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Stable carbon and nitrogen isotopes of human dental calculus: a potentially new non-destructive proxy for paleodietary analysis

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ABSTRACT

Fifty-eight dental calculus samples from medieval and post-medieval skeletons from Vitoria, Spain, and a single sample from an Alaskan Inuit were tested for stable carbon and nitrogen isotope compositions. There was sufficient carbon and nitrogen concentrations to obtain $\delta^{13}C$ and $\delta^{15}N$ values, and the samples from Spain produced results that were replicable and comparable to European isotope values based on bone collagen collected from literature sources. The Alaskan Inuit calculus sample yielded a $\delta^{15}N$ value of +17.5‰, well beyond the range of the Spanish samples, but consistent with literature data for modern Greenlandic Inuit consuming a diet rich in marine food. There are several potential sources for carbon and nitrogen in calculus. The results of this study yield stable isotope values consistent with those obtained from other biomaterials used as isotope proxies for paleodietary research, including bone collagen, hair, and fingernails, although further work is necessary to verify the fidelity of calculus as an isotope proxy. Many studies in bioarchaeology are precluded by curatorial concerns regarding the destructive analysis of primary biomaterials. However, calculus is an "add-on", or secondary biomaterial, that is not an integral part of the dental or skeletal system. Hence, its consumption during analysis is technically not destructive. Therefore, isotope analysis of dental calculus may provide a potential new avenue for paleodietary analysis where the use of other primary biomaterials is precluded.

1. Introduction

Stable isotope analysis (typically $\delta^{13}C$ and $\delta^{15}N$) of biomaterials, such as bone, teeth, fingernails, and hair, has become a common technique for paleodietary analysis in bioarchaeology (cf. Schwarcz and Schoeninger, 1991; Schoeninger, 2009, 2010). However, stable isotope analysis is destructive (i.e. sample material is consumed during analysis), and because of this, curatorial concerns sometime prohibit this analysis from being performed. In contrast to primary biomaterials, dental calculus is not an inherent part of the skeleton or dentition, but is a secondary biomaterial, or "add-on". Therefore, sampling calculus from a tooth's surface for stable isotope analysis could be considered non-destructive analysis, which has the potential to overcome curatorial concerns regarding sample preservation.

Dental calculus is produced from plaque, a biofilm that forms on human teeth as a product of microbial activity in the mouth. If not removed by brushing or flossing, plaque begins to harden after 10 days to form calculus, or tartar. Calculus can buildup over a period of time, with the rate of accumulation varying by individual differences in diet, salivary flow, local pH, genetic factors, and degree of dental care (Hardy et al., 2009). Heavy calculus production is associated with relatively high levels of dietary protein, which increases the alkalinity of the oral cavity (Hillson, 1979; Lieverse, 1999).

In modern times, the accumulation of calculus necessitates tooth cleaning and scaling by a dental hygienist, but in earlier human populations, where dental hygienists were not available, the accretion of calculus on teeth was common if not ubiquitous. In some instances, calculus deposits are so pronounced that they represent many years of buildup. It is difficult to discern whether or not individuals in the 'pre-dental hygiene era' simply let calculus accumulate during the course of their entire life or occasionally removed it by some means when its presence led to discomfort. Based on uniformitarian principles, pronounced calculus deposits would represent accretion over a few years to possibly a few decades.

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X-ray diffraction analysis shows calculus is composed of several calcium phosphate minerals, including brushite, whitlockite, octacalcium phosphate, and hydroxyapatite (Hayashizaki et al., 2008; White, 1997). Organic components (e.g., amino acids, proteins, carbohydrates, lipids, glycoproteins) from a variety of sources make up 15–20% of the dry weight of calculus (Lieverse, 1999).

