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 & 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 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; 2013; 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 &

[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.

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 & Lee-Thorp 1999). The increased reactivity of the small, less well-recrystallized crystallites may render them more prone to post-burial alteration. 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

104 will in turn be useful for the radiocarbon dating and study of funerary practices of sites where
105 cremation was practiced.
106
107 **2. MATERIALS AND METHODS**
108 ***2.1. Samples***
109 The samples investigated here (Table 1) have been previously analysed by FTIR (Snoeck et
110 al. 2014b) with the exception of 15 additional samples (labelled LAB 2). To investigate the
111 relative contributions of the different carbon sources (endogenous bone apatite carbonates,
112 organic matter, fuel) to the isotope composition of calcined bone apatite carbonates, modern
113 animal joints were heated in different conditions. A first set of experiments (LAB 1) in which
114 different fragments of the same defleshed cow tibia were heated at different temperatures
115 (500 to 900°C) for 0.5 to 24h aimed at assessing the impact of time and temperature. In the
116 second experiment (LAB 2), samples were heated together with either barley (C_3), millet
117 seeds, or corn flour (the latter both C_4) to test the impact of fuels with differing carbon
118 isotope composition. A further experiment with wood and coal, conducted outdoors (OUT)
119 also aimed at assessing this. Finally, in the last experiment, some samples were treated with
120 hydrazine hydrate (95%) following the protocol designed by Termine et al. (1973) to remove
121 organic matter prior to heating, to assess the contribution of the latter.
122 Next, fully calcined (white – colour codes 5 and 6 from Stiner et al. 1995)
123 archaeological samples of cremated human remains were compared to modern samples to
124 assess the possible effects of post-burial alterations or different heating/burning conditions.
125 The sites, all from Ireland, range in date from the Neolithic to the Middle Bronze Age (ca.
126 5500 to 3500 cal BP). They present a wide range of geological contexts from chalk and
127 limestone (high-carbonate) to granite and basalt (non/low-carbonate). For Ballymacaldrack,
128 Co. Antrim, two calcined bone fragments were selected from the single individuals
129 represented in three separate urns (BM1a/b, BM2a/b and BM3a/b respectively) (Tomb &
130 Davies 1938). Three fragments of the same bone were also analysed for a sample from
131 Parknabinnia, Co. Clare (P3a/b/c).
132
133 Table 1 – List of samples
134
135 ***2.2. Isotope analyses of apatite carbonates***
136 Before isotope analyses, the calcined bone fragments were treated with sodium hypochlorite
137 (1% NaOCl) for one hour to remove any remaining organic matter followed by three rinses

with MilliQ water and then a four-hour treatment with 1M 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 5mg 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 indicator of analytical precision over the period of analysis.

In order to determine the isotope composition of the unaltered bioapatite, prior to calcination a fragment of each modern sample was kept aside. These samples were pre-treated with hydrazine hydrate ($N_2H_4.H_2O$ 95%) following the protocol designed by Termine et al. (1973) to remove all organic matter. This was followed by 30 minutes in a 1M solution of calcium acetate buffered acetic acid.

2.3. Isotope analyses of collagen and fuel proxies

For collagen $\delta^{13}C$ measurements of modern unburned bone, skin and flesh were removed mechanically. Lipids were removed by soaking the bone in a mixture of methanol and chloroform (2:1), and ultrasonication. Following O'Connell et al. (2001), the bone was demineralised with HCl (0.5M at 4°C), and collagen extracted by heating in pH3 water at 75°C. The supernatant solution containing the dissolved collagen was freeze-dried. Fuel proxies, such as barley and millet seeds, were measured directly without prior pre-treatment other than crushing with a mortar and pestle. For isotope measurements, two or three aliquots of approximately 1 mg of dried collagen or crushed fuel were placed in a tin capsule and analysed by mass spectrometry (Sercon Geo 2022 IRMS coupled to Sercon Europa EA–GSL running with continuous flow mode with helium carrier gas 80 mL/min).

3. RESULTS

[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):

$$\delta^{13}C = \frac{\left({}^{13}C/_{12}C\right)_{sample} - \left({}^{13}C/_{12}C\right)_{vPDB}}{\left({}^{13}C/_{12}C\right)_{vPDB}} \times 1000 \quad \& \quad \delta^{18}O = \frac{\left({}^{18}O/_{16}O\right)_{sample} - \left({}^{18}O/_{16}O\right)_{vPDB}}{\left({}^{18}O/_{16}O\right)_{vPDB}} \times 1000$$

3.1. Isotopic results for apatite, collagen and fuels

The isotope values of apatite carbonate and collagen of each modern sample were measured prior to heating (Table 2) as well as the isotope values of the various fuels and fuel proxies used during the experiments (Table 3).

Table 2 – Isotope analyses results for modern unburned bone samples

Table 3 – Isotope analyses results for fuels and fuel proxies

3.2. Laboratory heating experiments

The results for the LAB 1 samples (Table 4) show a strong correlation between the carbon and oxygen isotope ratios ($R^2 = 0.73$) (Figure 1) independently of the degree of calcination (charred and calcined bone fragments together). The results are presented as $\Delta^{13}C$ and $\Delta^{18}O$ ($\Delta = \delta_{sample} - \delta_{unburned\ apatite}$) as we are more interested in the isotopic variation than the absolute values. All heated samples are depleted in both ^{13}C and ^{18}O compared to unburned bone, with calcined samples being more depleted than charred specimens. Two calcined samples (out of 80) have $\delta^{13}C$ values only slightly more negative than unburned bone collagen $\delta^{13}C$ value.

Table 4 – Isotope analyses results for LAB 1 samples and colour code (Stiner et al. 1995)

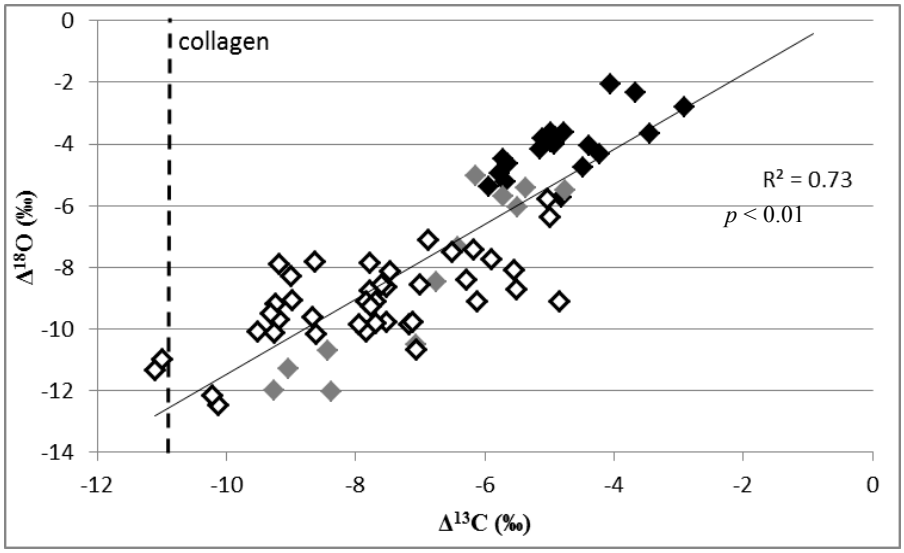


Figure 1 – Variations in carbon ($\Delta^{13}C$; $\Delta = \delta_{sample} - \delta_{unburned\ apatite}$) and oxygen ($\Delta^{18}O$) isotope ratios for LAB 1 samples; black, grey and white diamond correspond to charred (colour codes 3 and 3.5), intermediate (4, 4.5 and 5) and calcined (5.5 and 6) bone respectively; the dotted line represents the difference in carbon isotope composition ($\Delta^{13}C$) between collagen and unburned bone apatite

