



HAL
open science

Intra-and inter-individual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in human dental calculus and comparison to bone collagen and apatite isotopes

Jelmer W Eerkens, Alex de Voogt, Tosha L Dupras, Samuel C Rose, Eric J Bartelink, Francigny Vincent

► To cite this version:

Jelmer W Eerkens, Alex de Voogt, Tosha L Dupras, Samuel C Rose, Eric J Bartelink, et al.. Intra-and inter-individual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in human dental calculus and comparison to bone collagen and apatite isotopes. *Journal of Archaeological Science*, 2014, 52, p. 64-71. 10.1016/j.jas.2014.08.020 . halshs-02539279

HAL Id: halshs-02539279

<https://shs.hal.science/halshs-02539279v1>

Submitted on 14 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Intra- and inter-individual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in human dental calculus and comparison to bone collagen and apatite isotopes



Jelmer W. Eerkens^{a,*}, Alex de Voogt^b, Tosha L. Dupras^c, Samuel C. Rose^a,
Eric J. Bartelink^d, Vincent Francigny^b

^a Department of Anthropology, University of California Davis, USA

^b Division of Anthropology, American Museum of Natural History, USA

^c Department of Anthropology, University of Central Florida, USA

^d Department of Anthropology, California State University, Chico, USA

ARTICLE INFO

Article history:

Received 24 December 2013

Received in revised form

13 August 2014

Accepted 14 August 2014

Available online 23 August 2014

Keywords:

Stable isotope analysis

Nubia

Central California

Paleodiet

Dental calculus

ABSTRACT

There are mixed opinions on the suitability of dental calculus for paleodietary reconstruction using stable isotope analysis. We examine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of calculus samples from two regions, central California in the USA and Sai Island in the Sudan. When atomic C/N ratios are less than 12 in calculus, results show positive correlations at both the regional and individual level between stable isotopes of bone collagen and calculus, suggesting these materials track similar dietary behaviors. Correlations are still positive but lower between $\delta^{13}\text{C}$ values of calculus and bone apatite. Stable isotope ratios of calculus show between 30% and 50% greater variation than bone, are typically enriched in ^{15}N (mean = 2.1‰ higher), and are depleted in ^{13}C relative to bone collagen (mean = 0.8‰ lower) and apatite (mean = 6.4‰ lower). Calculus from multiple teeth was analyzed separately for seven individuals to examine intra-individual variation. Results show that within an individual $\delta^{13}\text{C}$ varies up to 1.8‰, and $\delta^{15}\text{N}$ up to 2.1‰, which may explain some of the weak bone-calculus correlations previously reported in the literature. When atomic C/N ratios are greater than 12, calculus correlates more poorly with bone collagen, suggesting these samples should be treated with caution.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Archaeological teeth often contain adhering dental calculus, but this material is often overlooked by researchers as a material for archaeometric analysis (Lieverse, 1999). Only recently has the potential of calculus been highlighted in research, most notably through the extraction of plant starch grains from the calculus of archaeological humans, and more recently Neandertals (Hardy et al., 2012; Henry et al., 2010). Other recent analyses focus on recovering bacterial DNA in calculus (Preus et al., 2011), characterizing organic compounds by chromatography (Buckley et al., 2014), and measuring the concentration and distribution of particular elements such as calcium, phosphorus, and manganese (Charlier et al., 2010; Klepinger et al., 1977).

Unlike faunal and botanical remains from archaeological middens, which inform on the behaviors of groups of people, the analysis of calculus has the potential to inform on individual

behavior. Such data, by extension, can then be compared between the sexes, between individuals of different statures, and compared to health and social status, among other interesting sub-groupings of people within populations. As such, there exists the potential to link dietary behaviors to a wide range of biological and/or cultural categories in ways that other approaches often cannot. Such individual-level data are especially relevant in evolutionary anthropology, which focuses on the behaviors of individuals, and aids in the reconstruction of life histories.

Dental calculus is composed of mineralized plaque (Hillson, 1996). It consists of a range of inorganic minerals, including hydroxyapatite, octacalcium phosphate, and whitlockite, but can also encompass a suite of organic components, including plant fibers, glycoproteins, proteins, lipids, and carbohydrates (Kani et al., 1983; Lieverse, 1999). Deposits of silicates, including quartz, calcium silicates, and calcium–aluminum silicates can also precipitate into the porous structure of calculus in archaeological samples when teeth are in contact with ground water (Klepinger et al., 1977). In clinical studies, supragingival calculus most commonly forms on the lingual surfaces of anterior mandibular teeth and the buccal surfaces of maxillary molars adjacent to salivary ducts (White,

* Corresponding author. Tel.: +1 530 752 1348; fax: +1 530 752 8885.

E-mail address: jweerkens@ucdavis.edu (J.W. Eerkens).

1997:513). Progression can begin after tooth eruption, and may take a decade or more to form. In teeth that are not regularly cleaned of plaque, calculus can peak in volume around 30 years of age (White, 1997:510). The organic component provides the source material for stable carbon and nitrogen isotope analysis of dental calculus (Poulson et al., 2013).

Stable isotope analysis of collagen and apatite from bone and teeth provide important windows on our understanding of ancient diet and mobility practices. Such analyses have highlighted differences and/or similarities in diet of elites vs. commoners in complex societies (e.g., Ambrose et al., 2003; Somerville et al., 2013; Voutsaki et al., 2013), differential mobility practices among adults suggesting a societal preference for matrilineal or patrilineal post-marital residence (e.g., Dupras and Schwarcz, 2001; Scheeres et al., 2013; Toyne et al., 2014), and age at weaning and dietary differences by sex (e.g., Dupras et al., 2001; Dupras and Tocheri, 2007; Eerkens and Bartelink, 2013).

A recent analysis by Scott and Poulson (2012) employed stable isotope analysis of cleaned but otherwise untreated bulk dental calculus samples. They showed that stable isotope signatures in the calculus were within the range of what would be expected of bone collagen, though they did not analyze bone collagen from the same set of individuals. From this, they concluded that calculus may be a suitable material for reconstructing paleodiet. In a second study, Poulson et al. (2013) examined stable carbon and nitrogen isotopes in ancient dental calculus from northern Chile, and found, again, that results matched what was expected from bone collagen. For seven of the 28 burials studied, they examined calculus from two different areas of the dentition to evaluate intra-individual variation (Poulson et al., 2013:4578). However, as in Scott and Poulson (2012), their sample did not include isotopic data on paired calculus and collagen from the same individuals.

Contrasting these results, Salazar-García et al. (2014) concluded that $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ values of dental calculus showed little correlation to values from bone collagen from the same individuals in a sample of 35 burials from Medieval Spain. While calculus and bone $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were found to be similar for some individuals, for others they differed up to 10‰ in $\delta^{13}\text{C}$ and 3.5‰ in $\delta^{15}\text{N}$. As a result, they did not recommend stable isotope analysis of calculus as a means to reconstruct paleodiet.

