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Impact of heating conditions on the carbon and oxygen isotope composition of calcined bone

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ABSTRACT

Only the inorganic fraction of bone survives the high temperatures reached during cremation, so that it remains the sole material available for isotopic analyses. In order to assess the amount of information that can be extracted from such material, we measured carbon and oxygen isotope ratios in the remaining carbonate fraction of experimentally heated modern bone, and cremated bone from several archaeological sites. The results show that the isotope composition of cremated bone is strongly altered, but some information can nevertheless be extracted. First, we find very little evidence of post-burial alterations on the isotope composition of calcined bone. More importantly, it appears possible to obtain information about the way bodies were burned (with or without fuel, oxygen availability) giving the opportunity to improve our knowledge regarding funerary practices in places and times where cremation was practiced.

1. Introduction

Cremated remains are found all over the world but they are usually excluded from isotopic analyses, leaving many blank pages in the archaeological record. It is generally thought that the high temperatures reached during cremation have obliterated any biogenic signal. Calcined bone is, however, used for radiocarbon dating (e.g. Lanting et al., 2001) and has been shown to provide a reliable substrate for strontium isotope analyses (Snoeck et al., 2015). Here we aim to understand what type of information is contained within the stable carbon and oxygen isotope composition of the carbonate fraction of calcined bone.

Fresh bone is composed of water, an organic, and an inorganic fraction known as bioapatite (Vaughan, 1970; Posner et al., 1984). All water and organic components, including collagen, the standard substrate for isotopic analyses for detecting dietary information, are obliterated during calcination. In other words, bioapatite represents the only possibility for isotope analysis of calcined bone. However, it is known that the isotope composition of its carbonate fraction (CO_3^{2-}) is strongly modified by heating and that consequently $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios obtained from carbonates in calcined bone are unlikely to yield any in vivo signal (Lanting et al., 2001; Van Strydonck et al., 2005; Olsen et al., 2008; Zazzo et al., 2009). Nevertheless, there have been hints that it may still be possible to extract some useful information from the carbon and oxygen isotope variations in bioapatite carbonate after cremation (e.g. Munro et al., 2008). While it is unlikely that dietary and/or hydrological information survives, information about the way bones were burned or otherwise treated could perhaps be obtainable from the isotope composition of calcined bone (Zazzo et al., 2013).

After it was shown that comparable ^{14}C ages could be obtained from paired dates of charcoal and cremated bone from the same contexts (Lanting and Brindley, 1998; Lanting et al., 2001), the carbon isotope composition of calcined bone apatite carbonates has been intensively studied. Because of the variations observed in the $\delta^{13}C$ values between raw bone and calcined bone apatite carbonates, however, some doubts remain regarding the validity of radiocarbon dating (Lanting et al., 2001; Naysmith et al., 2007; Olsen et al., 2008). The results of efforts to better understand the carbon isotope

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composition of calcined bone apatite carbonates suggest three different explanatory models: (1) carbon exchanges between bone apatite carbonates and the fuel combustion gases, (2) loss of endogenous apatite carbonates accompanied by a potential time/temperature dependent fractionation, and (3) admixture of carbon originating from the combustion of the organic fraction of bone (e.g. collagen,[1] flesh, fats, etc.). It has been suggested that a combination of these three possibilities is required to explain the observed modification of the carbon isotope composition of bone apatite carbonates after heating (Olsen et al., 2008, 2012; Zazzo et al., 2009, 2012, 2013; Van Strydonck et al., 2005, 2010, 2015; Hüls et al., 2010; Harbeck et al., 2011; Snoeck et al., 2014a).

The oxygen isotope composition of calcined bone carbonates has received less attention (Harbeck et al., 2011), partly because it reflects even more complex exchange pathways. First, carbonate oxygen is more susceptible to exchange than carbonate carbon as oxygen has a known propensity to exchange with water (Shemesh et al., 1988; Wang and Cerling, 1994), and secondly, there is more oxygen than carbon present in the surroundings of bone during heating (e.g. atmospheric dioxygen and water, oxygen in organic compounds such as collagen, lipids, etc.). Consequently, the $\delta^{13}C$ values of structural carbonates in bone apatite should be less affected by external changes than $\delta^{18}O$ values (Hu et al., 2006).