Combining the results of all samples heated at the same temperature (for 30 minutes to 24 hours) it appears that both $\delta^{13}C$ and $\delta^{18}O$ values decrease with temperatures approaching 700-800ºC as previously observed (Van Strydonck et al. 2005; Munro et al. 2008; Harbeck et al. 2011) and then $\delta^{13}C$ values increase again at 900ºC (Figure 2a) regardless of heating duration. These variations have been tested statistically (ANOVA – Table 5) and show a significant decrease in both $\delta^{13}C$ and $\delta^{18}O$ values between samples heated at 500-600ºC and those heated at higher temperatures. The $\delta^{18}O$ values remain similar between 700 and 900ºC while $\delta^{13}C$ values increase again significantly at 900ºC. Harbeck et al. (2011), observed a similar pattern when heating cow tibia samples to 1000ºC (Figure 2b). No increase between at higher temperature could be observed in the data from Van Strydonck et al. (2005) but no data is available for 800ºC, which seem to be the temperature at which the samples have the lowest $\delta^{13}C$ values.

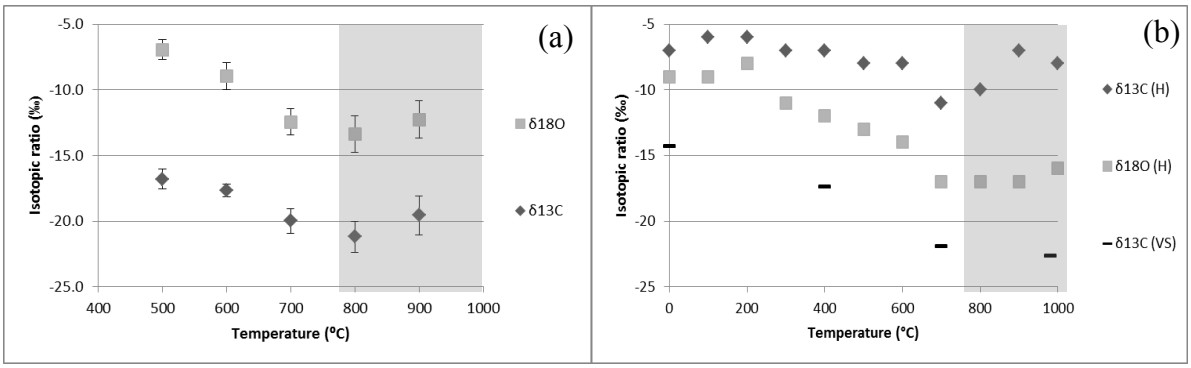


Figure 2 – Changes in carbon and oxygen isotopic ratios with temperature (a) for LAB 1 and (b) from Harbeck et al. (2011 – H) for one hour & Van Strydonck et al. (2005 – VS) for five hours; the grey shaded area represents the expected range of temperature for an outdoor pyre (DeHaan 2008)

Table 5 – ANOVA test comparing the $\delta^{13}C$ and $\delta^{18}O$ values of the LAB 1 samples heated at different temperature regardless of duration

The impact of fuel proxies on the $\delta^{13}C$ values of calcined bone is clear, shifting bone $\delta^{13}C$ values closer those of the proxy (LAB 2 – Table 6, Figure 3). The seal limb samples heated together with corn flour have $\delta^{13}C$ values even higher than the $\delta^{13}C$ values of unburned bioapatite and corn flour. The impact on the $\delta^{18}O$ values is less clear. Overall, the $\delta^{18}O$ values of samples heated with barley seeds and corn flour are enriched in ^{18}O if organic matter had been previously removed. Furthermore, for the dry seal limb, samples are more enriched in ^{18}O when burned without fuel proxies but this was not observed in the pig rib samples.

Table 6 – Isotope analyses results for LAB 2 samples

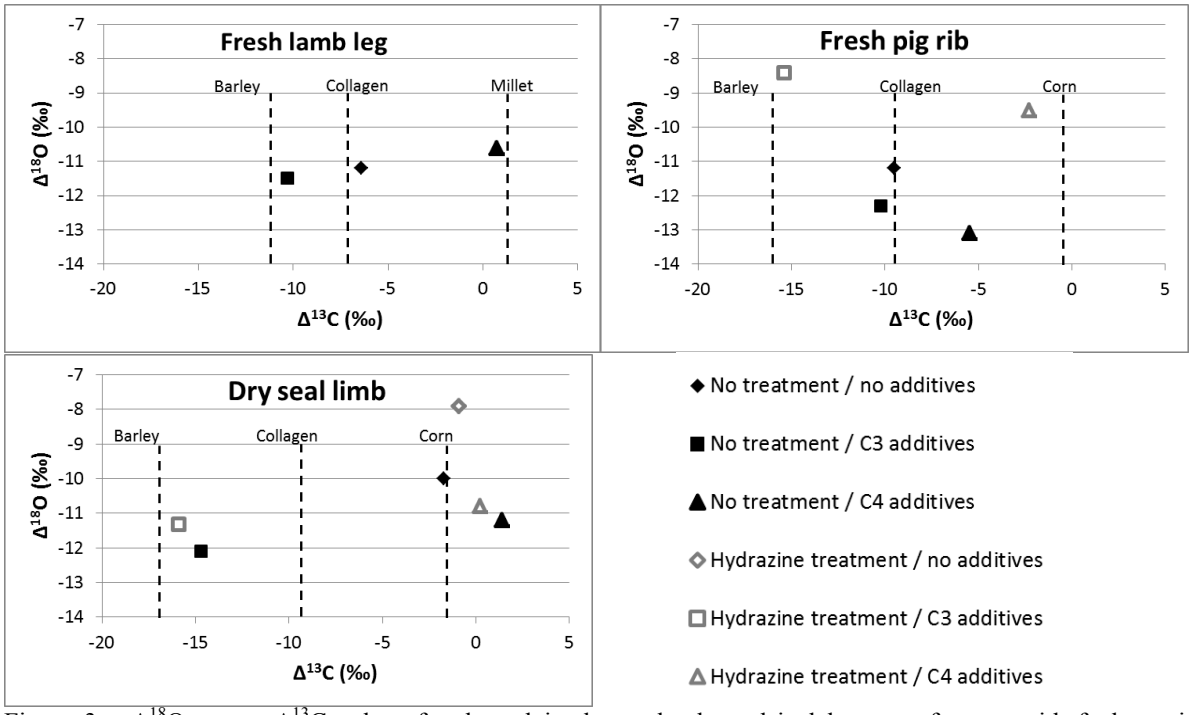


Figure 3 – $\Delta^{18}O$ versus $\Delta^{13}C$ values for the calcined samples heated in laboratory furnace with fuel proxies (LAB 2); some have been treated with hydrazine hydrate to remove all organic matter present prior to heating; the dotted lines represent the difference in carbon isotope composition ($\Delta^{13}C$) between fuel proxies or collagen and unburned bone apatite (e.g. in the case of the fresh lamb leg, there is a difference of 11.5‰ between barley and unburned bone apatite)