Given these contrasting results, we wanted to re-evaluate the question of using calculus stable isotope analysis as a proxy for diet by conducting additional analyses on paired calculus and bone collagen samples, in this case from western North American hunter-gatherers and northeastern African agriculturalists. We also examine correlations with $\delta^{13}\text{C}$ in bone apatite for a subsample of these individuals, on which no previous research has been conducted. Finally, like Poulson et al. (2013), we extracted and analyzed separately calculus samples from multiple teeth for seven individuals, to estimate the degree of intra-individual variation, which we compare to intra-site and inter-site variation.

2. Samples

Supragingival calculus samples were taken from 46 individuals from two disparate regions. All sites were occupied during the latter half of the Holocene. Table 1 shows the samples included in the analysis, listed by site.

The North American sample includes 36 individuals from five Middle and Late Holocene sites in central California (see Fig. 1). Sites are included from a range of environmental zones, from the southern San Francisco Bay estuary, to brackish-water Suisun Marsh, to more inland locations, in an attempt to capture a range of potential isotope values. Although not all the burials included in this analysis were directly dated, all the cemeteries have multiple

Table 1
Distribution of samples included in this study.

Geographic region	Site	Calculus only	# calculus-collagen pairs	# calculus-apatite pairs	# indiv. with multiple calculus
California, USA	CA-ALA-554	0	4	0	0
California, USA	CA-CCO-548	1	6	6	2
California, USA	CA-SOL-270	0	21	8	0
California, USA	CA-SOL-11	0	2	2	0
California, USA	CA-SCL-134	0	1	1	0
California, USA	CA-SCL-287	0	1	1	0
Republic of the Sudan	Sai Island	5	5	5	5
Total		6	40	23	7

bone collagen radiocarbon dates indicating that each was in use during a relatively narrow window of time (see Eerkens et al., 2013; Estes et al., 2012; Leventhal et al., 2010). The sites (with number of burials and age range) include CA-ALA-554 ($n = 4$; 2000–600 cal BP), CA-CCO-548 ($n = 7$; 4000–3000 cal BP), CA-SOL-270 ($n = 21$; 2500–1700 cal BP), CA-SOL-11 ($n = 2$; 2300–1700 cal BP), CA-SCL-134 ($n = 1$; 2800–2300 cal BP), and CA-SCL-287 ($n = 1$; 2000–1300 BP). Previous analyses indicate the individuals represented in this study were hunter-fisher-gatherers, relying on a wide range of plant and animal resources. Data indicate that all cemeteries are associated with relatively permanent (i.e., sedentary) villages. For two individuals from CCO-548, multiple calculus samples were taken from different teeth. In total, 39 calculus samples were analyzed from California.

The African sample includes ten individuals from a single site on Sai Island in the Republic of the Sudan. Sai Island is the largest island of the Nile valley located between the 2nd and 3rd cataracts of Nubia (Fig. 2). The ten individuals were buried in an elite necropolis belonging to the Meroitic Kingdom (2400 BP to 1600 BP; de Voogt and Francigny, 2012), though the skeletal material for this study dates to a more narrow temporal window between the first and the third century CE (1950–1750 BP). The Meroites from the northern part of the Nubian kingdom, where Sai Island is located, mostly relied on riverine agriculture supported by the alluvium deposited during the annual flood. Herding seems to have been widespread but very limited compared to the southern half of the kingdom and the capital, where rainland agriculture was also developed. Though archaeobotanical data are limited, it is generally assumed that millet, sorghum, wheat and barley were cultivated by the Meroites (Thompson et al., 2008; Welsby, 1998:155–161). Bone samples were only available for five individuals. In addition, for five individuals, multiple calculus samples from different teeth were analyzed. In total, 26 calculus samples were analyzed from Sai Island.

3. Methods

Calculus preparation methods followed Scott and Poulson (2012). Samples were removed from the tooth surface using a scalpel, and were cleaned but not chemically treated. Calculus was rinsed in deionized water and non-calculus material, such as sediment, was removed by hand with a set of tweezers under a low-power microscope. Calculus was then powdered in a mortar and pestle. For individuals with multiple calculus samples, the resulting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were averaged (separately for C and N) to compare to bone collagen and apatite values.

Between 1 and 12 mg of powdered calculus was submitted to the Stable Isotope Facility at UC Davis, and measured for total C and N (in μg), and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, using a PDZ Europa ANCA-GSL

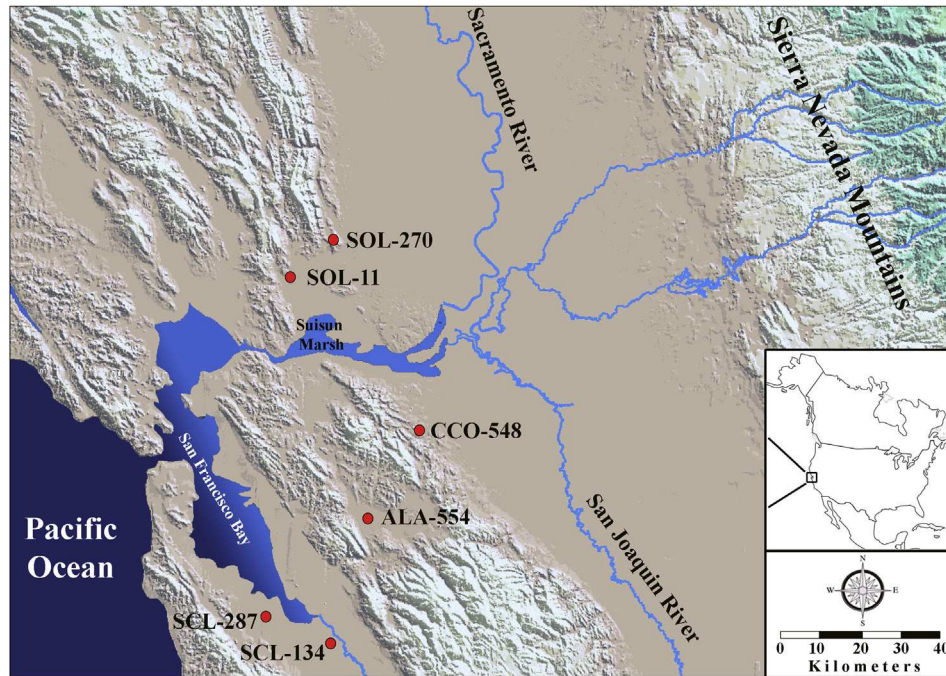


Fig. 1. Map of central California showing locations of sites included in this study.

elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). For samples with sufficient C (ca. 100 μg), the long-term standard deviation on this instrument is 0.2‰ for $\delta^{13}\text{C}$, and for samples with greater than 20 μg N, the long-term standard deviation is 0.3‰ for $\delta^{15}\text{N}$. Instrument precision decreases for smaller samples, and we only report values for samples containing greater than 20 μg C or

10 μg N. Analyses were limited by the amount of calculus present on a tooth and some of the samples in this analysis weighed less than 1 mg but were submitted nevertheless. Several of these produced enough C but not enough N for analysis. As well, for some individuals in the California sample, we combined calculus from more than one tooth to generate enough material for stable isotope analysis (see Supplementary information).

