CAN WE USE CALCINED BONES FOR ^{14}C DATING THE PALEOLITHIC?

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ABSTRACT. This work aims to test the reliability of calcined bones for radiocarbon dating of the Paleolithic. Fifty-five calcined bone samples coming from Aurignacian and Gravettian layers at Abri Pataud (Dordogne, France) were selected based on their macroscopic features. For each sample, the heating state was estimated on the basis of bone crystallinity (splitting factor [SF] using FTIR) and $\delta^{13}C$ value. Twenty-seven bone samples (3 unburnt and 24 calcined) from 5 different levels were prepared for ^{14}C dating. The majority (15/24) of the calcined samples had to undergo a sulfix treatment prior to graphitization, probably due to the presence of cyanamide ion in these samples. The comparison between our results and recently published dates on bone collagen for the same levels shows that unburned bone apatite is systematically too young, while a third of the calcined bones fall within or very near the range of expected age. No clear correlation was found between ^{14}C age offset and $\delta^{13}C$ value or SF. Most of the sulfixed samples (14/16) yielded ages that were too young, while almost all of the non-sulfixed samples (8/9) gave ages similar or <0.2 pMC from the expected minimum age. Although preliminary, these results suggest that sulfix should be avoided if possible and that clean CO_2 gas from well-calcined Paleolithic bones can provide reliable ^{14}C ages.

INTRODUCTION

Over the past decade, calcined bones (i.e. bones heated at high temperatures) have been recognized as a reliable support for radiocarbon dating (Lanting et al. 2001). Unlike biological (unburnt) apatites, which are poorly crystallized and prone to postdepositional chemical and structural exchanges, calcination of bones (i.e. burning >600 °C) induces a suite of modifications that enhance their preservation during burial. During heating, bone organic matter is progressively destroyed from 200 to 500 °C (Shipman et al. 1984; Chadefaux and Reiche 2009). The mineral fraction is also affected at low temperature with a progressive decrease of the carbonate content up to 600 °C, but the main modifications occur between 600 and 700 °C with the loss of more than 70% of the structural carbonates and the simultaneous increase of mineral crystallinity (size and perfection of crystals) (Shipman et al. 1984; Holden et al. 1995). These modifications are accompanied by a sharp decrease in the $\delta^{13}C$ values due to a combination of kinetic effects and carbon isotope exchange with the environment during heating (Zazzo et al. 2009, 2012; Hüls et al. 2010; Van Strydonck et al. 2010). As a result of these changes, calcined bone presents crystallinity values (crystal size and atomic order) at least equivalent or higher than tooth enamel, yet is considered a stable material over time (Person et al. 1996; Pasteris et al. 2004). The apatite crystal properties acquired by calcined bone make them very resistant to postdepositional contamination of the remaining inorganic carbon by exogeneous sources because of their lower specific surface (Reiche 2010). Several authors have considered calcined bones as a reliable alternative for ^{14}C dating in archaeological contexts where collagen was degraded (De Mulder et al. 2007, 2009, 2012; Olsen et al. 2008). However, the vast majority of these

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studies considered only young bones dating from the Bronze Age to Historic periods (but see Veil et al. 2012 for an example in the Late Glacial). To our knowledge, there has been no systematic attempt at dating calcined bones clearly predating the Holocene in well-dated archaeological contexts. To test the reliability of calcined bones through time, we dated a series of calcined bones coming from the site of the Abri Pataud (Dordogne, France). The best candidates for ^{14}C dating were selected using spectroscopic (FTIR) and isotopic ($\delta^{13}C$) prescreening techniques (Olsen et al. 2008). The Abri Pataud provides a key archaeological sequence for the Upper Paleolithic in Europe. This site is perfectly suited for our purpose because it presents a rich stratigraphic sequence containing 14 archaeological layers from the Aurignacian to the Gravettian. A recent ^{14}C dating program has provided a coherent and reliable chronology for this site (Table 1). A comparison between the dates obtained in this study and the recently published dates for the same levels will allow us to assess the potential of calcined bones for ^{14}C dating of the Upper Paleolithic.

Table 1 Recent bone collagen dates at Abri Pataud for the levels sampled in this study.

Lab #	Level	^{14}C age BP	Reference
GrA-45013	2	21,800 ± 90	Henry-Gambier et al. (2013)
GrA-45133	2	21,910 ± 90	Henry-Gambier et al. (2013)
GrA-45132	2	22,360 ± 90	Henry-Gambier et al. (2013)
GrA-45016	2	22,470 ± 90	Henry-Gambier et al. (2013)
OxA-21585	5	28,180 ± 270	Higham et al. (2011)
OxA-21585	5	28,230 ± 290	Higham et al. (2011)
OxA-21585	5	28,150 ± 290	Higham et al. (2011)
OxA-21588	5	28,250 ± 280	Higham et al. (2011)
OxA-X 2225-38	5	26,780 ± 280	Higham et al. (2011)
OxA-21601	11	34,150 ± 550	Higham et al. (2011)
OxA-21602	11	33,500 ± 500	Higham et al. (2011)
OxA-21580	11	33,550 ± 550	Higham et al. (2011)
OxA-21581	11	33,550 ± 550	Higham et al. (2011)
OxA-21670	12	33,450 ± 500	Higham et al. (2011)
OxA-21671	12	34,300 ± 600	Higham et al. (2011)
OxA-21672	12	34,050 ± 550	Higham et al. (2011)
OxA-21578	14	35,750 ± 700	Higham et al. (2011)
OxA-21579	14	35,000 ± 600	Higham et al. (2011)
OxA-21596	14	34,500 ± 600	Higham et al. (2011)
OxA-21597	14	35,000 ± 650	Higham et al. (2011)