3.3. Outdoor experiments

As seen for the samples heated in the presence of fuel proxies, the $\delta^{13}C$ values of calcined samples burned on outdoor pyres are close to those of the fuel on which they were burned (Table 7). The sample OUT 12 burned with manufactured 'coal' 1 ($\delta^{13}C_{coal}$ = -24.3‰) seems to have exchanged all its carbon with fuel-derived carbon. When the bone was left longer on the fire and C_4 millets seeds added (OUT 13), its $\delta^{13}C$ value increased significantly (by 4.6‰) in the direction of the millet seeds ($\delta^{13}C_{millet}$ = -12.9 ‰). The corn fed chicken also has a high $\delta^{13}C$ value compared to the other samples (except OUT 13 burned together with millet). While its unburned apatite $\delta^{13}C$ value (-11.5 ‰) was close to that of the other, its collagen $\delta^{13}C$ value was much higher (-12.2 ‰) than in the others. Looking at the $\delta^{18}O$ values, the samples are depleted in ^{18}O after burning.

Turning to the variation between unburned and calcined bone $\delta^{13}C$ values (expressed as a difference, or $\Delta^{13}C = \delta^{13}C_{calcined} - \delta^{13}C_{unburned}$), it is positively correlated with the difference between the $\delta^{13}C$ values of the fuel used and unburned apatite ($R^2 = 0.57$; $p < 0.01$ – Figure 4a). Variation in oxygen isotope ratio during burning ($\Delta^{18}O$) is inversely correlated with $\Delta^{13}C$, with $\Delta^{18}O$ increasing when $\Delta^{13}C$ decreases. This correlation is even stronger when

clustering the samples according to the fuel with which they were burned (R^2 = 0.71 & 0.83 – figure 4b). Small sample size in this instance means that only one of the trends is statistically significant at α = 0.05, although we would argue that both nevertheless can be considered as meaningful.

Table 7 – Isotope analyses results for OUT samples

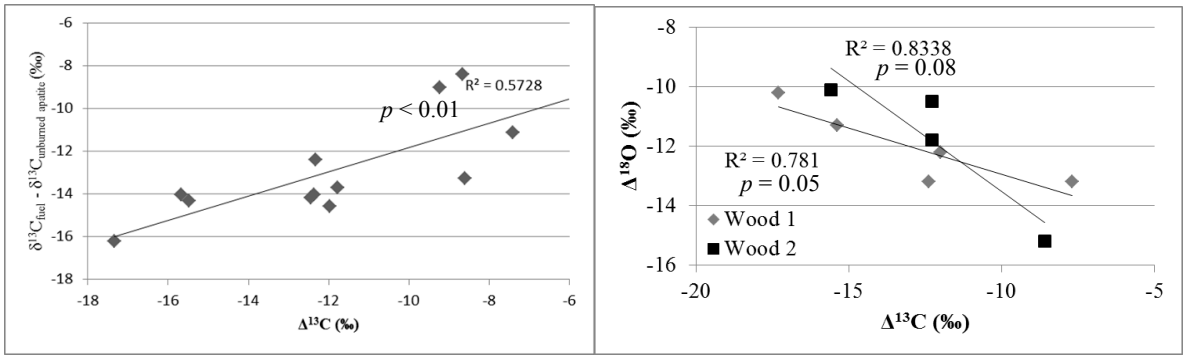


Figure 4 – (a) The difference in carbon isotopic ratios between fuels and unburned bone apatite versus the variation in the carbon isotopic ratios ($\Delta^{13}C$); (b) $\Delta^{18}O$ versus $\Delta^{13}C$ values for the calcined samples burned on outdoor wood fires 1 and 2

3.4. Archaeological samples

The $\delta^{13}C$ and $\delta^{18}O$ values of archaeological samples (Table 8) vary widely ranging from -29 to -18‰ and from -12 to -22‰, respectively. No statistical different in $\delta^{13}C$ and $\delta^{18}O$ values between samples with high (BPI > 0.30) and low (BPI < 0.30) carbonate content was found. However, samples containing cyanamide (CN/P > 0.25) have generally higher $\delta^{13}C$ values (Mann-Whitney U-test, Z = 4.87, $p < 0.001$) but no significant difference in $\delta^{18}O$ values. In some cases, several fragments of the same individual were analysed (BM1, 2, 3, and P3), showing that variations in $\delta^{13}C$ and $\delta^{18}O$ values for different fragments of a single cremated individual can reach to 3.1 and 4.6‰ (BM1) respectively.

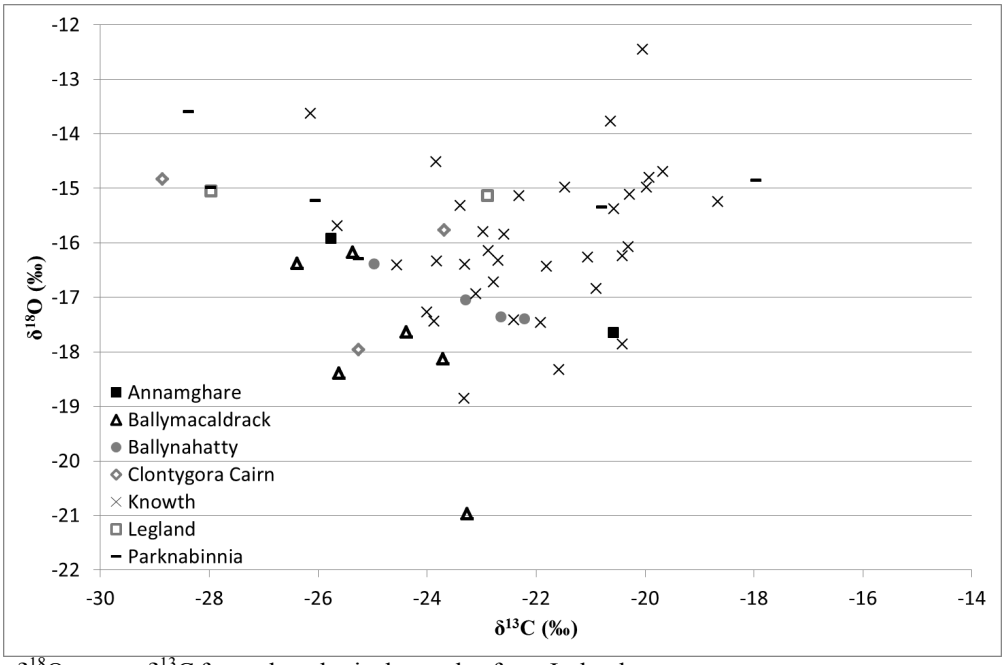


Figure 5 – $\delta^{18}O$ versus $\delta^{13}C$ for archaeological samples from Ireland

Table 8 – Isotope analyses data for archaeological calcined bone samples and colour code (Stiner et al. 1995)

4. DISCUSSION

4.1. Comparison between different samples

4.1.1. Modern heated and burned bone

The results of the laboratory heating and outdoor pyre experiments demonstrated that the presence of fuel or fuel proxies impacts strongly on the carbon and oxygen isotope composition of calcined bone apatite, whether or not organic matter had been previously removed chemically. Samples burned with fuel/fuel proxies are more depleted in ^{13}C and ^{18}O than those heated without (Figure 6); for the latter, $\delta^{13}C$ and $\delta^{18}O$ values both decrease up to 700-800°C before $\delta^{13}C$ values increase at higher temperature (Figure 2a). There is a strong correlation ($R^2 = 0.73$) observed between the $\delta^{13}C$ and $\delta^{18}O$ values of heated bone (LAB 1 – Figure 1). The variations in oxygen isotope composition of samples burned with fuel are lower when the carbon exchanges with fuel evaluated from $\delta^{13}C$ values are higher, and the presence of collagen limits exchanges with fuel as an additional source of carbon is present. Indeed, samples from which collagen had been removed prior to heating have $\delta^{13}C$ values closer to that of the fuel proxies they were burned with compared to samples still containing collagen (Figure 3). Finally samples heated without collagen are generally enriched in ^{18}O.