Bone collagen extraction for all samples followed a modified Longin procedure (Longin 1971). Approximately 1–2 g of cortical bone was cleaned of any surface contamination by drilling exposed surfaces with a diamond-studded bit and then sonicating the sample in deionized H_2O . The sample was demineralized with a solution of .5 M hydrochloric acid (HCl). The bone was then washed and soaked in .125 M NaOH (sodium hydroxide) to remove humic acids. The sample was then placed in slightly acidic water in a 70–90 °C oven for approximately 24 h to solubilize collagen and freeze dried to remove the water and isolate the collagen fraction. While the Californian collagen samples were analyzed on the same mass spectrometer as the calculus samples above, the Nubian collagen samples were analyzed on a Delta V Advantage Mass Spectrometer with a Carlo Erba NC2100 Elemental Analyzer at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University.

Bone apatite preparation started with the same cleaned bone, which was powdered in an agate mortar and pestle. Organics were removed by adding a 1.5% sodium hypochlorite at a ratio of 0.04 ml solution/mg sample (Koch et al., 1997), followed by a diagenetic wash composed of a 1 M acetic acid solution (at the same ratio of .04 ml solution/mg sample). The sample was then rinsed with dH_2O and dried. Apatite samples from California were analyzed on a GVI Optima Stable Isotope Ratio Mass Spectrometer at the Stable Isotope Lab in the Geology Department at University of California, Davis, with $\delta^{13}\text{C}$ measured on the carbonate component of bone apatite. Bone apatite for the Nubian samples were prepared using the same method as previously described, and analyzed on a Thermo-Finnigan Gasbench II and a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University.



Fig. 2. Map of northeast Africa, showing location of Sai Island.

4. Results

Complete data are available as [Supplementary Materials](#) to this article. While there was enough C ($\geq 20 \mu\text{g}$) in all calculus samples, ten samples did not produce enough N ($\geq 10 \mu\text{g}$) to be included in the analysis. In general, there was a strong and linear correlation between the sample weight and the recovered total C ($r^2 = 0.86$) and N ($r^2 = 0.77$), suggesting roughly similar elemental composition across the majority of samples. Overall, calculus samples averaged $50 \mu\text{g}$ of C per mg of calculus (i.e., 5% of calculus by weight), but just $7 \mu\text{g}$ of N per mg of calculus (0.7% of calculus by weight). These figures are very similar to [Salazar-García et al. \(2014\)](#), who report figures of 4.7% and 0.7% (C and N respectively), and [Scott and Poulson \(2012\)](#) at 4.9% and 0.7% for Medieval Spanish burials, but about half that reported by [Poulson et al. \(2013\)](#) for Late Holocene burials from Chile (11.7% C and 1.4% N). We note that there was some variation in our sample by region. The Nubian samples, on average, produced slightly more carbon (mean = 5.9%) than the California samples (mean = 4.5%), but nearly double the nitrogen (mean = 0.94% vs. 0.47%).

[Fig. 3](#) shows the correlations between isotopic values from calculus and bone with symbols used to denote different sites. The top and bottom panels show $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for calculus vs. bone collagen, respectively, while the middle panel shows $\delta^{13}\text{C}$ values for calculus vs. bone apatite. Also shown are best-fit regression lines and Pearson's r^2 correlation statistics for all individuals in the study together (heavy dashed line), as well as regression lines for individual sites (light dotted lines).

The data points in [Fig. 3](#) clearly separate the Californian from Nubian samples, for both calculus and bone. Our main goal here is not to reconstruct regional or individual differences in dietary behaviors from calculus, but rather, to explore patterns in calculus isotopes relative to those recorded in bone. Nevertheless, if dental calculus stable isotope signatures reflect diet, as bone does, these differences suggest quite different diets in the two continents. Of course, it is not surprising to find such dietary differences in sites representing hunter-gatherers in California, on the one hand, and agriculturalists in Nubia, on the other. However, if the differences are due to other, non-dietary factors such as environment, population genetic history, or the composition of oral bacteria communities, we would expect similar differences in such widely divergent samples. This study is currently unable to address this issue, but note that the different sites within California, populations with more similar genetic and environmental histories, also tend to cluster in the graph. In any case, these data point to unique calculus isotopic values from region to region and site to site, and that these patterns tend to mimic those in bone collagen. We note three additional patterns below.

First, when all individuals are examined collectively, the calculus isotopes are positively correlated with bone collagen isotope values for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, but are more weakly correlated with bone apatite $\delta^{13}\text{C}$ values. The correlation is strongest for $\delta^{15}\text{N}$ ([Fig. 3](#), lower panel), where there is an obvious and positive relationship ($r^2 = 0.62$). The correlation between calculus and bone collagen $\delta^{13}\text{C}$ is weaker ($r^2 = 0.13$), due primarily to elevated $\delta^{13}\text{C}$ calculus values from one site, CCO-548. If the CCO-548 samples are excluded, the collective correlation for $\delta^{13}\text{C}$ is much stronger ($r^2 = 0.61$) and nearly equal to the correlation with $\delta^{15}\text{N}$. On the other hand the figure suggests a positive but weak correlation between calculus and bone apatite $\delta^{13}\text{C}$ ($r^2 = 0.03$). Removing the CCO-548 samples again improves the correlation with bone apatite $\delta^{13}\text{C}$ ($r^2 = 0.36$), but it is still notably lower than correlations with bone collagen.

Note further that these relationships also tend to hold on a site-by-site basis. [Table 2](#) shows correlation statistics, and differences in