SITE AND SAMPLE SELECTION

The Upper Paleolithic site of Abri Pataud is located in the town of Les Eyzies (Dordogne, France) and corresponds to a rockshelter overlooking the Vézère River valley. The collapsed rockshelter was extensively excavated by H L Movius from 1953 to 1964 who revealed the major part of the stratigraphic sequence. Since 2005, a new program of excavation has been conducted on the archaeological Layer 2 attributed to the Final Gravettian (20–22 kyr BP). These excavations have exposed a 9-m-thick sequence containing 14 archaeological layers (Movius 1977; Lenoble and Agsous 2012). The chronology of the site was recently reassessed using state-of-the-art protocols of sample preparation and data treatment (Higham et al. 2011; Henry-Gambier et al. 2013). The results show that occupation at the Abri Pataud began during the millennium prior to 40 kyr cal BP and ended at 26 kyr cal BP, spanning the Aurignacian and the Gravettian.

Special care was taken during sample selection prior to analysis. Calcined bones can be recognized by a color change from light gray to white occurring between 600 and 700 °C, but sediments adhering to the surface of the samples can hamper identification of the calcined bones and the selection of poorly recrystallized samples may reduce our chances to get good results. A total of 145 samples were preselected at the Abri Pataud Museum collection based on visual inspection (color, presence of calcination cracks). After cleaning in demineralized water, only 55 calcined samples looked good enough to undergo further analytical work. Surfaces and cracks were drilled to remove any trace of sediments, and parts suspected to be partially calcined were removed. Three unburned samples were also selected for comparison. Samples were finely crushed and treated in 1N acid acetic under vacuum for 24 hr prior to infrared and isotopic analysis.

The samples were then analyzed by Fourier transform infrared (FTIR) spectroscopy and stable isotope mass spectrometry to evaluate the heating state of the sample based on bone crystallinity (splitting factor, SF) and $\delta^{13}C$ value. Samples showing high SF and low $\delta^{13}C$ values were considered to have been exposed to high temperatures, and were thus expected to be the best candidates for ^{14}C dating. Unburned samples and samples with intermediate SF and $\delta^{13}C$ values were also selected for comparison. In total, 27 bones were ultimately chosen for ^{14}C dating. These samples come from archaeological layers 2, 5, 11, 12, and 14 and range in age between 21.8 and 35.8 kyr BP (Table 1). The age boundaries for each layer have been defined according to recently published ultrafiltrated bone collagen results.

METHODS

Stable Isotope Analysis

Pretreated unburned bone apatite samples weighing 0.6 mg and calcined bone samples weighing 1.8–2.7 mg were reacted with 100% orthophosphoric acid at 70 °C in individual vessels in an automated cryogenic distillation system (Kiel IV device), interfaced with a Delta V Advantage isotope ratio mass spectrometer. Over the period of analysis of the bioapatite samples, the analytical precision estimated from 16 samples of the laboratory internal carbonate standard (LM Marble, calibrated against NBS-19) was ±0.03‰ (1σ).

Infrared Analysis

FTIR was performed in transmission mode to evaluate mineral crystallinity and carbonate relative content. For each sample, ~10 mg were ground in acetone to obtain a grain size smaller than 2 µm. KBr pellets were prepared by mixing 2.5 ± 0.05 mg of sample with 1 g of KBr. Some 300 mg of the mixture was compressed at 11 t/cm^{-1} for 1.5 min. FTIR analysis was performed on a Vertor 22 FTIR spectrometer (Bruker) by the accumulation of 64 scans with a spectral resolution of 2 cm^{-1}. Mineral crystallinity was quantified using the infrared splitting factor (IRSF) according to the calculation procedures defined by Weiner and Bar-Yosef (1990). Carbonate relative content (C/P) was estimated by the absorbance carbonate band at 1415 cm^{-1} ratioed to the absorbance of the main phosphate band at 1045 cm^{-1}, following the procedure described in Lebon et al. (2010). Cyanamide relative content (CN_2/P) was estimated by absorbance of the specific absorption band at 2012 cm^{-1} ratioed to the same phosphate band at 1045 cm^{-1}.