Traditionally, calculus has been more of a nuisance than a blessing in anthropological research as it has to be removed to make morphological observations and precise tooth measurements (Fig. 1). Until recently, calculus rarely served as a primary medium for research (Hillson, 1986). Upon closer examination, however, it has been shown to contain inclusions that provide insights into the cultural and dietary behavior of earlier populations (cf. Boyadjian et al., 2007; Fox et al., 1996; Wesolowski et al., 2010). Henry and Piperno (2008) isolated a number of plant macrofossils from human dental calculus, including starches and phytoliths. The researchers found that starches were more abundant than phytoliths and the individuals at a Neolithic site in Syria consumed fewer cereal starches (barley and wheat in particular) than expected. Hardy et al. (2009) observed starch granules in calculus, providing direct evidence for the consumption of starchy foods. Blatt et al. (2010) found not only phytoliths and mineralized bacteria, but also cotton embedded in the calculus matrix, which provided the first direct evidence for prehistoric cotton in Ohio (Late Woodland, A.D. 900–1000). Recently, Preus et al. (2011) have shown it is possible to analyze ancient bacterial DNA from calculus.

The presence of nitrogen-bearing organic compounds (e.g. amino acids, proteins, glycoproteins: Lieverse, 1999) in dental calculus indicates the potential for $\delta^{13}C$ and $\delta^{15}N$ analyses to be conducted using this material. This study performed stable carbon and nitrogen isotope analysis of human calculus as a first attempt to identify the utility of this material as a new, non-destructive isotope proxy for paleodietary research, in the same manner that stable isotope analysis of bone, teeth, fingernails, or hair can be used as isotope proxies for paleodietary study.

2. Materials and methods

2.1. Materials

Calculus samples were collected during a dental analysis of over 400 Basque and Spanish skeletons that dated from the 12th to 19th centuries. All burials were associated with the Cathedral of Santa Maria in Vitoria, Spain, which is currently undergoing major renovation. Most burials came from under the floor of the massive cathedral. The only exceptions to burying the dead under the cathedral's floors were medieval graves from area 17 that were placed outside, adjacent to the cathedral. For the most part, it is not possible to determine age precisely because bodies were stacked up over the course of many centuries, making temporal sorting difficult. Area 17 is known to be medieval in age (12th to 15th century), but there is no way to determine which burials under the cathedral's floors were also medieval in age. Although ^{14}C dating of individual skeletons could rectify the problems associated with temporal context, most of the funds raised to date have been devoted to stabilizing the cathedral's buckling walls and disinterring over 2000 sets of remains. Precise dating of individual skeletons has not been a priority.

Fig. 1. Two lower molars of an individual buried at the Cathedral of Santa Maria, Vitoria, Spain, with pronounced dental calculus deposits on buccal surfaces. Note how the 'ledge' of calculus extended to the individual's gum line during life.

The skeletal remains were not subject to any preservative agent. Although most individuals showed some calculus formation, focus was on individuals that showed above average deposits. In all instances, supragingival calculus was sampled from the buccal or lingual surfaces of maxillary and/or mandibular teeth and was never removed in its entirety from any single individual. To obtain samples, a dental pick was used to peel back or flake off a 10+ mg sample of calculus. Calculus was easily removed and the surfaces of the teeth were neither scratched nor scraped during the removal process. In most instances, calculus was sampled from a single tooth. For some individuals, calculus was removed from 2 to 3 teeth to obtain a sufficient quantity for testing.

Dental calculus was obtained from 58 individuals associated with seven defined areas of the Cathedral of Santa Maria. The areas and samples sizes are: area 12 ($n = 15$); area 15 ($n = 4$); area 17 ($n = 5$); area 19 ($n = 12$); area 20 ($n = 1$); area 29 ($n = 17$); area 62 ($n = 1$); and unspecified area ($n = 3$). In addition to the Spanish samples, we obtained calculus from a single prehistoric Alaskan Inuit skeleton that served as an additional test to compare a measured calculus isotope composition vs. an isotope composition expected to have a high $\delta^{15}N$ ratio. The Inuit skeleton was collected during the early 20th century and does not have exact provenance. "Bering Sea coast" was noted on the specimen so the presumption is the individual subsisted on some combination of marine and/or riverine resources, a characterization that would apply to most Inuit groups.