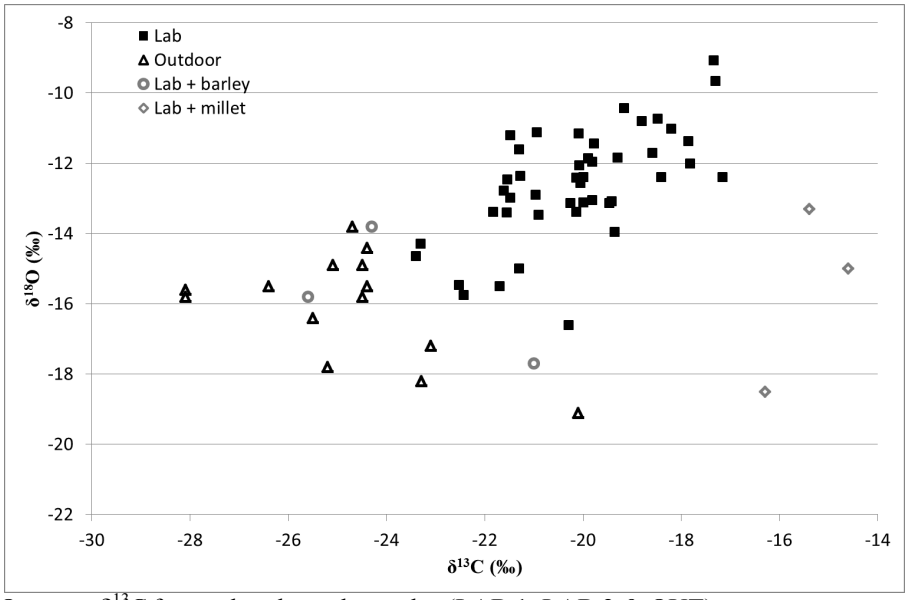


Figure 6 – $\delta^{18}O$ versus $\delta^{13}C$ for modern heated samples (LAB 1, LAB 2 & OUT)

The correlation observed between the carbon and oxygen isotope ratios of bone heated without fuel proxies (LAB 1) suggests that the exchanges and losses of carbon and oxygen occur as part of a similar process, most likely through the loss of carbon dioxide and exchanges with carbon dioxide from the combustion atmosphere. Also, the observation that two samples (out of 80) are more depleted in ^{13}C than their collagen values suggests either that some fractionation occurs between the carbon dioxide emitted by the combustion of collagen and bone apatite carbonate or that there is some exchange with ^{13}C-depleted lipids (Tieszen & Farge 1993). However, these are likely to burn away before bone apatite starts to exchange carbon with the combustion atmosphere. Furthermore, the fact that the samples heated at 800°C are more depleted in both ^{13}C compared to those heated at 700 and 900°C may suggest – as for a calcite-CO_2 system (Scheele & Hoefs 1992) – that the fractionation between bone apatite carbonates and carbon dioxide present in the combustion atmosphere is temperature-dependent. However, as observed in the calcite-CO_2 system, fractionation should decrease with temperature, from -4‰ at 500°C to -3‰ at 800°C. The fact that the amount of carbonates (evaluated by infrared spectroscopy) is very similar in calcined samples heated at different temperature suggests that the differences seen in isotope ratios are due to different exchanges happening or to a combination of different exchanges and fractionations.

The isotopic results of the samples heated with C_3 and C_4 fuel and fuel proxies (LAB 2) confirm their impact on both carbon and oxygen isotope ratios. The observation that some calcined seal samples have isotopes values higher than unburned bone strongly suggests that some fractionation occurs between bone apatite carbonates and the carbon dioxide released

by its heating, leaving heavier carbon isotopes in bone apatite. At 800ºC, it has been suggested that such fractionation could be around 3‰ (Hüls et al. 2010). This is consistent with our results where the maximum enrichment observed between unburned and burned bone apatite carbon is 1.4‰ when a seal limb is burned at 800ºC in the presence of corn flour (the latter being depleted in ^{13}C compared to unburned bone apatite carbonates).

Looking at the oxygen isotopes, it is difficult to detect possible fractionation as more sources of oxygen (including large amounts of atmospheric dioxygen) are available for exchange and the exact $\delta^{18}O$ values of bone collagen and fuels could not be measured. It is however likely that, if there is a carbon fractionation, this would also occur for oxygen.

4.1.2. Archaeological and modern calcined bone

All human bone samples in this study, dating from the Irish Neolithic and Middle Bronze Age, are expected to have typical C_3 terrestrial-based diet values prior to calcination as indicated by isotopic measurements carried out on unburned human bone from the same period (e.g. Schulting et al. 2012). At Knowth, for example, the $\delta^{13}C$ values of the unburned human bone collagen (n = 14) fall between -22.5 and -21.0‰, a very narrow range of 1.5‰. In calcined bone, however, the range observed in bioapatite carbonates $\delta^{13}C$ values is much higher (ca. 9‰ – Figure 5). This difference appears to support the large impact of fuels and fuel proxies on the isotope composition of cremated bone apatite carbonates.

As would be expected, most of the archaeological samples have $\delta^{13}C$ and $\delta^{18}O$ values similar to those of experimental samples burned with C_3 fuel (Figure 7a). Some archaeological samples, however, are more ^{13}C-enriched than others. We can discount the use of both C_4 foods and fuels in Ireland at this time. Most of these samples show evidence for cyanamide (Figure 7b) suggesting that either (1) the presence of cyanamide is limiting the exchanges and incorporation of fuel carbon into the structure of bone apatite, the latter being the most ^{13}C depleted carbon source, or (2) the lack of carbon dioxide in the combustion atmosphere could favour the incorporation of cyanamide into bone apatite. The latter could occur when bone is used as fuel as less organic carbon is present for combustion. However, there is no evidence for the use of bone as fuel in prehistoric Ireland, nor would this be expected given the plentiful wood supplies. Another possibility that could explain the incorporation of cyanamide in cremated bone is a low ratio of oxygen to fuel. This would induce more reducing conditions around the bone. The lack of oxygen during combustion leads to incomplete oxidization of organic matter emitting CO and other components instead of CO_2 (González-Pérez et al. 2004). Such conditions are also used to produce charcoal. In

any case, there seem to be a competition between cyanamide and carbon dioxide from the combustion atmosphere.