AMS Analysis

Pretreated bioapatite samples were reacted in 100% orthophosphoric acid at 70 °C for 20 min. The evolved CO_2 was purified (water and non-condensable removal), trapped cryogenetically, and sealed in a glass tube for ^{14}C dating at the Muséum national d'Histoire naturelle (MNHN) lab. Reading of the pressure of CO_2 evolved from the sample during the acid reaction was used to calculate the amount of carbon (mgC_{MNHN}) and estimate the bone inorganic carbon content (%C), with a precision of ±0.03% (1σ). Graphitization and ^{14}C accelerator mass spectrometry (AMS) measurements were performed at the Artemis facility (LMC14, Saclay, France). The graphitization process is described in Cottereau et al. (2007). Graphitization was problematic for the majority of the calcined samples (15/24), and CO_2 samples needed an additional pretreatment step using a mixture of cobalt oxide and silver called sulfix (Wako Chemicals Ltd). Sulfix is generally employed to remove SOx, NOx, and halide species that poison the iron catalyst used during graphitization. When the graphitization stops, the pressure in the reactor does not decrease any more, so the gas is cryogenically trapped at –196 °C in a glass ampoule hooked up to the reactor. The H_2 gas added to reduce the CO_2 is pumped away. The quartz tube containing the iron powder is replaced by another tube with ~500 mg of sulfix reagent and a plug of silver wool. The reactor is pumped until pressure reaches 10^{-5} mbar and the reagent is degassed at 650 °C for 45 min. The pumping access is closed and the CO_2 is transferred from the storage ampoule to the reactor where it is trapped at –200 °C. Sulfix is heated again at 650 °C and the CO_2 is expanded in the reactor. These conditions are maintained for 2 hr. The tube is then allowed to cool and the CO_2 gas is trapped in the ampoule. The amount of CO_2 is measured (mgC_{LMC14}) and graphitization can proceed normally. The same backgrounds (Icelandic spar hydrolyzed and not sulfixed) were used for either non-sulfixed apatite and sulfixed apatite ^{14}C measurements.

Statistics

We used PAST v 2.17 software to perform statistical tests (Hammer et al. 2001).

RESULTS

Unburned and calcined bones and can be easily separated based on their IRSF and $\delta^{13}C$ values. Unburned bones are characterized by low IRSF, varying from 3.4 to 4.0, and high $\delta^{13}C$ values, from –9.0‰ to –11.8‰. On the other hand, calcined bones display high IRSF values, varying from 4.9 to 9.9, and low $\delta^{13}C$ values, from –15.8‰ to –28.5‰ (Table 2, Figure 1). The majority (48 out of 55) of the calcined samples show an IRSF higher than 6, suggesting heating at 650 °C or above (Lebon et al. 2010). No correlation was found between the IRSF and $\delta^{13}C$ values measured in calcined bones ($r^2 = 0.03$). We note a significant ($p < 0.001$, Kruskall Wallis test) decrease in average $\delta^{13}C$ values of calcined bone through time (Figure 2). Values decrease from the Aurignacian (–19.6 ± 2.6‰ in level 12, $n = 19$) to the Gravettian (–24.2 ± 2.5‰ in level 2, $n = 13$). Mann-Whitney pairwise comparisons indicate that bone $\delta^{13}C$ values in level 2 are significantly lower than in levels 5, 11, and 12. Finally, several calcined bones spectra showed the presence of 2 bands at 2012 and 700 cm^{-1} (Table 2, Figure 3). These bands were attributed to cyanamide ions (CN_2^{2-}) substituted in the apatite lattice (Dowker and Elliott 1979; Habelitz et al. 1999, 2001).

^{14}C results are presented in Table 2 and Figure 4. Unheated bones display ages that are 5300 to 8600 ^{14}C yr younger than the minimum age estimated for the layer to which they belong. Among calcined bones, 2 groups of results can be observed. Almost all of the samples requiring sulfix pretreatment (14/16) returned ages that were too young. Age differences can be significant and reach 8000 to 9000 ^{14}C yr (Figure 4). The opposite situation was observed when sulfix pretreatment was not necessary, and most (8/9) of the samples provided coherent ^{14}C ages, with only 1 sample (Pat64a) more than 0.2 pMC apart from the minimum age for the corresponding layer. Five samples were dated twice. Reproducibility was good for the 2 unburned samples that did not need sulfix, but poor for the 3 calcined samples requiring sulfix (Table 3).

Table 2 Stable isotope, ^{14}C and FTIR results obtained from unburned and calcined bones at Abri Pataud.