2.2. Analytical methods

Calculus material was powdered in a steel mortar and pestle. It was not chemically purified or pre-treated in any fashion. Approximately 5–10 mg of calculus was used for each analysis. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) and elemental concentrations (weight % C and weight % N) were performed using a Eurovector elemental analyzer (which liberates all C and N in the calculus as CO_2 and N_2, respectively) interfaced to an Isoprime stable isotope ratio mass spectrometer, after the method of Werner et al. (1999). Stable isotope results are reported in units of ‰ in the usual δ notation vs. VPDB for carbon, and vs. air for nitrogen. Replicate analyses of samples (i.e. multiple analysis of material from a single powdered sample) indicate approximate reproducibilities (one standard deviation, which includes any sample heterogeneity that may be present) of 0.1‰ for $\delta^{13}C$, 0.2‰ for $\delta^{15}N$, 0.11% for weight % C, and 0.02% for weight % N. An acetanilide elemental analysis standard (Costech Analytical Technologies Inc., Valencia, CA) that had previously been characterized vs. NIST and IAEA standards was used as a standard for $\delta^{13}C$, $\delta^{15}N$, weight % C, and 0.02% for weight % N analyses.

3. Results

Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and elemental concentration (weight % C and weight % N) results for all dental calculus samples are presented in Table 1.

Table 1
Stable isotope compositions and elemental concentrations of dental calculus sampled from the Cathedral of Santa Maria, Vitoria, Spain, and for an Alaskan Inuit. For the Spanish samples, sample numbers given as XX-YY, where XX refers to the area that the sample was collected and YY refers to a unique individual sample number. For samples which were analyzed in replicate, the mean values are presented (see Table 1).

Sample #	$\delta^{15}N$ (‰)	wt. % N	$\delta^{13}C$ (‰)	wt. % C
Alaskan Inuit	17.5	0.84	−19.5	5.05
Spanish				
12–6	11.3	0.25	−19.9	1.74
12–8	12.6	0.65	−22.4	3.83
12–9	15.1	0.77	−24.1	6.33
12–16	13.1	0.38	−21.8	2.71
12–19	12.2	0.61	−22.0	3.49
12–20	10.8	0.73	−21.5	4.38
12–26	11.8	0.94	−21.5	4.96
12–28	11.9	0.74	−22.1	4.43
12–29	11.0	0.67	−21.2	3.88
12–35	9.4	0.81	−20.9	5.62
12–36	13.0	0.88	−21.0	5.09
12–39	12.3	1.14	−21.1	6.71
12–43	12.2	0.55	−22.2	4.31
12–50	11.4	0.51	−22.1	3.04
12–59	12.2	0.50	−21.2	2.81
15–14	10.1	0.64	−21.0	4.42
15–17	10.2	0.57	−21.4	5.23
15–20	9.8	0.70	−21.2	5.30
15–53	10.3	0.75	−20.4	5.65
17–70	11.0	0.69	−21.2	5.28
17–77	11.0	0.70	−21.2	5.42
17–86	10.7	0.57	−21.1	4.71
17–208	11.1	0.65	−21.0	4.73
17–209	11.7	0.67	−21.7	5.15
19–7	12.7	0.94	−20.4	4.87
19–15	12.7	0.92	−21.1	5.83
19–17	12.8	0.53	−22.4	5.19
19–23	13.6	0.76	−21.8	4.38
19–24	12.8	0.67	−21.6	4.15
19–26	12.6	0.79	−21.2	5.06
19–32	9.9	0.73	−21.4	4.42
19–45	12.2	0.98	−20.8	4.86
19–57	11.0	0.90	−20.9	5.16
19–107	12.3	0.63	−20.7	5.01
19–112	10.6	0.97	−20.9	5.96
19–113	9.7	0.70	−21.8	4.66
20–2	14.6	1.02	−20.8	6.16
29–4	11.8	0.97	−20.7	5.95
29–27	11.0	1.05	−21.0	4.79
29–48	13.4	1.33	−20.4	5.55
29–62	11.9	0.70	−20.5	4.09
29–66	10.4	1.08	−20.6	4.93
29–67	11.7	1.38	−20.1	5.70
29–81	12.2	0.75	−20.6	4.04
29-149B	12.9	0.64	−21.8	3.91
29–150	13.6	0.95	−20.6	5.93
29–198	12.2	1.05	−20.1	5.35
29–214	10.1	1.04	−22.3	5.63
29–431	12.0	0.67	−21.9	4.30
29–588	13.1	0.79	−21.2	5.15
29–658	11.7	0.68	−17.4	4.95
29–673	11.9	0.83	−22.2	5.16
29–683	13.2	0.62	−22.4	4.72
29–685	12.5	0.61	−20.5	5.21
62–71	10.3	0.74	−20.7	3.52
70	10.6	0.82	−23.3	8.49
73	10.8	0.61	−20.1	5.46
116	11.9	0.95	−19.6	6.35

