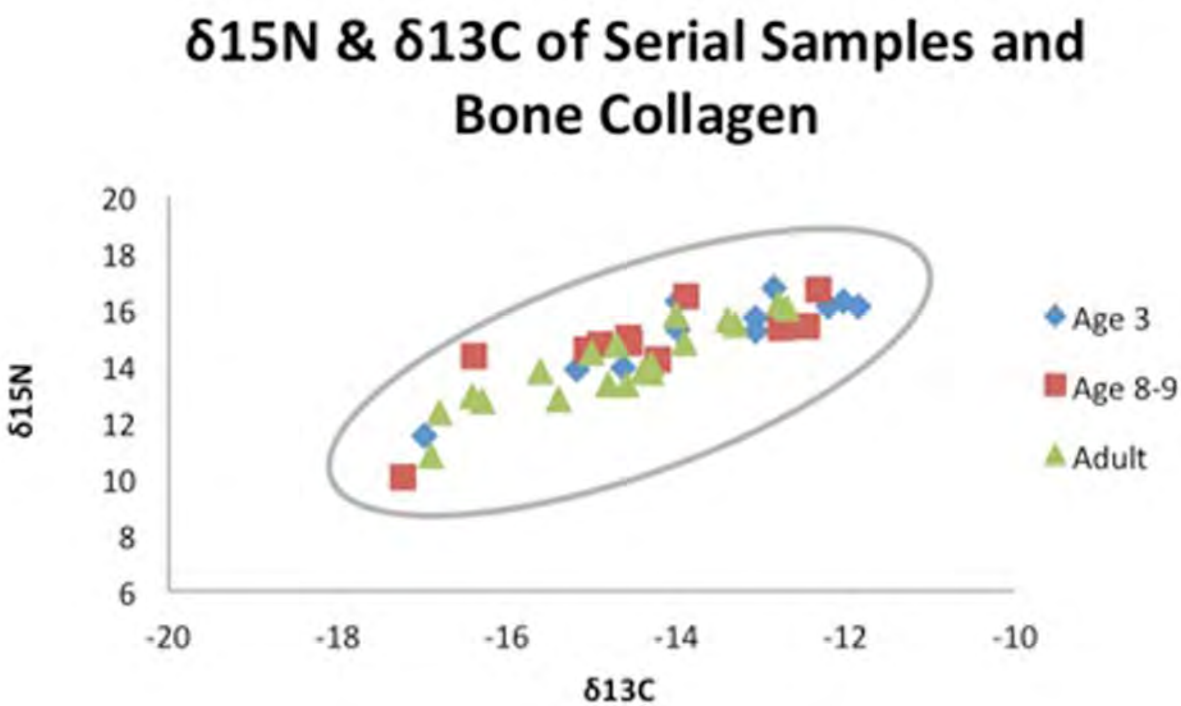

Figure 5. A scatterplot depicting the isotopic values of age landmark samples and adult bone collagen.

marine fish (especially herring and sardine), and marine mammals (especially otters) in the zooarchaeological assemblage (DeGeorgey 2013).

In conclusion, the relatively early weaning age may reflect lower levels of parental investment in individuals at CCO-297 compared to other sites in central California. $\delta^{13}C$ enrichment reflects consistent inclusion of marine resources from early childhood through adulthood. We aim to explore potential causes for these in future research and publications.

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