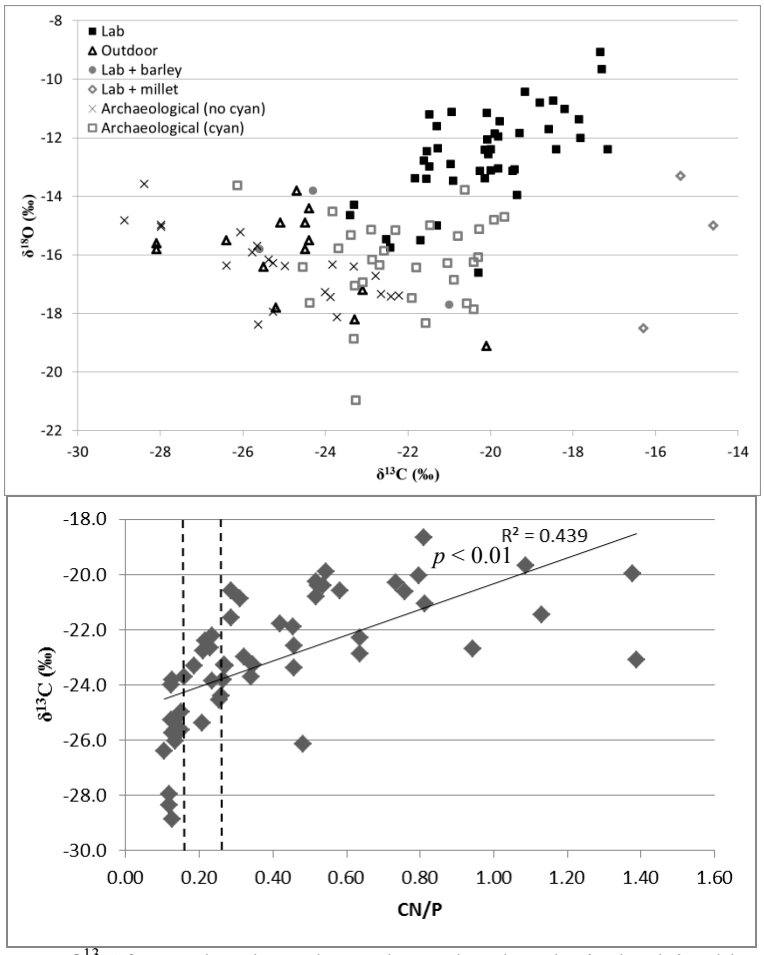


Figure 7 – (a) $\delta^{18}O$ versus $\delta^{13}C$ for modern heated samples and archaeological calcined bone highlighting those having cyanamide (cyan – CN/P ≥ 0.25); (b) $\delta^{13}C$ value versus CN/P ratio for archaeological calcined bone

4.1.3. Archaeological calcined bone from different sites

Comparing the isotope results of samples from archaeological sites where more than three measurements were carried out (Figures 8a–f), there seems to be an inverse correlation between $\delta^{13}C$ and $\delta^{18}O$ values as observed in modern samples burned on outdoor pyres (Figure 4b). Most of these correlations are not significant ($p > 0.05$) due to the small number of samples. Their presence at each site, however, warrants further discussion.

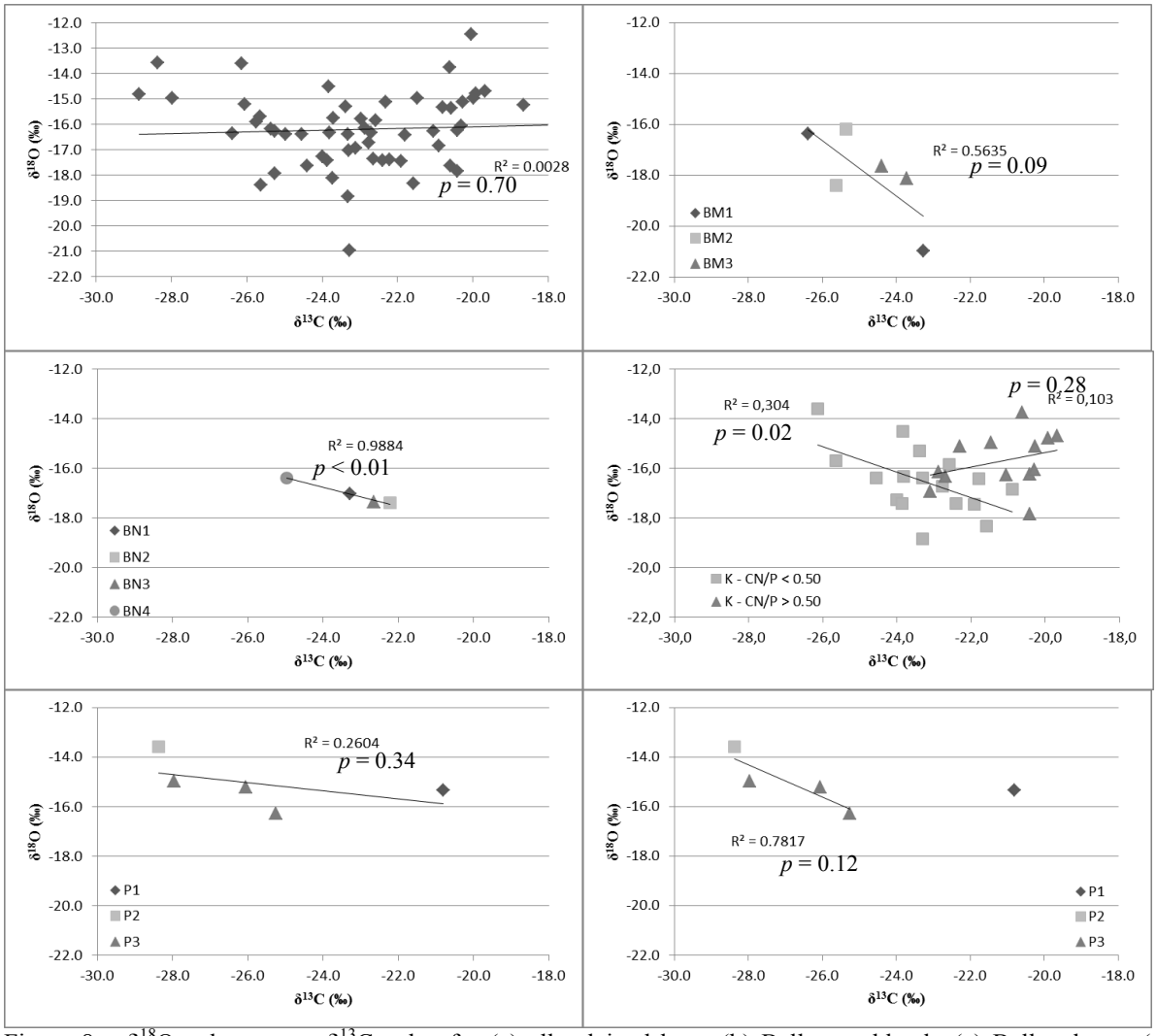


Figure 8 – $\delta^{18}O$ value versus $\delta^{13}C$ value for (a) all calcined bone (b) Ballymacaldrack; (c) Ballynahatty; (d) Knowth; (e) Parknabinnia; (f) Parknabinnia excluding P1 (see text)