Sample #	Level	Type	$\delta^{13}C$ MNHN	$\delta^{13}C$ LMC14	%C	IRSF	CO_3/PO_4	CN_2/PO_4	Lab ID	Sulfix	mgC MNHN	mgC LMC14	Yield[a]	Code SacA-	pMC	^{14}C age
PAT-58	2	Calcined	–20.3			6.05	0.04	0.098								
PAT-59	2	Calcined	–24.4	–24.2	0.41	7.14	0.04	0.069	Muse68	yes	1.08	0.76	0.71	27061	9.90 ± 0.11	18,570 ± 90
PAT-59	2	Calcined	–24.4	–25.1	0.34	7.14	0.04	0.069	Muse168	yes	0.59	0.59	1.00	29143	10.37 ± 0.07	18,200 ± 50
PAT-60	2	Calcined	–20.8			8.13	0.02	0.073								
PAT-61	2	Calcined	–28.1	–31.6	0.14	8.77	0.02	0.008	Muse69	no	0.77	0.80	1.05	27062	6.17 ± 0.09	22,370 ± 110
PAT-62	2	Calcined	–21.3			5.95	0.06	0.131								
PAT-63	2	Calcined	–25.0	–26.7	0.24	7.56	0.03	0.005	Muse70	no	1.00	0.92	0.92	27063	6.40 ± 0.06	22,080 ± 110
PAT-64a	2	Calcined	–22.9	–20.6	0.28	7.47	0.03	0.032	Muse67	yes	0.94	0.62	0.66	27060	9.84 ± 0.10	18,630 ± 80
PAT-64a	2	Calcined	–22.9	–14.8	0.27	7.47	0.03	0.032	Muse166	no	1.26	1.25	0.99	29141	8.48 ± 0.06	19,820 ± 60
PAT-66	2	Calcined	–28.5	–29.7	0.17	9.08	0.01	0.004	Muse79	yes	1.02	0.80	0.78	27070	6.65 ± 0.09	21,770 ± 110
PAT-67	2	Calcined	–25.4	–24.4	0.35	7.22	0.04	0.007	Muse80	no	1.11	1.02	0.92	27071	6.03 ± 0.08	22,560 ± 110
PAT-70a	2	Calcined	–24.7	4.8	0.39	7.50	0.04	0.009	Muse73	yes	0.96	0.20	0.21	27066	8.86 ± 0.11	19,470 ± 100
PAT-71	2	Calcined	–25.5	–18.3	0.29	8.37	0.02	0.007	Muse81	yes	1.00	0.54	0.54	27072	7.36 ± 0.09	20,960 ± 100
PAT-74	2	Unburned	–11.5	–12.5	0.85	3.42	0.17		Muse88	no	0.86	0.79	0.92	27079	13.05 ± 0.10	16,360 ± 60
PAT-2	3	Unburned	–10.6	–11.7	0.94	3.99	0.12	0.003	Muse78	no	1.04	1.05	1.01	27069	14.13 ± 0.11	15,720 ± 60
PAT-12a	5	Calcined	–23.0	–22.0	0.57	7.01	0.05	0.005	Muse71	no	1.18	1.13	0.96	27064	3.69 ± 0.08	26,500 ± 170
PAT-17a	5	Calcined	–21.9	–23.9	0.15	8.56	0.02	0.014	Muse82	no	0.90	0.84	0.94	27073	3.13 ± 0.07	27,820 ± 190
PAT-25a	5	Calcined	–21.5			6.57	0.04	0.050								
PAT-28a	5	Calcined	–19.4			7.44	0.05	0.053								
PAT-29a	5	Calcined	–23.6	–23.7	0.17	8.68	0.02	0.009	Muse72	yes	1.03	0.67	0.65	27065	4.07 ± 0.08	25,720 ± 150
PAT-29a	5	Calcined	–23.6	–19.7	0.17	8.68	0.02	0.009	Muse167	yes	0.42	0.34	0.80	29142	8.60 ± 0.06	19,710 ± 60
PAT-29b	5	Calcined	–18.6			7.32	0.02	0.063								
PAT-30	5	Calcined	–21.1			6.96	0.03	0.086								
PAT-33a	5	Calcined	–21.3			7.23	0.05	0.064								
PAT-33b	5	Calcined	–22.9	–24.4	0.32	9.64	0.04	0.008	Muse83	yes	1.06	0.92	0.87	27074	3.64 ± 0.08	26,620 ± 170
PAT-34a	5	Calcined	–20.5			7.91	0.04	0.088								
PAT-34b	5	Calcined	–21.9			8.85	0.04	0.078								
PAT-34c	5	Calcined	–19.4	–19.1	0.27	10.02	0.03	0.140	Muse84	yes	1.00	0.95	0.95	27075	5.32 ± 0.08	23,560 ± 120
PAT-34d	5	Calcined	–21.1													
PAT-5a	5	Calcined	–22.5	–21.1	0.29	6.76	0.04	0.081	Muse169	yes	1.12	0.94	0.84	29144	5.86 ± 0.05	22,780 ± 70
PAT-78b	5	Unburned	–11.8	–8.3	1.09	3.77	0.18		Muse89	no	0.96	0.93	0.97	27080	8.54 ± 0.09	19,770 ± 80
PAT-78b	5	Unburned	–11.8	–12.1	1.09	3.77	0.18		Muse163	no	0.93	0.94	1.01	29138	8.39 ± 0.06	19,900 ± 60
PAT-77	8	Calcined				6.58	0.06	0.007								
PAT-39	11	Calcined	–19.6			6.55	0.03	0.210								
PAT-40a	11	Calcined	–17.9			5.76	0.05	0.207								

Table 2 Stable isotope, ^{14}C and FTIR results obtained from unburned and calcined bones at Abri Pataud. *(Continued)*