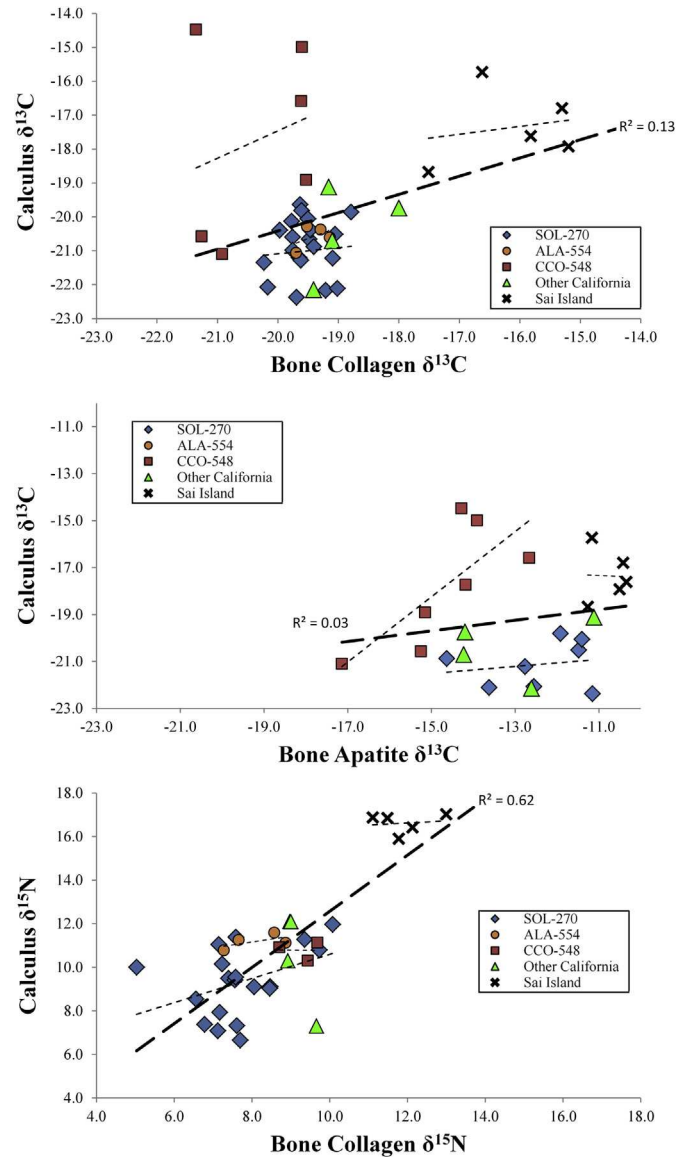


Fig. 3. Comparison of $\delta^{13}\text{C}$ in bone collagen (top) and apatite (middle) and $\delta^{15}\text{N}$ from bone collagen (bottom) against isotopes from calculus, by individual.

the statistical range of isotopic values for calculus vs. bone collagen, and apatite, for sites with more than three paired measurements. The strength of the correlation within sites is lower, as measured by r^2 , but still positive for calculus and bone within each site. The only exception is the calculus-bone apatite $\delta^{13}\text{C}$ values from Sai Island, where there is no correlation ($r^2 = 0$).

Second, calculus isotopes typically show greater variation, as measured by the range, than bone collagen. This, again, holds

Table 2
Correlation statistics, and the magnitude of the increase in range for calculus.

Calculus vs.		SOL-270	CCO-548	ALA-554	Sai Island	Overall
Collagen $\delta^{13}\text{C}$	r^2	0.01	0.06	0.23	0.04	0.13
	Range	+2.4‰	+4.8‰	+0.2‰	+0.6‰	+2.8‰
Apatite $\delta^{13}\text{C}$	r^2	0.03	0.56	n/a	0.00	0.03
	Range	+0.4‰	+2.2‰	n/a	+2.0‰	+1.1‰
Collagen $\delta^{15}\text{N}$	r^2	0.17	n/a	0.36	0.02	0.62
	Range	+0.3‰	-0.2‰	-0.8‰	-0.8‰	+2.4‰

Notes: n/a = no data available or data set too small for correlation ($n \leq 3$).

within sites and overall (Table 2). When considered as a collective data set, calculus $\delta^{13}\text{C}$ has a 2.8‰ greater range (46% higher) than bone collagen $\delta^{13}\text{C}$ and 1.1‰ greater range (16% higher) than bone apatite $\delta^{13}\text{C}$. Likewise, calculus $\delta^{15}\text{N}$ has a 2.4‰ greater range (30% greater) than bone collagen $\delta^{15}\text{N}$. Similarly, when we examine data within sites, calculus $\delta^{13}\text{C}$ is between 27% (Sai Island) and 364% (CCO-548) more variable than bone collagen $\delta^{13}\text{C}$, and between 10% (SOL-270) and 48% (CCO-548) more variable than bone apatite $\delta^{13}\text{C}$. On the other hand, calculus $\delta^{15}\text{N}$ is less variable than bone collagen at CCO-548, ALA-554 and Sai Island, but is more variable at SOL-270.

Third, the data also show that the absolute $\delta^{13}\text{C}$ values in calculus tend to be lower (i.e., more negative), but $\delta^{15}\text{N}$ elevated, relative to bone. Thus, individuals tend to fall in the lower right (i.e., below the diagonal) in the plots for $\delta^{13}\text{C}$, but in the upper left (above the diagonal) for $\delta^{15}\text{N}$ in Fig. 3. The only exception to this pattern are the individuals from CCO-548, where calculus $\delta^{13}\text{C}$ is elevated relative to bone collagen.

Because human bone collagen has a relatively fixed chemical composition, the atomic C/N ratio is a useful indicator of sample quality in ancient bone collagen (DeNiro, 1985; Weber et al., 2005). For bone collagen, studies show that samples with C/N between 2.7 and 3.3 produce $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within expected ranges, while C/N falling above or below this range more often produces unusual or outlying isotopic values. All bone collagen samples in this study produced C/N within this range. No such accepted C/N measure exists for calculus, and the more variable biomolecular composition of calculus makes it difficult to predict an acceptable range for “good” samples. Calculus samples in this study vary from 29.0 to 5.9 in C/N, with a mean of 9.9 ± 3.2 (1 SD).

However, our data show that there are notable patterns in C/N ratios and the calculus-bone offset. Fig. 4 plots C/N in calculus against the $\delta^{13}\text{C}$ value of calculus minus the $\delta^{13}\text{C}$ value of bone collagen (the calculus-bone collagen offset). For comparison, we also include data published by Salazar-García et al. (2014) from a medieval period site in Spain. The figure shows, first, that $\delta^{13}\text{C}$ is generally lower in calculus than in bone collagen, as discussed above, resulting in negative values on the y-axis. Second, the calculus-bone collagen $\delta^{13}\text{C}$ offset is increasingly variable for samples where the calculus C/N ratio is greater than 12. One site in particular (CCO-548) has consistently high C/N, and unlike other sites, the calculus-bone collagen offset for $\delta^{13}\text{C}$ is notably positive for nearly all samples at this site (see Fig. 3, top panel). For calculus samples with C/N less than 12, the mean calculus-bone collagen offset for $\delta^{13}\text{C}$ was -1.3‰ ($0.9 = 1$ SD). Likewise, for these samples

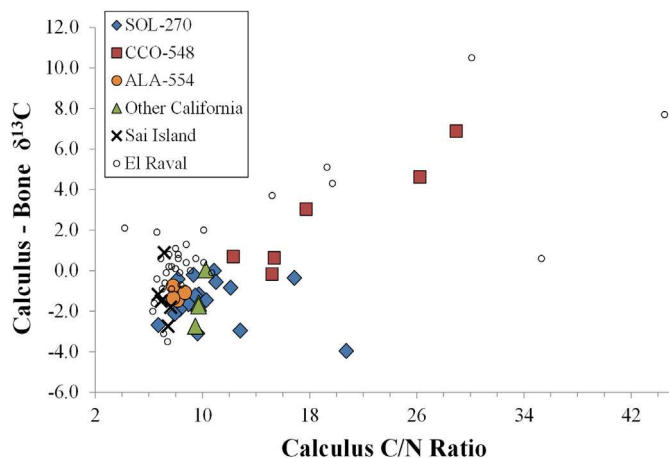


Fig. 4. Calculus – Bone collagen offset for $\delta^{13}\text{C}$ vs. calculus C/N ratio.

the mean calculus-bone apatite $\delta^{13}\text{C}$ offset was -7.8‰ ($1.6 = 1$ SD) and the mean calculus-bone offset for $\delta^{15}\text{N}$ was 2.1‰ ($1.8 = 1$ SD). Note that this pattern among calculus samples with high C/N ratios also holds among the Medieval Spain samples reported by Salazar-García et al. (2014) from El Raval, and may explain some of their reservations about using calculus to predict bone collagen values.