Such a correlation is not observed in the combined dataset (Figure 8a), suggesting that it only exists in samples that have been cremated with similar fuel and/or on pyres of similar size, and/or built in similar ways (i.e. similar oxygen to fuel ratios). At Parknabinnia (Figure 8e), the correlation is weaker ($R^2 = 0.26$) than that observed in Ballymacaldrack (Figure 8b: $R^2 = 0.56$) and Ballynahatty (Figure 8c: $R^2 = 0.99$). However, strontium isotope evidence strongly suggests that sample P1 at Parknabinnia did not originate from the same place as P2 and P3, so that this individual could have been cremated under different conditions. Excluding P1, the correlation is stronger (Figure 8f: $R^2 = 0.78$). For Knowth, where relatively high cyanamide content is observed in the calcined bone, there seem to be no pattern, but when excluding samples having particularly high cyanamide content there is a moderate but significant correlation ($R^2 = 0.30$; $p = 0.02$) between $\delta^{18}O$ and $\delta^{13}C$ values (Figure 8d). The fact that the correlation is weaker than observed for other sites may again be due to different

385 cremation conditions for different individuals. This can be expected for a site such as
386 Knowth, which is one of the largest passage tomb complexes in Western Europe.
387 Nevertheless, it seems that for samples with low or no cyanamide content (CN/P < 0.5) the
388 correlation between $\delta^{13}O$ and $\delta^{18}O$ indicate broadly similar cremation conditions.
389 The interpretation of cremation practices remains, however, complex. Indeed, two
390 bone samples originating from a single cremated individual can exhibit different isotopic,
391 compositional and structural characteristics (e.g. BM1a and BM1b from Ballymacaldrack).
392 This highlights the extreme variability in conditions (temperature, oxygen availability, carbon
393 exchange rates) around the body during heating. Nevertheless, the good inverse correlation
394 observed between $\delta^{13}C$ and $\delta^{18}O$ ($R^2 > 0.55$) at Ballynahatty and Ballymacaldrack may
395 suggest similar cremation practices (e.g. similar pyre size, fuel, length) for the individuals
396 represented at those sites. The weaker correlation observed at Knowth ($R^2 = 0.3$) may
397 indicate more variable cremation practices. Furthermore, a high number of samples having
398 high CN/P (> 0.50) at a particular site (e.g. Knowth) could suggest the use of larger pyres for
399 cremation with more reducing conditions (lower oxygen to fuel ratio). Another possibility
400 explaining lower oxygen availability during cremation could be that cremation took place in a
401 badly ventilated area (e.g. within a building or in a pit) or that the body was not place on top
402 of the pyre but within it.
403
404 ***4.2. Isotopic changes during calcination***
405 The difference in carbon isotope ratios between samples heated without additional fuel and
406 those burned on an outdoor fire clearly shows that the presence of fuels has a significant
407 impact on the isotope composition of calcined bone. There is still a question concerning the
408 origin of the rest of the carbon present in bone apatite carbonates: endogenous bone apatite
409 carbonates and/or collagen carbon. All but two samples in the laboratory experiments carried
410 out without fuel proxies and prior treatment have $\delta^{13}C$ values between those of unburned
411 apatite carbonates and collagen suggesting that some endogenous carbonates remain in bone
412 apatite after calcination (with potential fractionation) but that there is a significant
413 incorporation of collagen carbon into the carbonate fraction. As suggested by radiocarbon
414 dating results, it appears that the carbon composition of calcined bone apatite carbonates is
415 made of endogenous bone apatite carbon as well as carbon originating from the fuel and
416 collagen in varying proportions (Hüls et al. 2010; Van Strydonck et al. 2010; Zazzo et al.
417 2012; Snoeck et al. 2014a). The lack of directional variation in the isotope ratios of the LAB
418 1 samples heated at the same temperature for different amounts of time suggests limited

adsorption-desorption. However, some exchanges are still possible when the combustion environment is modified, as demonstrated by the change in $\delta^{13}C$ seen in the lamb leg burned outdoor first without and then with millet seeds (OUT 12 & 13).

Shifts in $\delta^{18}O$ composition of calcined bone are more difficult to assign to particular pathways. Samples heated with fuel of whatever kind have lower $\delta^{18}O$ values than those heated without (-16.4±1.6‰ vs. -12.4±1.4‰; t-test $p < 0.0001$). Additionally, samples that exchanged more ^{13}C with fuel (and are therefore depleted in ^{13}C) are more enriched in ^{18}O than the others. This correlations between the carbon and oxygen isotope values, suggests that the source(s) of carbon and oxygen are largely the same, or at least that the carbonates present in bone apatite can only exchange with carbon dioxide and not with dioxygen or water. Furthermore, the inverse correlation is likely due to the fact that the oxygen isotope composition of cellulose (main component of wood) is significantly enriched in ^{18}O (c. 0 to -5‰ – Roden & Farquhar 2012) compared to bone apatite and collagen and even slightly enriched compared to atmospheric oxygen (c. -7‰ – Kroopnick & Craig 1972).

In summary, the carbon present in calcined bone apatite is comprised of a combination of endogenous bone apatite carbonate, endogenous collagen carbon and fuel carbon. The oxygen originates mostly from endogenous carbonates, fuel, collagen and atmospheric oxygen. The proportion in which each of these sources contributes to the isotope composition of calcined bone apatite is variable and, at present, still difficult to evaluate.

4.3. Diagenesis

The infrared results showed that some calcined bone samples had higher carbonate content (Snoeck et al. 2014b). It was suggested that this could be the consequence of the incorporation of exogenous carbonates from the soil as all samples with higher carbonate content originated from high-carbonate marine geological contexts. Such contamination is usually significantly enriched in ^{13}C compared to calcined bone apatite ($\delta^{13}C \approx 0$ ‰ vs. < -20 ‰) and small amounts should be easily detected. However, neither $\delta^{13}C$ values nor $\delta^{18}O$ values show a statistically significant difference between samples with high (BPI > 0.30) and low (BPI < 0.30) carbonate content, suggesting that the large concentrations of carbonates are not diagenetic but require another explanation. Very limited (if any) post-burial intake of strontium in calcined bone was also highlighted through artificial contamination experiments (Snoeck et al. 2015), confirming calcined bone's high resistance to diagenesis. The observation that only samples from high carbonate geological context present high carbonate content could be the consequence that such contexts are less acid compared to granitic and

other geological contexts where more leaching and loss of sample is possible. This difference, however, would only destroy part of the sample and not add diagenetic contamination.

5. CONCLUSION

The results of this study, together with the existing body of radiocarbon studies, indicate that the majority of carbonates present in bone apatite after cremation originates from the fuel used in the pyre. Furthermore, it appears possible to identify bones heated under different conditions. More specifically the following distinctions can be made: (1) samples heated with and without fuel, the former being enriched in both ^{13}C and ^{18}O; (2) samples containing cyanamide that have generally higher $\delta^{13}C$ values than those without cyanamide. This could be the consequence of reducing conditions potentially reflecting different cremation conditions (size of the pyre, position of the corpse, degree of ventilation, etc.); in this case, $\delta^{18}O$ values can be used to differentiate between samples heated without fuel and those presenting high amounts of cyanamide; and (3) samples heated with C_3 and C_4 fuels, the latter being enriched in ^{13}C (though C_4 plants, being mainly grasses, are unlikely to have ever been used for pyre material). These results can also be applied to calcined faunal remains to discriminate samples that were discarded as waste and burned compared to those used as fuel when other resources were scarce such as during the Middle- and Upper Palaeolithic (e.g. Théry-Parisot 2002; Münzel & Conard 2004; Morin 2010).