3.1. Elemental concentration

For the Spanish samples: weight % C ranged from 1.74 to 8.49%, with an average of 4.90% and a standard deviation of 1.06%; weight % N ranged from 0.25 to 1.38%, with an average of 0.74% and a standard deviation of 0.21%. The single sample of Inuit calculus had 5.05 weight % C and 0.84 weight % N.

3.2. Stable isotope composition

For the Spanish samples: $\delta^{13}C$ ranged from −24.1 to −17.4‰, with an average of −21.2‰ and a standard deviation of 1.0‰; $\delta^{15}N$ ranged from +9.4 to +15.1‰, with an average of 11.8‰ and a standard deviation of 1.2‰. The single sample of Alaskan Inuit dental calculus had $\delta^{13}C = -19.5$‰ and $\delta^{15}N = +17.5$‰. Calculus $\delta^{13}C$ vs. $\delta^{15}N$ is plotted for all samples in Fig. 2.

4. Discussion

In northern Spain during the medieval period, staples were bread and porridge, made primarily from wheat. Barley and rye were the next most commonly used grains. Other important foods included peas, lentils, chickpeas, and fava beans. While the wealthy had greater access to meat, commoners could obtain meat in special shops with sheep and beef the most common sources. Milk, cheese and eggs also contributed to the diet, along with fish as dictated by church law in this strictly Catholic country. In the Basque region, other important foodstuffs included almonds, walnuts, hazel nuts, garlic, onions, carrots, and spinach. Although New World domesticates were introduced after A.D. 1500, some major imports, including maize, potatoes, and tomatoes were not widely adopted until the 18th century or later (Flandrin and Montanari, 2006; Llopis, 2007). Based on the carbon isotope values of the Spanish sample, the distinctive C_4 signature associated with maize was not yet evident. The distribution of $\delta^{13}C$ and $\delta^{15}N$ values in Fig. 2 are consistent with a diet that centered on temperate grasses with moderate protein consumption.

The fundamental issue we are addressing is whether or not dental calculus can serve as an effective proxy for the measurement

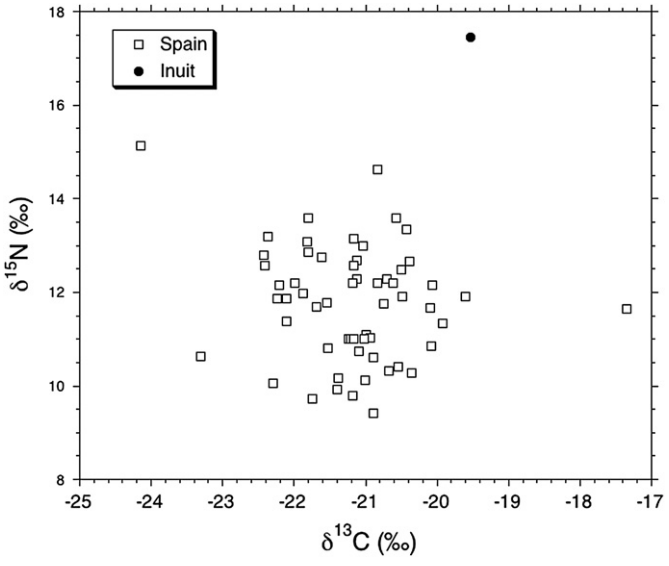

Fig. 2. Stable carbon and nitrogen isotopic values based on analysis of dental calculus from samples in northern Spain (n = 58) and Alaska (n = 1).

of stable carbon and nitrogen isotopes. Although the exact chemical form and sources of carbon and nitrogen preserved in calculus have yet to be determined (e.g., from some combination of oral microbiota, salivary constituents, food particles, oral mucosa), there are two lines of evidence that support dental calculus as a potentially valuable biomaterial for isotope analysis.