Fig. 5 shows the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results of repeated sampling of calculus from different teeth within the same individual for two CCO-548 and five Sai Island burials. The x-axis denotes different individuals, reproduced in the same order for the upper and lower panes, and the small black circles represent all samples from that particular site. The figure shows that calculus samples from different teeth within an individual do not produce identical isotope values, but differ by up to 1.8‰ for $\delta^{13}\text{C}$ and 2.1‰ for $\delta^{15}\text{N}$. This is slightly greater than the maximum intra-individual differences of 1.0‰ and 2.0‰ found by Poulson and colleagues for calculus samples in Chile (Poulson et al., 2013). As well, it is much greater than the degree of instrument precision (0.2–0.3‰).

At the same time, the figure also shows that the ranges and standard deviations of values within an individual are less than the range and standard deviation of values within each site as a whole. Thus, the standard deviation of $\delta^{13}\text{C}$ values in all Sai Island samples is 1.3‰, while standard deviations for T035-individual 2 and T030-individual 5 are 0.6‰ and 0.3‰, respectively (the only individuals with more than three samples). In spite of small sample sizes and a lack of information about the underlying statistical distribution of our isotope data, two-sample F-tests comparing equality of variance for intra-tooth vs. intra-population values for $\delta^{13}\text{C}$ are significant for both individuals ($p = 0.01$ and 0.04 , respectively). Likewise, the standard deviation in $\delta^{13}\text{C}$ for all CCO-548 samples is 7.2‰, while burial 59 has a standard deviation of just 1.2‰ (F-test comparing equality of variance is not significant, with $p = 0.10$, but the sample size is much smaller). The same general pattern holds

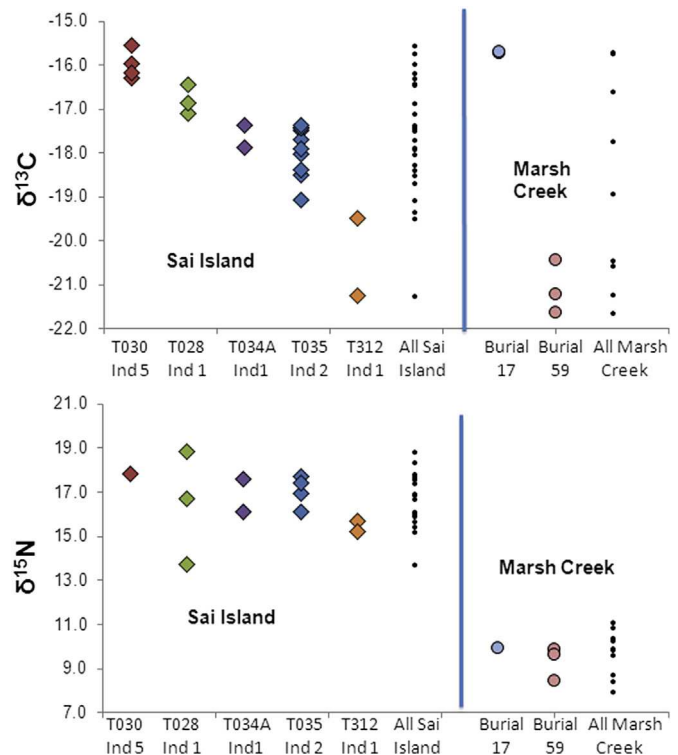


Fig. 5. Intra-individual variation in dental calculus $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at Sai Island and CCO-548.

more weakly for $\delta^{15}\text{N}$ values, though there are fewer samples available for analysis, precluding performance of F-tests. Thus, intra-individual variation for $\delta^{15}\text{N}$ is closer to the inter-individual variation for a particular site. While we do not place great interpretational weight on the results from the statistical tests, they do support our general argument that the degree of isotopic variation is less within individuals than within the population as a whole.

We also examined isotopic variation by tooth type and position in the mouth. All but one of our repeat samples are from mandibular teeth, thus we are unable to compare teeth from the upper and lower oral cavity. Teeth from the left and right side of the mandibular dentition overlap completely, suggesting no difference. However, the data do indicate differences between posterior and anterior teeth. For example, the incisors and canines of T035-individual 2 at Sai Island have slightly more negative $\delta^{13}\text{C}$ values (mean = -18.5‰ ; $n = 4$), relative to premolars and molars (mean = -17.6‰ ; $n = 6$). Even though the sample size is small, a *t*-test comparing calculus from anterior vs. posterior teeth (2-tailed, assuming unequal variance) is statistically significant ($p = 0.02$) for this individual. On the other hand, three other individuals show the opposite pattern, though the differences are not significant. $\delta^{13}\text{C}$ values of calculus from a molar from T312 is more negative (-21.3‰) relative to the canine (-19.5‰), calculus from the molar of T034A-Individual 1 is more negative (-17.9‰) relative to the canine (-17.4‰), and the molar and premolar are more negative (mean = -16.2‰) than the incisor and canine (mean = -15.8‰) from T030-Individual 5. There are not enough $\delta^{15}\text{N}$ values to make a similar comparison for nitrogen. These results, particularly as they pertain to intra-individual variation, are discussed further below.

5. Discussion

Our results suggest several items of interest for isotopic analyses of human dental calculus. First, calculus C and N isotope values appear to conserve the between-site differences that are present in bone collagen and bone apatite isotopes. This is consistent with the studies of Scott and Poulson (2012) and Poulson et al. (2013), who found that stable isotopes of calculus were similar to previously published studies of bone collagen. In support of those studies, we agree that isotopic analysis of calculus may be a useful tool for estimating general dietary differences between regions or over time in a single area when bone is not available.

Second, while preserving inter-regional signals, there appears to be an offset in our samples between calculus and bone in that the former are often depleted in ^{13}C but enriched in ^{15}N . If calculus isotopes reflect diet, this finding is consistent with the notion that a significant portion of the carbon in calculus is derived from organic plant matter, such as starch and cellulose. These biomolecules are high in carbon but low in nitrogen, and would therefore contribute mainly to the $\delta^{13}\text{C}$ signatures in calculus. Terrestrial C_3 plants typically have lower $\delta^{13}\text{C}$ values than tissues derived from fish or other animal sources, especially proteins which contribute the majority of carbon to bone collagen (Fernandes et al., 2012). This could account for the lower $\delta^{13}\text{C}$ values in calculus relative to bone.