Sample #	Level	Type	$\delta^{13}C_{MNHN}$	$\delta^{13}C_{LMC14}$	%C	IRSF	CO_3/PO_4	CN_2/PO_4	Lab ID	Sulfix	mgC_{MNHN}	mgC_{LMC14}	Yield[a]	Code SacA-	pMC	^{14}C age
PAT-40b	11	Calcined	–19.1			6.73	0.04	0.153								
PAT-40c	11	Calcined	–18.3			6.73	0.05	0.139								
PAT-42a	11	Calcined	–21.6			5.88	0.04	0.154								
PAT-50a	11	Calcined	–20.9	–21.1	0.44	5.98	0.06	0.137	Muse172	yes	1.27	1.13	0.89	29147	2.43 ± 0.04	29,850 ± 140
PAT-50b	11	Calcined	–16.1	–17.2	0.23	8.64	0.02	0.227	Muse85	yes	1.03	0.90	0.88	27076	4.22 ± 0.09	25,430 ± 160
PAT-51b	11	Calcined	–18.1			7.89	0.02	0.196								
PAT-80a	11	Calcined	–19.2			7.30	0.03	0.146								
PAT-80b	11	Calcined	–18.9	–21.0	0.14	8.61	0.01	0.040	Muse86	yes	0.92	0.88	0.96	27077	4.63 ± 0.08	24,690 ± 140
PAT-81	11	Calcined	–21.8	–19.2	0.25	6.72	0.04	0.137	Muse165	yes	0.73	0.64	0.87	29140	2.41 ± 0.04	29,940 ± 140
PAT-43a	12	Calcined	–16.8													
PAT-43c	12	Calcined	–18.6			7.25	0.05	0.13								
PAT-45b	12	Calcined	–22.3			5.44	0.10	0.013								
PAT-46a	12	Calcined	–16.3			7.75	0.01	0.027								
PAT-46b	12	Calcined	–19.3			5.97	0.08	0.051								
PAT-46c	12	Calcined	–23.5	–24.2	0.29	4.84	0.06	0.022	Muse170	yes	1.11	1.04	0.94	29145	4.38 ± 0.05	25,130 ± 90
PAT-46d	12	Calcined	–19.3			6.64	0.04	0.025								
PAT-46e	12	Calcined	–20.7			6.98	0.04	0.10								
PAT-47a	12	Calcined	–15.8			6.78	0.02	0.011								
PAT-47b	12	Calcined	–19.4			7.59	0.03	0.061								
PAT-48a	12	Calcined	–18.3			5.93	0.06	0.171								
PAT-48b	12	Calcined	–24.0	–20.7	0.29	6.69	0.04	0.024	Muse171	yes	1.17	1.01	0.86	29146	3.93 ± 0.05	26,000 ± 90
PAT-82a	12	Calcined	–21.2	–21.2	0.26	6.65	0.04	0.038	Muse66	no	0.92	0.93	1.01	27059	1.84 ± 0.07	32,080 ± 310
PAT-82b	12	Calcined	–21.4			6.33	0.05	0.087								
PAT-83a	12	Calcined	–17.5			6.67	0.04	0.098								
PAT-83b	12	Calcined	–19.1			7.15	0.04	0.10								
PAT-83c	12	Calcined	–17.1			7.13	0.02	0.013								
PAT-83d	12	Calcined	–17.8	–20.4	0.14	7.69	0.01	0.186	Muse87	yes	0.82	0.81	0.99	27078	2.89 ± 0.07	28,460 ± 200
PAT85	12	Unburned	–9.0	–11.3	0.95	3.65	0.13		Muse90	no	0.96	0.90	0.94	27081	4.81 ± 0.08	24,380 ± 130
PAT85	12	Unburned	–9.0	–8.8	1.01	3.65	0.13		Muse164	no	1.02	0.94	0.92	29139	4.85 ± 0.05	24,310 ± 80
PAT-89	12	Calcined	–24.4	–23.5	0.38	6.78	0.05	0.005	Muse65	no	1.00	1.00	1.00	27058	1.67 ± 0.07	32,860 ± 340
PAT-38a	14	Calcined	–22.7	–23.6	0.2	6.90	0.03	0.007	Muse173	no	0.80	0.83	1.04	29148	1.54 ± 0.04	33,530 ± 190

[a]The yield is defined as the amount of C measured at LMC14 (mgC_{LMC14}) divided by the amount of C measured at MNHN (mgC_{MNHN}).

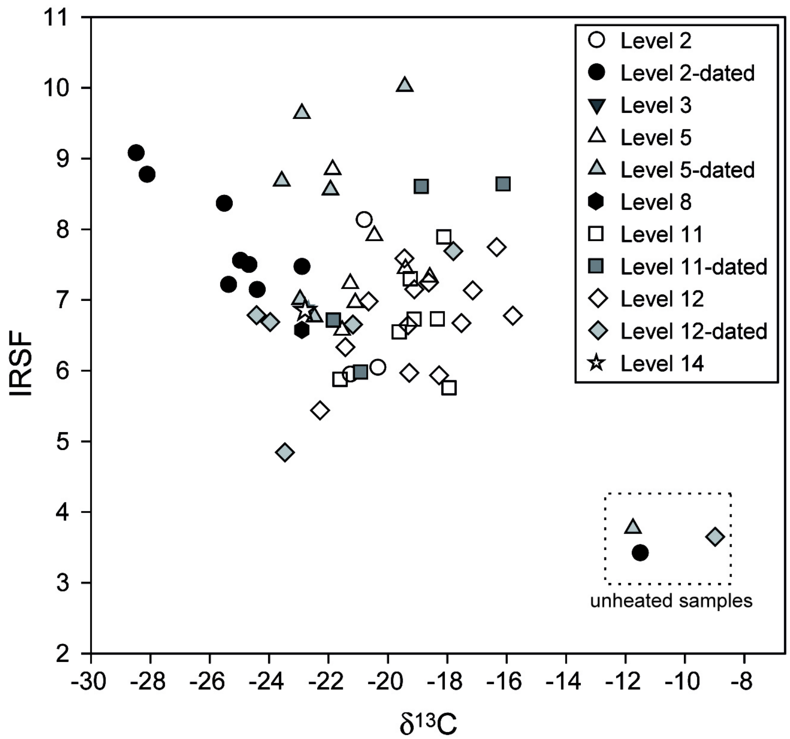

Figure 1 IRSF and $\delta^{13}C$ values of unburned and calcined bones from Abri Pataud