In parallel, the structure and chemical composition of calcined bone has been analysed by Fourier Transform Infrared spectroscopy (FTIR), confirming that, when heated, all organic matter in bone is destroyed and the only remaining carbon is contained within the carbonate fraction of bone apatite (Thompson et al., 2009; Lebon et al., 2010, 2014). The carbonate content decreases significantly during heating, and the composition and structure of calcined bone have been shown to be temperature-dependent (Munro et al., 2008; Thompson et al., 2009; Lebon et al., 2010, 2014; Snoeck et al., 2014b). This introduces the possibility that the carbon and oxygen isotope compositions of calcined bone could also be temperature-dependent. In modern experimentally heated bone, smaller, less crystalline crystallites are present compared to archaeological calcined bone (Snoeck et al., 2014b). Over long periods of time these become more stable and less reactive, as was observed in unburned bone (Lee-Thorp and van der Merwe, 1991; Sponheimer and Lee-Thorp, 1999). The increased reactivity of the small, less well-recrystallized crystallites may render them more prone to post-burial alterations. In an earlier study, we observed that archaeological calcined samples with the highest carbonate content were all retrieved from high-carbonate geological contexts (e.g. limestone, chalk). This may suggest that carbonates are incorporated into the structure of calcined apatite from the burial environment. Pre-treatment of these samples with acetic acid, however, had almost no effect on their carbonate content, which indicates that the carbonates were either endogenous or permanently incorporated into bone apatite crystallites (Snoeck et al., 2014b). Nevertheless, it is difficult to ascertain the origin of the carbon left in bone apatite after calcination using infrared analyses alone.

Isotopic analyses present an opportunity to investigate diagenetic exchanges (during and after calcination) and to shed light on whether the differences observed in the infrared data between modern and archaeological calcined bone are the result of post-burial alterations and/or the heating processes themselves. If the latter, then there is the potential to gain information regarding the conditions under which cremation occurred. Here, using isotope ratio mass spectrometry (IRMS), we explore this possibility by analysing the isotopic differences between modern bone heated in different experimental conditions, and cremated archaeological bone originating from carbonate and non-carbonate geological contexts. This will in turn be useful for the radiocarbon dating and study of funerary practices of sites where cremation was practiced.

2. Materials and methods

2.1. Samples

The samples investigated here (Table 1) have been previously analysed by FTIR (Snoeck et al., 2014b) with the exception of 15 additional samples (labelled LAB 2). To investigate the relative contributions of the different carbon sources (endogenous bone apatite carbonates, organic matter, fuel) to the isotope composition of calcined bone apatite carbonates, modern animal joints were heated in different conditions. A first set of experiments (LAB 1) in which different fragments of the same defleshed cow tibia were heated at different temperatures (500–900 °C) for 0.5–24 h aimed at assessing the impact of time and temperature. In the second experiment (LAB 2), samples were heated together with either barley (C_3), millet seeds, or corn flour (the latter both C_4) to test the impact of fuels with differing carbon isotope compositions. A further experiment with wood and coal, conducted outdoors (OUT) also aimed at assessing this. Finally, in the last experiment, some samples were treated with hydrazine hydrate (95%) following the protocol designed by Termine et al. (1973) to remove organic matter (Snoeck and Pellegrini, 2015) prior to heating, to assess the contribution of the latter.

Next, fully calcined (white – colour codes 5 and 6 from Stiner et al., 1995) archaeological samples of cremated human remains were compared to modern samples to assess the possible effects of post-burial alterations or different heating/burning conditions. The sites, all from Ireland, range in date from the Neolithic to the Middle Bronze Age (ca. 5500 to 3500 cal BP). They present a wide range of geological contexts from chalk and limestone (high-carbonate) to granite and basalt (non/low-carbonate). For Ballymacaldrack, Co. Antrim, two calcined bone fragments were selected from the single individuals represented in three separate urns (BM1a/b, BM2a/b and BM3a/b respectively) (Tomb and Davies, 1938). Three fragments of the same bone were also analysed for a sample from Parknabinnia, Co. Clare (P3a/b/c).

2.2. Isotope analyses of apatite carbonates

Before isotope analyses, the calcined bone fragments were treated with sodium hypochlorite (1% NaOCl) for 1 h to remove any remaining organic matter followed by three rinses with MilliQ water and then a 4-h treatment with 1 M acetic acid (CH_3COOH) to remove any adsorbed carbonates and diagenetic apatites. The samples were then rinsed three times with MilliQ water and left in the freeze-dryer overnight. Between 3 and 5 mg were reacted with 100% phosphoric acid at 70 °C for 4 min in a Kiel IV autocarbonate device interfaced with a Delta V Advantage isotope ratio mass spectrometer at the SSMIM (Service de Spectrométrie de Masse Isotopique du Muséum National d'Histoire Naturelle), Paris, France. A laboratory carbonate standard (LM marble) normalized with NBS 19 and giving mean $\delta^{13}C$ and $\delta^{18}O$ values of +2.08 ± 0.04‰ and +1.70 ± 0.05‰ respectively (n = 78) was used to check the accuracy of the data.[2] We used these standard deviations as an

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[1] Collagen is used here as a shorthand term for the acid-insoluble protein content of bone, which mostly comprises collagen (90–95%) but also includes non-collagenous proteins.

[2] The carbon and oxygen isotope ratios are, by convention, expressed relative to a standard (vPDB – Vienna Pee Dee Belemnite). Both are expressed in per mil (‰ – 1/1000): $\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{vPDB}}{(^{13}C/^{12}C)_{vPDB}} \times 1000$ & $\delta^{18}O = \frac{(^{18}O/^{16}O)_{sample} - (^{18}O/^{16}O)_{vPDB}}{(^{18}O/^{16}O)_{vPDB}}$.