The results obtained over the last decade on the structural and chemical changes occurring in bone during heating (Thompson et al. 2009; Lebon et al. 2010; 2014; Snoeck et al. 2014b) together with the increasing pool of knowledge on the carbon, oxygen and strontium isotope compositions of calcined bone (Hüls et al. 2010; Van Strydonck et al. 2010; Zazzo et al. 2012; 2013; Snoeck et al. 2015) confirm the high resistance to post-burial alterations of calcined bone. We now have an improved basis to better understand mobility patterns and funerary practices in places and times where cremation was the dominant burial practice and/or where conservation of unburned remains is very poor.

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583

584

585
586 **TABLES**

587 Table 1 – List of samples

Samples (#)	Age	Location
LAB 1 (80)	Modern	Oxford, England (Heated in a muffle furnace)
LAB 2 (15)		
OUT (13)		Oxford, England (Cremated on outdoor pyres)
K (36)	Neolithic	Knowth, Ireland (sandstone and limestone)
A (2)		Annaghmare, N. Ireland (sandstone and shale)
BN (4)		Ballynahatty, N. Ireland (sandstone and shale)
C (3)		Clontygora Cairn, N. Ireland (granite)
L (2)		Legland, N. Ireland (sandstone and shale)
P (6)		Parknabinnia, Ireland (limestone)
BM (6)	Middle Bronze Age	Ballymacaldrack, N. Ireland (basalt)

588
589 Table 2 – Isotope analyses results for modern unburned bone samples

#	Corresponding heated fragments	$\delta^{13}C$ (‰) – collagen	$\delta^{13}C$ (‰) – apatite	$\delta^{18}O$ (‰) – apatite
L1	LAB 2 (lamb leg), OUT 9, 12, 13	-22.6	-15.3	-4.3
L2	OUT 8, 10	-22.4	-15.9	-5.4
P1	LAB 2 (pig rib), OUT 1, 2, 14	-20.3	-10.8	-5.4
P2	OUT 6	-23.1	-12.7	-4.5
P3	OUT 7	-23.5	-12.8	-4.6
C	LAB 1, OUT 3, 4	-23.2	-12.4	-3.3
Ch	OUT 11	-12.2	-11.5	-3.9
S	LAB 2 (seal bone)	-19.0	-9.6	-1.7

590
591 Table 3 – Isotope analyses results for fuels and fuel proxies

Fuel/fuel proxy	Type	$\delta^{13}C$ (‰)
Wood 1	Old wood	-24.8
Wood 2	Old wood	-27.0
Wood 3	Modern wood	/
Coal 1	Manufactured coal briquettes	-24.3
BAR	Barley seeds	-26.8
MIL	Millet seeds	-12.9
CORN	Corn flour	-11.5

592
593 Table 4 – Isotope analyses results for LAB 1 samples and colour code (Stiner et al. 1995)

T (°C)	Time (h)	Sample	Colour Code	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
0	0	/	0	-12.4	-3.3
500	0.5	05_3	3	-16.4	-5.4
		10_4	3	-16.0	-5.6
	1	13_2	3	-15.2	-6.1
		15_1	3	-17.4	-7.1
	2	05_4	4	-16.7	-7.4
		15_4	4	-17.2	-7.1
	4	13_3	3.5	-17.1	-6.9
		14_4	3.5	-17.3	-6.9
	8	01_2	3.5	-17.9	-7.9
		05_2	3.5	-17.2	-7.3
	12	07_2	3.5	-17.3	-7.2
		11_1	3.5	-17.4	-7.5
	18	01_4	3.5	-15.7	-7.0
		20_2	3.5	-16.5	-7.6
	24	04_4	*failed*		
		20_4			

594

595

600	0.5	03_1	3.5	-18.0	-7.8
		16_1	3.5	-16.8	-8.1
	1	01_1	4	-18.2	-8.7
		10_2	4	-17.1	-9.0
	2	02_4	4	-18.0	-8.5
		04_3	4	-18.1	-8.3
	4	14_1	5	-18.4	-8.3
		16_2	5	-17.1	-8.8
	8	09_4	*failed*		
		18_4			
	12	04_2	5	-18.0	-9.0
		08_2	5	-17.8	-9.4
	18	08_4	5.5	-17.3	-9.7
		14_3	5	-17.7	-8.7
	24	02_2	6	-17.3	-9.1
		18_1	6	-17.8	-12.0
700	0.5	08_1	4.5	-19.0	-11.8
		12_1	4.5	-18.7	-10.6
	1	05_1	5	-19.4	-13.8
		10_1	5	-21.3	-14.6
	2	06_3	6	-19.8	-12.0
		15_2	6	-20.1	-12.1
	4	12_2	6	-19.8	-13.1
		13_4	6	-20.0	-12.4
	8	12_3	6	-20.1	-12.4
		15_3	6	-20.1	-12.6
	12	17_2	6	-21.8	-13.4
		18_2	6	-21.3	-12.4
	18	17_4	*failed*		
		19_3			
	24	03_3	6	-18.6	-11.7
		03_4	6	-19.8	-11.4
800	0.5	02_1	4.5	-20.7	-14.0
		20_1	4.5	-20.7	-15.3
	1	07_3	5	-21.6	-15.3
		19_2	5.5	-23.4	-14.6
	2	09_2	6	-21.5	-13.0
		17_1	6	-22.5	-15.5
	4	02_3	6	-20.9	-11.1
		11_2	6	-19.9	-11.8
	8	01_3	6	-20.1	-13.4
		17_3	6	-23.3	-14.3
	12	16_3	6	-21.3	-11.6
		20_3	6	-20.9	-13.5
	18	03_2	6	-20.0	-13.1
		18_3	6	-21.6	-13.4
	24	06_4	6	-19.3	-11.8
		11_3	6	-21.5	-12.5

596

900	0.5	11_4	5.5	-17.2	-12.4
		19_4	5.5	-22.4	-15.8
	1	04_1	6	-19.5	-13.1
		13_1	6	-19.4	-14.0
	2	10_3	6	-19.4	-13.1
		14_2	6	-18.4	-12.4
	4	06_2	6	-18.8	-10.8
		07_4	6	-20.3	-13.1
	8	08_3	6	-21.0	-12.9
		09_3	6	-21.5	-11.2
	12	07_1	6	-19.2	-10.4
		09_1	6	-21.6	-12.8
	18	06_1	6	-20.1	-11.2
		19_1	6	-18.5	-10.7
	24	12_4	6	-17.9	-11.4
		16_4	6	-18.2	-11.0

Table 5 – ANOVA test comparing the $\delta^{13}C$ and $\delta^{18}O$ values of the LAB 1 samples heated at different temperature regardless of duration

