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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)

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ABSTRACT

Palaeodietary reconstruction using the carbon and nitrogen isotope values of bone and dentine collagen is a well-established method and the biochemical processes involved are well known. Researchers have recently explored using bulk samples of dental calculus as a substitute for bone and dentine collagen in dietary analyses, because calculus can be sampled without causing damage to the teeth, and may be useful in situations where more destructive analyses are not possible, or where collagen is poorly preserved. Several questions remain about the use of bulk calculus as a source of carbon and nitrogen isotope data, however. It is not yet clear how much of an individual's life span dental calculus represents, what portions of the diet it records, and how diagenesis effects the carbon and nitrogen isotope values of this material. Most importantly, there have been no comparative studies of collagen and calculus isotope values, which are necessary to establish the value of bulk calculus as a source of accurate isotope values. Here we report the comparison of carbon and nitrogen stable isotope analyses of bulk calculus to those from bone and dentine collagen. These analyses have been performed on individuals from the El Raval Mudéjar Medieval Cemetery (Eastern Iberia, 15th century A.D.). Although calculus isotope values may be broadly similar to expected values at the population level, we report here no correlation between collagen and bulk dental calculus values at the individual level. As a result, we recommend that carbon and nitrogen analysis on bulk dental calculus should only be used as a last resource archaeological dietary marker, if at all.

1. Introduction

Over the last several decades, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis of dentine and bone collagen has been established as a reliable tool to recover information on past human diets (e.g. Katzenberg, 2000; Lee-Thorp, 2008; Sealy, 2001). There are a few known limitations to using these sources for isotopic analysis. The collagen must be well preserved, and it represents only a broadly averaged, long-term, multi-year dietary protein intake (e.g. Salazar-García et al., 2014). Therefore, recent research has investigated whether there are other biological materials that can be used for isotope analysis, such as coprolites (Ghosh et al., 2003), carbonized sherd residues (Hart et al., 2009) and animals with human-like diets (Guiry and Grimes, 2013).

Dental calculus (mineralized plaque) has recently been identified as a substrate for multiple kinds of dietary analysis. Plant microremains (e.g. starch grains and phytoliths) recovered from calculus have provided information about plant consumption (e.g. Henry et al., 2011, 2012; Lalueza-Fox et al., 1996; Power et al., 2014; Salazar-García et al., 2013; Scott Cummings and Magennis, 1997).

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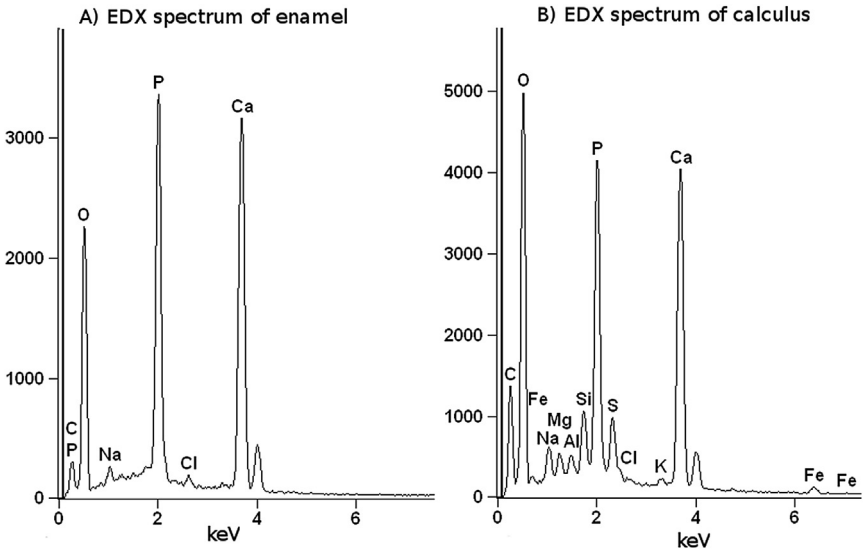

Fig. 1. Energy-dispersive X-ray spectroscopy (EDX) spectrum of (A) enamel and (B) dental calculus from a single tooth. The peaks in the spectra indicate the abundance of specific elements in the sample. Elements with greater mass are often indicated by several peaks (e.g. iron). In (B), the largest peak represents oxygen, and the two very small peaks just to the right of the oxygen peak are iron and sodium respectively.