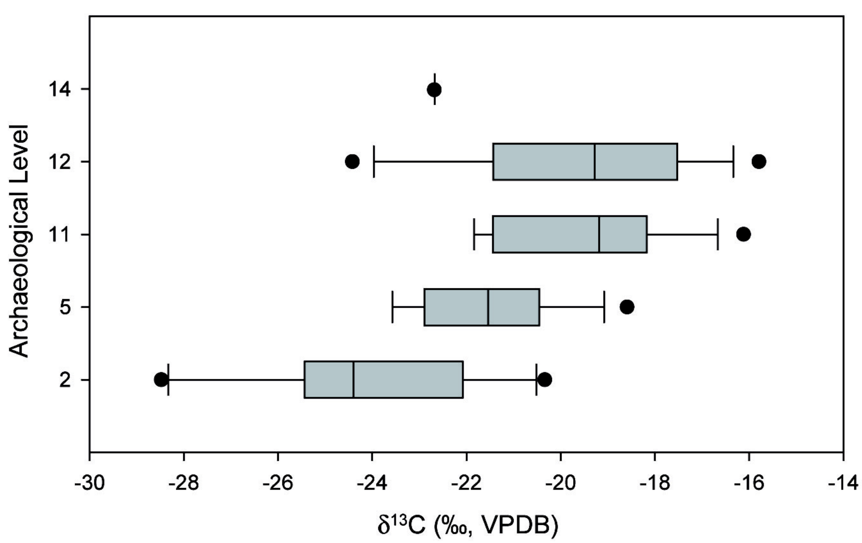

Figure 2 Boxplot displaying the change in average $\delta^{13}C$ values of calcined bone through time

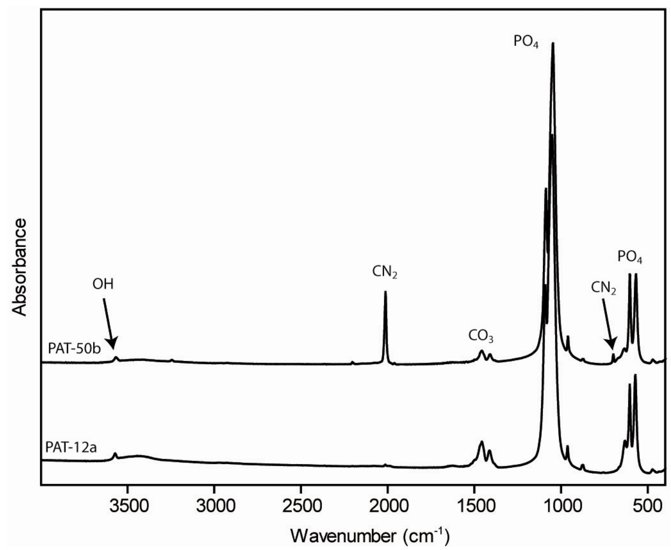

Figure 3 General FTIR spectra of 2 calcined bones from Abri Pataud (France)

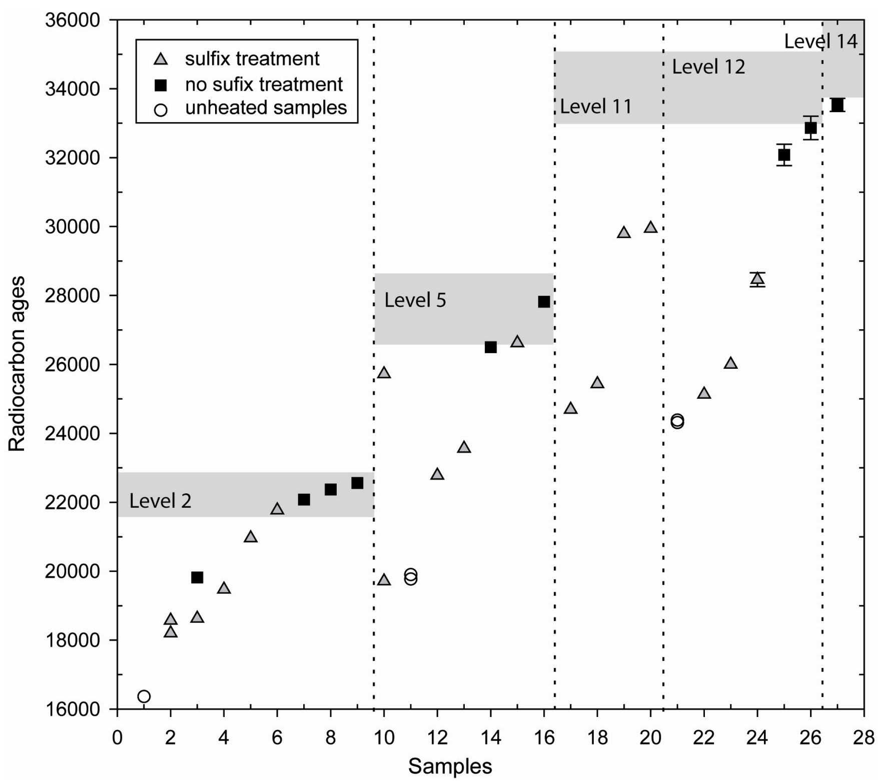

Figure 4 Comparison between ^{14}C ages obtained on unburned and calcined bones at Abri Pataud and expected ages for the corresponding levels. Expected age boundaries are based on recently published dates obtained on bone collagen (Higham et al. 2011; Henry Gambier et al. 2013).