Temp.	Isotope	Difference	Test Statistic	p
500 vs 600	$\delta^{13}C$	1.17	2.74	0.0780
	$\delta^{18}O$	2.27	4.96	< 0.0001
500 vs 700	$\delta^{13}C$	3.47	8.11	< 0.0001
	$\delta^{18}O$	5.76	12.59	< 0.0001
500 vs 800	$\delta^{13}C$	4.69	11.32	< 0.0001
	$\delta^{18}O$	6.70	15.13	< 0.0001
500 vs 900	$\delta^{13}C$	3.07	7.43	< 0.0001
	$\delta^{18}O$	5.58	12.61	< 0.0001
600 vs 700	$\delta^{13}C$	2.30	5.28	< 0.0001
	$\delta^{18}O$	3.49	7.50	< 0.0001
600 vs 800	$\delta^{13}C$	3.51	8.34	< 0.0001
	$\delta^{18}O$	4.43	9.83	< 0.0001
600 vs 900	$\delta^{13}C$	1.90	4.51	0.0003
	$\delta^{18}O$	3.31	7.35	< 0.0001
700 vs 800	$\delta^{13}C$	1.21	2.88	0.0525
	$\delta^{18}O$	0.94	2.08	0.4124
700 vs 900	$\delta^{13}C$	-0.40	0.94	1.0000
	$\delta^{18}O$	-0.18	0.40	1.0000
800 vs 900	$\delta^{13}C$	-1.61	3.96	0.0018
	$\delta^{18}O$	-1.12	2.57	0.1235

604
605 Table 6 – Isotope analyses results for LAB 2 samples

	Setting	Treatment	Fuel proxies	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
Fresh lamb leg	/	Unburned		-15.3	-4.1
	1h – 700°C	/	/	-21.7	-15.3
	4h – 700°C		BAR	-25.6	-15.6
			MIL	-14.6	-14.7
Fresh pig rib	/	Unburned		-10.8	-5.4
	4h – 800°C	/	/	-20.3	-16.6
			BAR	-21.0	-17.7
			CORN	-16.3	-18.5
		Collagen removed by hydrazine hydrate	/	/	/
			BAR	-26.2	-13.8
			CORN	-13.1	-14.9
Dry seal leg	/	Unburned		-9.6	-1.7
	4h – 800°C	/	/	-11.3	-11.7
			BAR	-24.3	-13.8
			CORN	-8.2	-12.9
		Collagen removed by hydrazine hydrate	/	-10.5	-9.6
			BAR	-25.5	-13.0
			CORN	-9.4	-12.5

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607 Table 7 – Isotope analyses results for OUT samples (see Table 2 and 3 for unburned and fuel/fuel proxy values)

#	Sample	Fuel	Time (h)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
OUT 1	Defleshed pig rib	Wood 2	1.25	-26.4	-15.5
OUT 2	Defleshed pig rib	Wood 1	1	-28.1	-15.6
OUT 3	Defleshed cow tibia	Wood 2	1.25	-24.7	-13.8
OUT 4	Defleshed cow tibia	Wood 1	1	-24.4	-15.5
OUT 6	Pig trotter with flesh and skin	Wood 1	1.5	-28.1	-15.8
OUT 7	Pig scapula with flesh and skin	Wood 1	1.5	-25.2	-17.8
OUT 8	Defleshed lamb leg	Coal 1	1.5	-24.5	-15.8
OUT 9	Defleshed lamb leg	Wood 3	1	-25.5	-16.4
OUT 10	Defleshed lamb leg	Wood 1	1.5	-23.3	-18.2
OUT 11	Whole corn-fed chicken	Wood 2	2.5	-20.1	-19.1
OUT 12	Defleshed lamb leg	Coal 1	0.83	-24.5	-14.9
OUT 13	Defleshed lamb leg	Coal 1 + millet seeds	2	-19.9	-17.7
OUT 14	Defleshed pig rib	Wood 2	2.5	-23.1	-17.2

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609 Table 8 – Isotope analyses data for archaeological calcined bone samples and colour code (Stiner et al. 1995)

#	Colour code	Geological carbonate content	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	BPI	CN/P
					from Snoeck et al. 2014b	
A1	5	Low	-25.8	-15.9	0.30	0.13
A2	5.5		-20.6	-17.6	0.22	0.29
BM1a	6	Low	-26.4	-16.4	0.17	0.10
BM1b	6		-23.3	-21.0	0.10	0.35
BM2a	6		-25.6	-18.4	0.15	0.15
BM2b	6		-25.4	-16.2	0.15	0.21
BM3a	6		-23.7	-18.1	0.13	0.16
BM3b	6		-24.4	-17.6	0.13	0.26
BN1	6	Low	-23.3	-17.0	0.16	0.27
BN2	5.5		-22.2	-17.4	0.24	0.24
BN3	6		-22.7	-17.4	0.27	0.23
BN4	6		-25.0	-16.4	0.31	0.15

C1	5.5		-25.3	-18.0	0.23	0.13
C2	6	Low	-28.9	-14.8	0.31	0.13
C3	5.5		-23.7	-15.8	0.24	0.34
K01	5.5		-21.0	-16.3	0.23	0.81
K02	6		-23.3	-16.4	0.32	0.19
K03	4.5		-20.0	-15.0	0.14	1.38
K04	4.5		-23.0	-15.8	0.37	0.32
K05	6		-21.6	-18.3	0.33	0.29
K06	6		-20.6	-13.8	0.20	0.76
K07	6		-20.3	-16.1	0.19	0.73
K08	5.5		-20.4	-16.2	0.30	0.54
K09	5.5		-21.9	-17.5	0.34	0.45
K10	5		-23.8	-14.5	0.47	0.27
K11	5		-22.7	-16.3	0.13	0.94
K12	5		-19.7	-14.7	0.26	1.09
K13	5		-23.3	-18.9	0.32	0.27
K14	5.5		-25.6	-15.7	0.38	0.13
K15	6		-19.9	-14.8	0.24	0.54
K16	5.5		-22.8	-16.7	0.30	0.21
K17	4.5	High	-18.7	-15.3	0.30	0.81
K18	6		-24.0	-17.3	0.35	0.12
K30	5		-21.5	-15.0	0.15	1.13
K31	5		-23.9	-17.4	0.29	0.24
K32	5		-21.8	-16.4	0.25	0.42
K35	4.5		-22.3	-15.1	0.15	0.64
K36	3.5		-20.0	-12.5	0.19	0.80
K37	4.5		-20.6	-15.4	0.45	0.58
K38	6		-23.1	-16.9	0.21	1.39
K39	5		-22.9	-16.1	0.13	0.64
K40	3.5		-23.4	-15.3	0.16	0.46
K41	5		-20.9	-16.8	0.51	0.31
K42	5.5		-23.8	-16.3	0.31	0.13
K43	5		-26.1	-13.6	0.22	0.48
K44	6		-22.4	-17.4	0.21	0.22
L1	5.5		-22.9	-15.1	0.19	0.40
L2	5.5		-28.0	-15.0	0.19	0.18
P1	5.5		-20.8	-15.3	0.21	0.52
P2	5.5		-28.4	-13.6	0.49	0.12
P3a	6	High	-25.3	-16.3	0.40	0.12
P3b	6		-26.1	-15.2	0.45	0.13
P3c	6		-28.0	-15.0	0.48	0.12
P4	4.5		-18.0	-14.9	0.18	1.34

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