4.1. Comparison to European samples based on isotope analysis of collagen

To our knowledge, there are no previous stable isotope measurements of dental calculus available in the literature. In the absence of previous data for calculus, $\delta^{13}C$ and $\delta^{15}N$ of bone collagen from selected paleodiet studies performed at European locations is compared to $\delta^{13}C$ and $\delta^{15}N$ of calculus measured in this study (Table 2). The literature values provide a representative, though not exhaustive, survey of $\delta^{13}C$ and $\delta^{15}N$ values in European samples distributed through both space (England to the Ukraine, Sweden to Greece) and time (late Upper Paleolithic to later medieval period). Subsisting primarily on temperate grasses and the animals that consume those grasses, the mean $\delta^{13}C$ values in Europe range from −22.6 to −18.8‰ while mean $\delta^{15}N$ values range from +8.4 to +13.6‰. For the measurements of stable isotopes obtained from calculus, the Spanish samples fall within the range for both $\delta^{13}C$ (−21.1‰) and $\delta^{15}N$ (+11.8‰). A direct comparison between calculus and bone collagen isotopic compositions is not strictly possible, as there may be a systematic difference between the isotopic compositions of carbon and nitrogen preserved in calculus vs. bone collagen, analogous to the small (≤1.4‰) but systematic differences between the isotope compositions of bone collagen vs. hair vs. fingernail measured in modern human samples (O'Connell et al., 2001). Indeed, it seems likely that a difference in the isotopic compositions of calculus vs. bone collagen does exist, and characterization of this difference will be necessary in order to perform direct comparisons of the isotope compositions of calculus vs. collagen (or hair, or fingernail). Nevertheless, the isotope compositions measured for calculus are consistent with values obtained from traditional paleodietary isotope analytical methods based on collagen extraction, providing a strong indication of the suitability of this material as an isotope proxy for paleodietary research.

4.2. Isotope values from the dental calculus of an Alaskan Inuit

Isotope values for the single Alaskan Inuit sample provide an additional test of the suitability of calculus as an isotope proxy. Inuit diets are typically rich in marine foods that are high in the trophic system (e.g. seals). Individuals consuming such foods are expected to have isotopically heavy $\delta^{15}N$ values. Isotope analysis of fingernails of modern Greenlandic Inuit (Buchardt et al., 2007) demonstrates this is the case, with a mean $\delta^{15}N = +16.0‰$ ($n = 73$, range = +12.2 to +19.1‰) and a mean $\delta^{13}C = -18.2‰$ ($n = 75$, range = −20.2 to −16.5‰). By contrast, four Danish samples, including an Inuit sample in Denmark, had $\delta^{15}N$ values ranging between +8.6 and +10.7‰. In no instance did the minimum and maximum $\delta^{15}N$ values for the Danish samples overlap the Greenlandic Inuit sample range (i.e., no single Danish individual had a $\delta^{15}N$ value equal to or above +12.2‰). Our single Alaskan Inuit dental calculus sample ($\delta^{15}N = +17.5‰$) is in agreement with the expectation for an exceptionally high $\delta^{15}N$ value, consistent with a marine food-rich diet, and lies within the $\delta^{15}N$ range for modern Greenlandic Inuit fingernails (+12.2 to +19.1‰; Buchardt et al., 2007). In addition, the $\delta^{13}C$ composition of calculus (−19.5‰) falls within the $\delta^{13}C$ range for modern Greenlandic Inuit (−20.2 to −16.5‰; Buchardt et al., 2007).