DISCUSSION AND CONCLUSION

This study represents the first attempt at ^{14}C dating calcined bones from the Paleolithic. The young ages measured on unburned bone apatite confirm that bone apatite cannot be used to date Paleolithic sites in temperate environments. This confirms earlier results obtained on samples coming from the Neolithic of France (Zazzo and Saliège 2011). Results obtained on calcined bones are much more encouraging but also less clear-cut. When sulfix was not necessary, calcined bones almost always returned coherent ^{14}C ages. This result demonstrates that calcined bone can behave as a closed system for more than 38,000 yr. However, when sulfix treatment was needed, calcined bones almost never returned the correct age, suggesting that these samples were contaminated by modern carbon. This contamination could either be present in the sample prior to its handling in the laboratory, or introduced later, during sample preparation. Finding an answer to this question is crucial because it determines the status of calcined bones as an alternative support to ^{14}C date the Paleolithic.

Table 3 Reproducibility of ^{14}C ages obtained on unburned and calcined bone samples.

Sample #	Level	Type	Lab ID	Sulfix	mgC MNHN	mgC LMC14	Yield	Code SacA-	pMC	^{14}C age
PAT-78b	5	Unburned	Muse89	no	0.96	0.93	0.97	27080	8.54 ± 0.09	19,770 ± 80
PAT-78b	5	Unburned	Muse163	no	0.93	0.94	1.01	29138	8.39 ± 0.06	19,900 ± 60
PAT85	12	Unburned	Muse90	no	0.96	0.90	0.94	27081	4.81 ± 0.08	24,380 ± 130
PAT85	12	Unburned	Muse164	no	1.02	0.94	0.92	29139	4.85 ± 0.05	24,310 ± 80
PAT-64a	2	Calcined	Muse67	yes	0.94	0.62	0.66	27060	9.84 ± 0.10	18,630 ± 80
PAT-64a	2	Calcined	Muse166	no	1.26	1.25	0.99	29141	8.48 ± 0.06	19,820 ± 60
PAT-59	2	Calcined	Muse68	yes	1.08	0.76	0.71	27061	9.90 ± 0.11	18,570 ± 90
PAT-59	2	Calcined	Muse168	yes	0.59	0.59	1.00	29143	10.37 ± 0.07	18,200 ± 50
PAT-29a	5	Calcined	Muse72	yes	1.03	0.67	0.65	27065	4.07 ± 0.08	25,720 ± 150
PAT-29a	5	Calcined	Muse167	yes	0.42	0.34	0.80	29142	8.60 ± 0.06	19,710 ± 60

Informal discussion with different participants at the 21st Radiocarbon Conference, where these results were presented, confirmed that calcined bones can be difficult to graphitize. This difficulty is commonly attributed to the presence of sulfur oxides, although other halogens like cyanamide ions are expected to behave similarly (Van Strydonck et al. 2010). Cyanamide ions were found to be substituted in the apatite lattice for the majority of the Abri Pataud samples. Figure 5 shows that when this band is present, samples often need a sulfix treatment, and this is usually associated with a large age deviation. The presence of cyanamide in apatite structure is attributed to the presence of ammonia in the heating environment (Dowker and Elliott 1979; Habelitz et al. 2001). Habelitz et al. (2001) showed that at temperatures above 900 °C, cyanamide molecules (H_2CN_2) are formed in ammonia atmosphere in the presence of graphite:

$$2NH_3 + C \rightarrow H_2CN_2 + 2H_2 \tag{1}$$

Cyanamide then interacts with hydroxyl in apatite to form cyanamide-apatite according to the following equation:

$$Ca_{10}(PO_4)_6(OH)_2 + H_2CN_2 \rightarrow Ca_{10}(PO_4)_6CN_2 + H_2O \tag{2}$$

Body tissues could provide a source of ammonia in the case of human cremation in a pyre (Hüls et al. 2010). Cremation experiments suggest that the source of ammonia could also be a byproduct of