The higher mean $\delta^{15}\text{N}$ values in calculus (averaging 2.1‰ higher in this study) could likewise be due to the presence of significant amounts of bacteria and bacterial byproducts preserved in calculus. The microbial mass preserved in calculus is expected to be enriched in ^{15}N relative to the proteins ingested by humans (and routed to synthesize bone collagen). Indeed, recent studies of human dental plaque show tremendous bacterial diversity, with well over 300 different species present in a single sample (Paster et al., 2001). We are not aware of isotopic studies specifically on human oral bacteria, but studies on other bacterial colonies indicate extremely complex fractionation processes that vary greatly depending on the

energy sources introduced (e.g., protein, glucose, cellulose, etc.) and interactions between different bacterial species (Wattiaux and Reed, 1995). Variation among different human populations, and/or individuals within populations, in the species diversity and number of bacteria in the oral cavity are likely to contribute to offsets between dietary $\delta^{15}\text{N}$, bone collagen $\delta^{15}\text{N}$, and calculus $\delta^{15}\text{N}$.

Our results also show that the magnitude of the offset in calculus relative to bone can vary by site and region. Even within California, CCO-548 appears to have elevated $\delta^{13}\text{C}$ values in calculus relative to bone while other sites show the opposite pattern. This suggests that it may not be possible to simply add or subtract a given amount from calculus $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to approximate what would be observed in bone. These inter-site differences in calculus-bone offsets point to either variation in the composition of calculus from site to site, which may be indicative of differences in paleodiet, or to preservational conditions at different sites in which case calculus isotopes are less useful for reconstructing paleodiet. Lacking additional information on the composition of calculus samples and/or a good index for estimating the state of preservation (as exists for bone collagen using collagen yield and C/N ratios), it is difficult to evaluate which of these scenarios accounts for the patterns reported (though see below for discussion on C/N ratios in calculus).

Third, while regional signals are preserved within sites, calculus isotope values only marginally preserve the inter-individual variation in C and N isotopes observed in bone collagen and apatite. Correlations between bone and calculus isotopes are positive within sites, suggesting a relationship exists between the two indices, but low, indicating that the nature of that relationship is more complex. Of course, this finding does not necessarily mean that calculus isotopes do not inform on inter-individual differences in diet. As we have shown, there is significant inter-tooth variation in the isotopes of calculus for a single individual, up to 1.8‰ for $\delta^{13}\text{C}$ and 2.1‰ for $\delta^{15}\text{N}$. In cases where Poulson et al. (2013) took multiple calculus samples from a single individual, they found a similar magnitude of isotopic variation. Moreover, this effect has been shown in isotopic studies of bone when multiple bone samples from the same individual are analyzed (Jørkov et al., 2009; Lucy et al., 2009), and for tissue samples from animals fed a monotonous diet (DeNiro and Schoeninger, 1983). All of this suggests that the particular bone sampled for collagen and apatite, and the exact tooth chosen for sampling calculus, may contribute to some of the discordance between bone and calculus isotopic signatures.

The source of inter-tooth isotopic variation may relate to the types of foods masticated with molars and premolars vs. incisors and canines, and hence, the organic material that accumulates in calculus on those teeth. It is not clear from our data that there are systematic differences between the location of a tooth in the oral cavity and isotopic values. Thus, while three of four individuals from Sai Island showed lower $\delta^{13}\text{C}$ in anterior teeth (incisors/canines), one showed elevated values. In addition, it is possible that the inter-tooth differences relate to the length of time represented by calculus deposits. Thus, calculus on incisors and canines may break off more readily than calculus on molars and premolars, particularly if an individual is using their anterior teeth as tools. If an individual shifted the isotopic composition of their diet over the course of seasons or years, this may be reflected in the isotopic composition of calculus on different teeth. In this respect, molars and premolars may be more similar to one another, and may be different from incisors and canines, because the calculus on those teeth accumulated during an overlapping window of time. In fact, SEM and TEM studies show that calculus forms accretionally, marked by periods of deposition 20–400 μm thick, followed by periods of quiescence (White, 1997:514). This means that bulk samples of calculus likely represent an aggregate of different

depositional episodes and thus may contain different dietary timeframes.

Fourth, we suggest that C/N ratios may be useful for identifying calculus samples that correlate poorly with bone collagen values, especially for $\delta^{13}\text{C}$. Empirically, we find that calculus-bone differences are much less variable when the C/N ratio from the calculus sample is less than 12. By contrast, calculus samples with C/N above 12 have $\delta^{13}\text{C}$ values that are often highly divergent from bone collagen, with calculus $\delta^{13}\text{C}$ values typically highly elevated (less negative) by up to 7‰. The effect is less pronounced for $\delta^{15}\text{N}$. Data published in Salazar-García et al. (2014) show a similar effect. In their study, calculus samples with C/N ratios above 12 are also highly variable in $\delta^{13}\text{C}$, and are typically elevated (by up to 10‰).

It is unclear if this pattern among calculus samples with high C/N is a result of poor preservation (diagenesis), or represents reasonably preserved calculus samples that have a different biomolecular makeup, and hence, unusual isotopic composition relative to bone collagen. We suspect the former, but additional research will be necessary to address this issue. In any case, empirical evidence suggests that if we are trying to estimate paleodiet using calculus, and are seeking to replicate values that would be obtained from bone collagen, that we should eliminate calculus samples with C/N ratios greater than 12.

6. Conclusions

Previous studies on the C and N stable isotope composition of dental calculus have resulted in mixed opinions on the utility of this material for reconstructing paleodiet, with some positive (Scott and Poulson, 2012; Poulson et al., 2013) and others negative (Salazar-García et al., 2014). Our independent analyses on paired calculus-bone samples from western North America and northeastern Africa suggest generally positive results. Isotopic signals in dental calculus effectively reproduce regional-level differences observed in bone. These patterns hold at the inter-individual level within sites as well, but the correlations are weaker, especially with respect to $\delta^{13}\text{C}$ values in bone apatite.

We suggest that the poor results obtained by Salazar-García et al. (2014) relate, in part, to three factors. First, they only analyzed samples from a single site and time period, the Medieval Spanish site of El Raval. We showed that correlations between calculus and bone isotopes are weaker within sites (vs. across sites) due to more similar diets among individuals, and hence, the more limited range of isotopic values observed. Second, and related, there is significant inter-tooth variation in dental calculus isotopes within a single individual, in our study up to 1.8‰ for $\delta^{13}\text{C}$ and up to 2.1‰ for $\delta^{15}\text{N}$. As a result, the particular tooth sampled can have an impact on calculus-bone isotope correlation coefficients, especially within a single site with a smaller range of values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Third, calculus samples with C/N ratios greater than 12 are often isotopic outliers. Including these samples decreases correlations between paired calculus and bone isotope samples considerably. Removing these samples from the El Raval study produces results that generally mimic those from bone collagen in the same individuals.

That a positive correlation in the isotopes of bone and calculus exists is not surprising. Like bone, the synthesis of calculus begins with much of the same basic building materials, that is, substances that are put into the mouths of individuals. Most of this, of course, will be food consumed by individuals, though calculus may be partially formed from other non-food materials present in the mouth (e.g., quids; Buckley et al., 2014). Bacteria in the oral cavity may fractionate C and N in food differently than bacteria in the gut, and human digestive systems, do. In other words, despite the same starting substances, C and N may be partitioned and fractionated in

different manners prior to incorporation into the structure of calculus and bone. This may partly account for the positive correlations, but also the offsets, we see for isotopes in dental calculus (lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ values), and may also add “noise” to the bone-calculus correlation.