Food and bacteria proteins and DNA are also preserved in calculus, which can give information about diet and health (Adler et al., 2013; Warinner et al., 2014). Some researchers have attempted to use dental calculus as a source for carbon and nitrogen isotopes (Poulson et al., 2013; Scott and Poulson, 2012). These studies assume that carbon and nitrogen isotope values from bulk dental calculus can be interpreted in the same way as those from bone or dentine collagen. Here we report on the results of tests that explore the relationship between collagen and bulk calculus carbon and nitrogen isotope values.

1.1. C and N isotope analysis and dietary reconstruction

Carbon and nitrogen stable isotope analysis relies on the principle that the isotopic composition of food eaten by both animals and humans is recorded in their body tissues after a predictable isotope fractionation (Schoeller, 1999). Collagen is usually the preferred substrate for these analyses, because it is the only considerable nitrogen source from skeletal remains, its biochemistry is well known, and it has accepted quality indicators (De Niro, 1985; Van Klinken, 1999). Stable isotope ratios of collagen reflect the isotopic signals of the main dietary protein sources, rather than that of a diet as a whole (Ambrose and Norr, 1993). Results obtained on bone collagen average the dietary protein consumed during several years prior to death, depending on the collagen turnover rate of the bone sampled (Hedges et al., 2007). In contrast, dentine collagen has almost no turnover, so the isotope values reflect protein diet consumed during the short interval in which the dentine of each tooth was formed (Beaumont et al., 2012).

The stable carbon isotope values can either distinguish between the consumption of C_4 (^{13}C enriched) and C_3 (^{13}C depleted) terrestrial resources (Van der Merwe and Vogel, 1978), or it can define the input of marine (^{13}C enriched) or terrestrial (^{13}C depleted) foods in the diet (Chisholm et al., 1982). The nitrogen stable isotope ratio is usually an indication of trophic level, since it increases by 3–5‰ each step up the food-chain (De Niro and Epstein, 1981; Schoeninger and De Niro, 1984). This allows us to generally distinguish diets rich in plant proteins from those rich in animal proteins (Minagawa and Wada, 1984), with some acknowledgement of inherent variation among these values (Hedges and Reynard, 2007). Nitrogen stable isotope ratios may also be useful to detect the consumption of ^{15}N enriched aquatic foods versus relatively ^{15}N depleted terrestrial food sources such as domestic animals (Schoeninger et al., 1983). When combined, nitrogen and carbon values can discriminate between the consumption of aquatic foods and C_4 terrestrial foods.

1.2. Composition and formation of dental calculus

Dental calculus is the mineralized plaque that forms on the surface of teeth (Jin and Yip, 2002; Lieverse, 1999). Saliva is saturated in calcium phosphate in order to prevent the dissolution of the teeth during consumption of acidic foods. Oral bacteria form a pellicle on the surface of the teeth and this roughened surface is a site for precipitation of calcium phosphate. Bacteria then grow through and on top of the calcium phosphate, causing the calculus to accumulate. During this process, food particles, bacteria, human proteins and enzymes, and other constituents become trapped in the mineral context. Environmental scanning electron microscopy analysis coupled with energy-dispersive X-ray spectroscopy (ESEM-EDX) of calculus indicates that its elemental constituents are very similar to enamel, containing a high percentage of calcium and phosphate, but also several other elements including carbon (Fig. 1). The enamel is almost exclusively calcium, phosphate and oxygen, indicating hydroxyapatite. The major components of the calculus are calcium, phosphate and oxygen, consistent with hydroxyapatite. Minor components include carbon, sulphur, iron, silicon, sodium, aluminium, magnesium and chlorine indicating the presence of organics, salts and other minerals. The strongly mineralized nature of calculus may provide a protected context for archaeologically relevant residues.

Research on modern calculi has demonstrated that traces of food and other remains can be found within the mineral phase. Therefore, dental calculus might be a useful source of information for dietary reconstruction. However, the nature of the formation of dental calculus seems to be highly individualistic and uncertain. The rate and amount of calculus formation depends on the specific diet, particularly on the relative proportion of meat and other urea-inducing and high-pH foods (Jin and Yip, 2002; Lieverse, 1999) as well as sugars (Scheie, 1994), on genetic variations in salivary