4.3. Future work

Our initial results indicate that calculus is a new potential material for paleodietary research, but additional work will be necessary to firmly establish calculus as a robust isotope proxy for paleodietary research.

A limited number of studies of calculus composition have been performed (e.g. Lieverse, 1999), so the distribution of C and N among various possible components within calculus is not well characterized. In the absence of this information, it was not possible to identify which (if any) components would be present in the necessary concentrations for isotope analysis, or which components

Table 2
Mean values of stable carbon and nitrogen isotopes for European samples based on bone collagen.

Region (site)	Time period (age range)	$\delta^{13}C$				$\delta^{15}N$			Reference
		n	Mean	sd	se	Mean	sd	se	
Western Europe (various)	Late Upper Paleolithic (10,200–15,800)	15	−18.95	0.800	0.207	9.09	3.116	0.804	Garcia-Guixé et al., 2009
Ukraine (Vasilyevka III)	Epipaleolithic (9200–10,400 calBC)	21	−22.38	0.325	0.071	12.48	0.626	0.137	Lillie et al., 2003
Ukraine (Dnieper)	Epipaleolithic–Neolithic (3640–10,400 calBC)	29	−22.56	1.098	0.204	13.14	1.135	0.211	Lillie et al., 2011
Bulgaria (Varna)	Neolithic–Eneolithic (mid-5th millenium BC)	55	−19.29	0.356	0.048	10.01	0.597	0.082	Honch et al., 2006
Bulgaria (Durankulak)	Neolithic–Eneolithic (mid-5th millenium BC)	78	−19.12	0.284	0.032	9.29	0.850	0.096	Honch et al., 2006
North Caucasus (various)	Eneolithic–Bronze Age (4000–2600 BC)	51	−18.83	0.905	0.127	11.84	1.570	0.220	Hollund et al., 2010
Greece (Lerna)	Middle Bronze Age (2100–1700 BC)	38	−19.55	0.329	0.053	8.42	0.743	0.121	Triantaphyllou et al., 2008
Western Europe (southern France)	Middle Neolithic (4500–3500 BC cal)	57	−19.70	0.728	0.096	8.75	1.200	0.159	Herrscher and Le Bras-Goude, 2010
Sweden (Birka)	Viking Age (800–1000 AD)	22	−19.96	0.589	0.125	13.62	1.082	0.231	Linderholm et al., 2008
England (Whithorn priests)	Medieval (13th–14th centuries AD)	6	−19.35	0.362	0.148	12.63	0.814	0.332	Müldner et al., 2009
England (Whithorn laity)	Medieval (13th–14th centuries AD)	7	−20.43	0.713	0.270	11.43	0.390	0.148	Müldner et al., 2009
England (Fishergate, York)	Late Medieval (13th–early 16th centuries)	155	−19.09	0.641	0.052	12.78	1.300	0.104	Müldner and Richards, 2007
North England (various)	Late Medieval (13th–early 16th centuries)	46	−19.46	0.613	0.090	12.34	0.897	0.132	Müldner and Richards, 2005
Spain (Vitoria)	Medieval to Postmedieval (11th–18th centuries)	58	−21.19	0.965	0.127	11.77	1.224	0.161	Present study

might best represent paleodiet and be most suitable for selective extraction and analysis, so isotope analyses of bulk calculus material were performed in this study. Better understanding of how C and N is incorporated into calculus, the source(s) of C and N to calculus and their relative importance, and how C and N occurs in calculus may lead to the identification of specific components within calculus that could be selectively extracted and analyzed. This would provide a more accurate representation of the isotopic composition of diet and the components that are more resistant to post-depositional alteration or contamination. In particular, characterizing the distribution of C between organic-C vs. carbonate-C components will be important. If $\delta^{13}C$ analysis of both organic-C and carbonate-C components is possible, this would have the potential to provide additional information regarding paleodiet.