The exact nature of the correlation and offset between calculus and bone $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is not well established. The results reported here suggest that the offset may vary between sites and regions, or may be dependent on calculus (and/or bone) preservation. For example, calculus $\delta^{13}\text{C}$ values are elevated relative to bone collagen at CCO-548, but are otherwise lower at sites in this study. Whether this variation is due to differences in ancient diet, differences in oral hygiene practices, the structure of calculus or bone collagen, or preservation conditions, is not yet clear. In this respect, it may be significant that CCO-548 is the oldest of the sites included in the study.

What seems clear is that further research is needed on the formation processes of dental calculus. In particular, we need a better understanding of how different macronutrients in foods contribute to the overall isotopic composition of calculus, much in the way that we know that approximately 75% of the isotopic composition of bone collagen relates to dietary protein (Fernandes et al., 2012; Froehle et al., 2010, 2012; Kellner and Schoeninger, 2007; Schoeninger et al., 1983). It is clear that plant macrofossils and other organic compounds are readily preserved in dental calculus (Buckley et al., 2014; Hardy et al., 2009, 2012; Piperno and Dillehay, 2008). Because plant products are high in carbon but low in nitrogen, it is possible that a significant component of the carbon in calculus derives from plant products. Because C_3 plants comprise much of the diets in California and Nubia, this may explain the lower $\delta^{13}\text{C}$ values we observed in our calculus samples relative to bone collagen. Future studies could investigate this by splitting calculus samples and treating portions with particular chemical washes (e.g., with acid to demineralize samples, or with solvents to remove organics), focusing isotopic analyses on particular organic fractions of calculus. The main drawback of such an approach is that calculus samples are often quite small to begin with and do not always produce enough nitrogen to make precise isotope ratio analysis possible. Advances in mass spectrometry instrumentation are rapidly lowering minimum sample size requirements and may promote such approaches in the future.

In any case, the greater range of variation in calculus $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the lower correlations between calculus and bone isotope values within sites is in many regards desirable. Assuming calculus isotopes are reflective of some aspect of paleodiet, it opens the door for complementing rather than duplicating bone isotopic analyses, which reflect primarily protein intake in the case of bone collagen and whole diet in the case of apatite. In this respect, isotopic analysis of calculus may hold the potential to inform on other aspects of diet, such as carbohydrate intake. Even if calculus and bone isotopes inform on largely the same aspects of paleodiet, they may still inform on dietary behaviors over different windows of time. Supplementing isotopic analyses of bone with those of calculus could then generate additional life history information for particular individuals, and dietary changes within their lives. In short, we believe there is still much potential in dental calculus to inform on the dietary histories of individuals.

Acknowledgments

Funding for the isotopic analyses was provided, in part, by a National Science Foundation grant to JWE and EJB (BCS 0819968 and BCS-0819975) and a UC Davis President's Undergraduate Fellowship awarded to SCR and JWE. Funding for T. Dupras' participation in this project was provided through a research grant

from The Institute for the Study of the Ancient World at New York University. We thank Howard Spero, Joy Matthews, and the research staff at the UC Davis Stable Isotope Facility for advice and assistance with running unusual samples. We also thank Ramona Garibay, most likely descendent, for supporting the scientific analysis of the California samples, and Randy Wiberg, Richard Fitzgerald, Gina Jorgenson, Melanie Beasley, Alan Leventhal, Jennifer Blake, Kari Lentz, Susan Talcott, Alex Greenwald, Jordon Munizzi, Katie Whitmore, and Katherine Page for assistance with collecting and analyzing samples. Lucas Barton and Seth Newsome generously provided some of their unpublished bone collagen data from CA-SOL-270. We thank the National Museums of Khartoum, Sudan, the American Museum of Natural History, New York, and Gisselle Garcia-Pack for their permission and assistance with the collection of the Sai Island material.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jas.2014.08.020>.

References

- Ambrose, S.H., Buikstra, J., Krueger, H., 2003. Status and gender difference in diet at Mound 72, Cahokia, revealed by isotopic analysis of bone. *J. Anthropol. Archaeol.* 22, 217–226.
- Buckley, S., Usai, D., Jakob, T., Radini, A., Hardy, K., 2014. Dental calculus reveals unique insights into food items, cooking and plant processing in prehistoric Central Sudan. *PLoS One* 9, e100800–e100808.
- Charlier, P., Huynh-Charlier, I., Munoz, O., Billard, M., Brun, L., Lorin de la Grandmaison, G., 2010. The microscopic (optical and SEM) examination of dental calculus deposits (DCD). Potential interest in forensic anthropology of a bioarchaeological method. *Leg. Med.* 12, 163–171.
- DeNiro, M.J., 1985. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317, 806–809.
- DeNiro, M.J., Schoeninger, M.J., 1983. Stable carbon and nitrogen isotope ratios of bone collagen: variations within individuals, between sexes, and within populations raised on monotonous diets. *J. Archaeol. Sci.* 10, 199–203.
- de Voogt, A., Francigny, V., 2012. Opening a grave in antiquity: formation and interpretation in the kingdom of Meroe. *J. Afr. Archaeol.* 10, 59–70.
- Dupras, T.L., Schwarcz, H.P., 2001. Strangers in a strange land: stable isotope evidence for migration in the Dakhleh Oasis, Egypt. *J. Archaeol. Sci.* 28, 629–636.
- Dupras, T.L., Schwarcz, H.P., Fairgrieve, S.I., 2001. Infant feeding and weaning practices in Roman Egypt. *Am. J. Phys. Anthropol.* 115, 204–212.
- Dupras, T.L., Tocheri, M.W., 2007. Reconstructing infant weaning histories at Roman period Kellis, Egypt, using stable isotope analysis of dentition. *Am. J. Phys. Anthropol.* 134, 63–74.
- Eerkens, J.W., Bartelink, E.J., 2013. Sex-biased weaning and early childhood diet among middle holocene hunter–gatherers in Central California. *Am. J. Phys. Anthropol.* 152, 471–483.
- Eerkens, J.W., Bartelink, E.J., Gardner, K.S., Wiberg, R.S., 2013. The evolution of a cemetery: rapid change in burial practices in a middle holocene site in Central Alta California. *Calif. Archaeol.* 5, 3–35.
- Estes, A., Buckley, D., Lentz, K., Sanchez, R., Wheelis, B., Wohlgenuth, E., Blake, J., Farnsworth, P., Price, H., Arrigoni, A., Fino, N., Young, T., 2012. Report on Archaeological Data Recovery at CA-ALA-554, Pleasanton, California. In: Data Recovery, vol. I. William Self Associates, Walnut Creek, CA.
- Fernandes, R., Nadeau, M.J., Grootes, P.M., 2012. Macronutrient-based model for dietary carbon routing in bone collagen and bioapatite. *Archaeological Anthropological Sci.* 4, 291–301.
- Froehle, A.W., Kellner, C.M., Schoeninger, M.J., 2010. FOCUS: effect of diet and protein source on carbon stable isotope ratios in collagen: follow up to Warinner and Tuross (2009). *J. Archaeol. Sci.* 37, 2662–2670.
- Froehle, A.W., Kellner, C.M., Schoeninger, M.J., 2012. Multivariate carbon and nitrogen isotope model for the reconstruction of prehistoric human diet. *Am. J. Phys. Anthropol.* 147, 352–369.
- Hardy, K., Blakeny, T., Copeland, L., Kirkham, J., Wrangham, R., Collins, M., 2009. Strach granules, dental calculus and new perspectives on ancient diet. *J. Archaeol. Sci.* 36, 248–255.
- Hardy, K., Buckley, S., Collins, M.J., Estalrich, A., Brothwell, D., Copeland, L., García-Taberero, A., García-Vargas, S., de la Rasilla, M., Lalueza-Fox, C., Huguet, R., Bastir, M., Santamaría, D., Madella, M., Wilson, J., Cortés, A.F., Rosas, A., 2012. Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus. *Naturwissenschaften* 99, 617–626.
- Henry, A.G., Brooks, A.S., Piperno, D.R., 2010. Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). *Proc. Natl. Acad. Sci.* 108, 486–491.
- Hillson, S., 1996. *Dental Anthropology*. Cambridge University Press, Cambridge.
- Jørkov, M.L.S., Heinemeier, J., Lynnerup, N., 2009. The petrous bone—a new sampling site for identifying early dietary patterns in stable isotopic studies. *Am. J. Phys. Anthropol.* 138, 199–209.
- Kani, T., Kani, M., Moriwaki, Y., Doi, Y., 1983. Microbeam X-ray diffraction analysis of dental calculus. *J. Dent. Res.* 62, 92–95.
- Kellner, C.M., Schoeninger, M.J., 2007. A simple carbon model for reconstructing prehistoric human diet. *Am. J. Phys. Anthropol.* 133, 1112–1127.
- Klepinger, L.L., Kuhn, J.K., Thomas, J., 1977. Prehistoric dental calculus gives evidence for coca in early coastal Ecuador. *Nature* 269, 506–507.
- Koch, P.L., Tuross, N., Fogel, M.L., 1997. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *J. Archaeol. Sci.* 24, 417–429.
- Leventhal, A., DiGiuseppe, D., Atwood, M., Grant, D., Morley, S., Cambra, R., Field, L., Nijmeh, C., Arellano, M.V., Rodriguez, S., Guzman-Schmidt, S., Gomez, G.E., Sanchez, N., 2010. Final Report on the Burial and Archaeological Data Recovery Program Conducted on a Portion of a Middle Period Ohlone Indian Cemetery, Yuki Kutsuimi Saatoš Inuxw [Sand Hill Road] Sites: CA-SCL-287 and CA-SMA-263. Stanford University, California (Report Prepared for Stanford University).
- Lieverse, A.R., 1999. Diet and the aetiology of dental calculus. *Int. J. Osteoarchaeol.* 9, 219–232.
- Lucy, S., Newman, R., Dodwell, N., Hills, C., Dekker, M., O'Connell, T., Riddler, I., Rogers, P.W., 2009. The burial of a princess? the later seventh century cemetery at Westfield Farm, Ely. *Antiq. J.* 89, 81–141.
- Paster, B.J., Boches, S.K., Galvin, J.L., Ericson, R.E., Lau, C.N., Levanos, V.A., Sahasrabudhe, A., Dewhirst, F.E., 2001. Bacterial diversity in human subgingival plaque. *J. Bacteriol.* 183, 3770–3783.
- Piperno, D.R., Dillehay, T.D., 2008. Starch grains on human teeth reveal early broad crop diet in northern Peru. *Proc. Natl. Acad. Sci.* 105, 19622–19627.
- Poulson, S.R., Kuzminsky, S.C., Scott, G.R., Standen, V.G., Arriaza, B., Muñoz, I., Dorio, L., 2013. Paleodiet in northern Chile through the Holocene: extremely heavy $\delta^{15}\text{N}$ values in dental calculus suggest a guano-derived signature? *J. Archaeol. Sci.* 40, 4576–4585.
- Preus, H.R., Marvik, O.J., Selvig, K.A., Bennike, P., 2011. Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. *J. Archaeol. Sci.* 38, 1827–1831.
- Salazar-García, D.C., Richards, M.P., Nehlich, O., Henry, A.G., 2014. Dental calculus is not equivalent to bone collagen for isotope analysis: a comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the Medieval site of El Raval (Alicante, Spain). *J. Archaeol. Sci.* 47, 70–77.
- Scheeres, M., Knipper, C., Hauschild, M., Schönfelder, M., Siebel, W., Vitali, D., Pare, C., Alt, K.W., 2013. Evidence for “Celtic migrations”? Strontium isotope analysis at the early La Tène (LT B) cemeteries of Nebringen (Germany) and Monte Biele (Italy). *J. Archaeol. Sci.* 40, 3614–3625.
- Schoeninger, M.J., DeNiro, M.J., Tauber, H., 1983. Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. *Science* 220, 1381–1383.
- Scott, G.R., Poulson, S.R., 2012. Stable carbon and nitrogen isotopes of human dental calculus: a potentially new non-destructive proxy for paleodietary analysis. *J. Archaeol. Sci.* 39, 1388–1393.
- Somerville, A.D., Fauvelle, M., Froehle, A.W., 2013. Applying new approaches to modeling diet and status: isotopic evidence for commoner resiliency and elite variability in the Classic Maya lowlands. *J. Archaeol. Sci.* 40, 1539–1553.
- Thompson, A.H., Chaix, L., Richards, M.P., 2008. Stable isotopes and diet at ancient Kerma, upper nubia (Sudan). *J. Archaeol. Sci.* 35, 376–387.
- Toyne, J.M., White, C.D., Verano, J.W., Castillo, S.U., Millaire, J.F., Longstaffe, F.J., 2014. Residential histories of elites and sacrificial victims at Huacas de Moche, Peru, as reconstructed from oxygen isotopes. *J. Archaeol. Sci.* 42, 15–28.
- Voutsaki, S., Milka, E., Triantaphyllou, S., Zerner, C., 2013. Middle Helladic Lerna: diet, economy and society. In: Voutsaki, S., Valamoti, S.-M. (Eds.), *Diet, Economy and Society in the Ancient Greek World*. Peeters Publishers, Leuven, NL, pp. 133–147.
- Wattiaux, M.A., Reed, J.D., 1995. Fractionation of nitrogen isotopes by mixed ruminal bacteria. *J. Anim. Sci.* 73, 257–266.
- Weber, A., McKenzie, H.G., Beukens, R., Gorunova, O.I., 2005. Evaluation of radiocarbon dates from the Middle Holocene hunter-gatherer cemetery Khuzhir-Nuge XIV, Lake Baikal, Siberia. *J. Archaeol. Sci.* 32, 1481–1500.
- Welsby, D.A., 1998. *The Kingdom of Kush: the Napatan and Meroitic Empires*. Markus Wiener Publishers, Princeton.
- White, D.J., 1997. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *Eur. J. Oral Sci.* 105, 508–522.