Our results to date are consistent with the hypothesis that the isotopic composition of dental calculus represents the isotopic composition of the diet, but this remains to be conclusively demonstrated. The difference between the isotopic compositions of calculus and diet needs to be characterized, as do the differences between the isotopic compositions of calculus and other isotope proxies (e.g. bone collagen, hair, fingernails) so that accurate and meaningful comparisons can be conducted between isotope studies performed using calculus and non-calculus biomaterials.

5. Conclusions

This reconnaissance study performed stable isotope analyses of dental calculus for a suite of medieval to post-medieval samples from Spain, and a single sample from an Alaskan Inuit. To our knowledge, dental calculus has not previously been analyzed for stable carbon and nitrogen isotope compositions, which may be due to the presumption of low carbon and nitrogen concentrations. This study has demonstrated the presence of significant concentrations of both carbon and nitrogen in dental calculus, sufficient for stable isotope analyses. Our results may not be directly comparable to isotope compositions taken from the literature for accepted isotope proxies for paleodietary research (e.g. bone collagen, hair, fingernails) due to possible systematic differences in isotope composition between calculus and other proxies. However, with this caveat in mind, our results are consistent with comparable isotope compositions taken from the literature, and strongly suggest that calculus is a new potential biomaterial suitable for paleodietary analysis, although additional work will be necessary to definitely identify if this is the case.

Presently, the most commonly used biomaterial sampled for stable isotope analyses is bone collagen, which requires destructive analysis of bone material. Consequently, curatorial concerns can often prevent the use of bone for isotope analysis. In contrast, dental calculus is not an inherent part of the skeleton or dentition, so sampling calculus for isotope analysis is not technically destructive. Although it is recognized that claimants in repatriation cases may not share this view, we believe the use of calculus for $\delta^{13}C$ and $\delta^{15}N$ measurements may represent a potential opportunity for paleodietary analysis by isotope techniques to be expanded to many samples where destructive analysis is prohibited.

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References

Blatt, S.H., Redmond, B.G., Cassman, V., Sciulli, P.W., 2010. Dirty teeth and ancient trade: evidence of human cotton fibres in human dental calculus from Late Woodland, Ohio. International Journal of Osteoarchaeology. doi:10.1002/oa.1173. www.interscience.wiley.com (early view).

Boyadjian, C.H.C., Eggers, S., Reinhard, K., 2007. Dental wash: a problematic method for extracting microfossils from teeth. Journal of Archaeological Science 34, 1622–1628.

Buchardt, B., Bunch, V., Helin, P., 2007. Fingernails and diet: stable isotope signatures of a marine hunting community from modern Uummannaq, north Greenland. Chemical Geology 244, 316–329.

Flandrin, J., Montanari, M., 2006. Historia de la Alimentacion (Spanish edition). Ediciones Trea, Gijon.

Fox, C.F., Juan, J., Albert, R.M., 1996. Phytolith analysis on dental calculus, enamel surface, and burial soil: information about diet and paleoenvironment. American Journal of Physical Anthropology 101, 101–113.

Garcia-Guixé, E., Martínez-Moreno, J., Mora, R., Núñez, M., Richards, M.P., 2009. Stable isotope analysis of human and animal remains from the late upper paleolithic site of Balma Guilanyà, southeastern Pre-Pyrenees, Spain. Journal of Archaeological Science 36, 1018–1026.

Hardy, K., Blakeney, T., Copeland, L., Kirkham, J., Wrangham, R., Collins, M., 2009. Starch granules, dental calculus and new perspectives on ancient diet. Journal of Archaeological Science 36, 248–255.

Hayashizaki, J., Ban, S., Nakagaki, H., Okumura, A., Yoshii, S., Robinson, C., 2008. Site specific mineral composition and microstructure of human supra-gingival dental calculus. Archives of Oral Biology 53, 168–174.

Henry, A.G., Piperno, D.R., 2008. Using plant microfossils from dental calculus to recover human diet: a case study from Tell Al-Raqā'i, Syria. Journal of Archaeological Science 35, 1943–1950.

Herrscher, E., Le Bras-Goude, G., 2010. Southern French Neolithic populations: isotopic evidence for regional specificities in environment and diet. American Journal of Physical Anthropology 141, 259–272.

Hillson, S.W., 1979. Diet and Dental Disease. World Archaeology 11, 147–162.

Hillson, S., 1986. Teeth. Cambridge University Press, Cambridge.

Hollund, H.I., Higham, T., Belinskij, A., Korenevskij, S., 2010. Investigation of paleodiets in the North Caucasus (South Russia) Bronze Age using stable isotope analysis and AMS dating of human and animal bones. Journal of Archaeological Science 37, 2971–2983.

Honch, N.V., Higham, T.F.G., Chapman, J., Gaydarska, B., Hedges, R.E.M., 2006. A palaeodietary investigation of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in human and faunal bones from the copper age cemeteries of Varna I and Durankulak, Bulgaria. Journal of Archaeological Science 33, 1493–1504.

Lieverse, A.R., 1999. Diet and the aetiology of dental calculus. International Journal of Osteoarchaeology 9, 219–232.

Lillie, M., Richards, M.P., Jacobs, K., 2003. Stable isotope analysis of 21 individuals from the epipalaeolithic cemetery of Vasilyevka III, Dnieper Rapids region, Ukraine. Journal of Archaeological Science 30, 743–752.

Lillie, M., Budd, C., Potekhina, I., 2011. Stable isotope analysis of prehistoric populations from the cemeteries of the Middle and Lower Dnieper Basin, Ukraine. Journal of Archaeological Science 38, 57–68.

Linderholm, A., Jonson, C.H., Svensk, O., Liden, K., 2008. Diet and status in Birka: stable isotopes and grave goods compared. Antiquity 82, 446–461.

Llopis, M.M., 2007. Historia de la gastronomia espanola. Alianza, Madrid.

Müldner, G., Richards, M.P., 2005. Fast or feast: reconstructing diet in later medieval England by stable isotope analysis. Journal of Archaeological Science 32, 39–48.

Müldner, G., Richards, M.P., 2007. Diet and diversity at later medieval fishergate: the isotopic evidence. American Journal of Physical Anthropology 134, 162–174.

Müldner, G., Montgomery, J., Cook, G., Ellam, R., Gledhill, A., Lowe, C., 2009. Isotopes and individuals: diet and mobility among the medieval bishops of Whithorn. Antiquity 83, 1119–1133.

O'Connell, T.C., Hedges, R.E.M., Healey, M.A., Simpson, A.H.R.W., 2001. Isotopic comparison of hair, nail, and bone: modern analyses. Journal of Archaeological Science 28, 1247–1255.

Preus, H.R., Marvik, O.J., Selvig, K.A., Bennike, P., 2011. Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. Journal of Archaeological Science. doi:10.1016/j.jas.2011.03.020.

Schoeninger, M.J., 2009. Stable isotope evidence for the adoption of maize agriculture. Current Anthropology 50, 633–640.

Schoeninger, M.J., 2010. Diet reconstruction and ecology using stable isotope ratios. In: Larsen, C.S. (Ed.), A Companion to Biological Anthropology. Wiley-Blackwell, Chichester, U.K, pp. 445–464.

Schwarcz, H.P., Schoeninger, M.J., 1991. Stable isotope analyses in human nutritional ecology. Yearbook of Physical Anthropology 34, 283–321.

Triantaphyllou, S., Richards, M.P., Zerner, C., Voutsaki, S., 2008. Isotopic dietary reconstruction of humans from Middle Bronze age Lerna, Argolid, Greece. Journal of Archaeological Science 35, 3028–3034.

Werner, R.A., Bruch, B.A., Brand, W.A., 1999. ConFlo III – an interface for high Precision $\delta^{13}C$ and $\delta^{15}N$ analysis with an extended dynamic range. Rapid Communications in Mass Spectrometry 13, 1237–1241.

Wesolowski, V., de Souza, S.M.F.M., Reinhard, K.J., 2010. Evaluating microfossil content of dental calculus from Brazilian sambaquis. Journal of Archaeological Science 37, 1326–1338.

White, D.J., 1997. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. European Journal of Oral Sciences 105, 508–522.