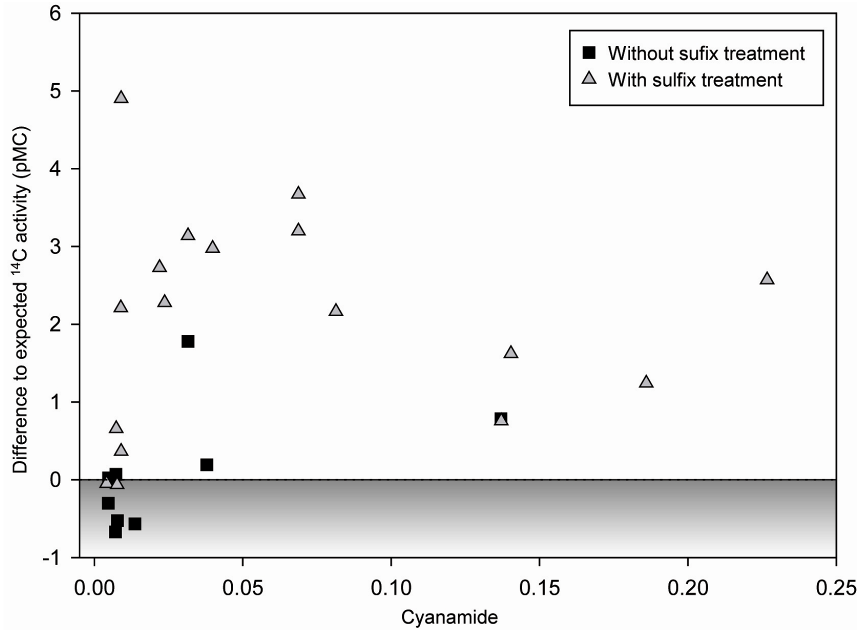

Figure 5 Relationship between the intensity of the cyanamide band at 2012 cm^{-1} and the deviation from expected age (in pMC) in sulfixed and non-sulfixed samples.

coal combustion (Van Strydonck et al. 2010). Experiments performed by Habelitz et al. (2001) and Van Strydonck et al. (2010) suggest that if graphite is essential, cyanamide-apatite formation is greatly improved by reducing conditions in the combustion atmosphere. This is in keeping with our observation that CN_2^{2-} substitution is higher when $\delta^{13}C$ values are high (Figure 6).

Laboratory and field experiments showed that the majority of the carbon left in a calcined bone originated from the atmosphere of combustion (Hüls et al. 2010; Van Strydonck et al. 2010; Zazzo et al. 2012). When no or little CO_2 is added to the calcination milieu, a limited (2–4‰) carbon isotopic shift occurs, caused by kinetic effects (Zazzo et al. 2009; Hüls et al. 2010). The high $\delta^{13}C$ values (between –16 and –22‰) measured in well-calcined bones (SF of 6 or above) could therefore correspond to a situation where little amounts of wood CO_2 were available for chemical exchange with bone apatite during calcination, thus promoting cyanamide-apatite formation. This could correspond to a situation where bones are used as a fuel source. In this case, cyanamide carbon and nitrogen would come preferentially from bone organic matter (collagen and fat). Théry-Parisot (1998) suggested that bone could indeed have been as fuel source during the Aurignacian at Abri Pataud. We note that bone $\delta^{13}C$ values are significantly higher in the oldest levels than in the youngest level, level 2 (Figure 2). This could indicate a diachronic change in fuel management between the Aurignacian and the Gravettian and confirm the hypothesis of Théry-Parisot (1998). Further experimental work is needed to establish whether calcined bone $\delta^{13}C$ values can be safely used as an indicator of the use of bone as fuel source.

Even if little is known about the source of cyanamide, its impact on the age obtained is evident. This impact could be direct and related to the origin of carbon in cyanamide. It could also be indirect, through contamination during gas purification by sulfix. As noted by Hüls et al. (2010), the incorporation of a cyanamide group delivers additional carbon to the resulting cremated apatite and could therefore affect the age of the sample. The source of carbon present in cyanamide ions could come

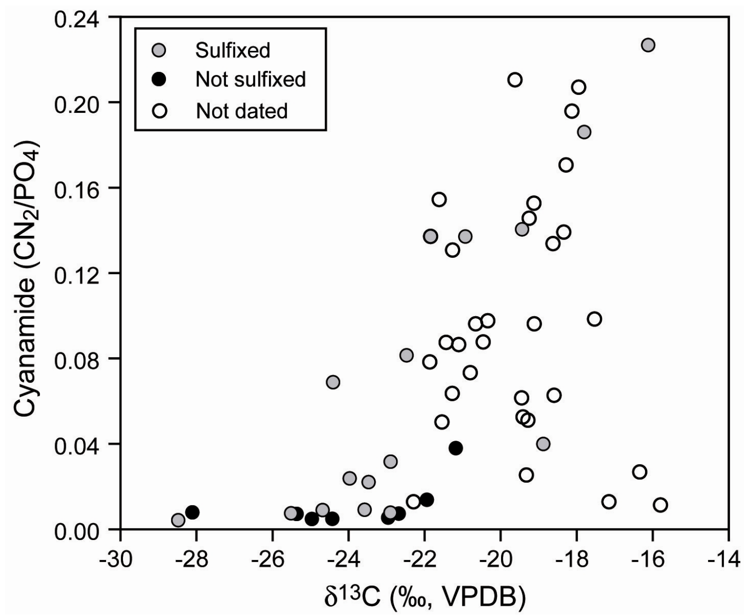

Figure 6 Relationship between the intensity of the cyanamide band at 2012 cm^{-1} and the $\delta^{13}C$ value of the Abri Pataud calcined bone samples.

from carbonized collagen and be present in charred bone as graphite between 300 and 550 °C. The other possible source of carbon is the wood used for cremation. Both sources of carbon cannot be younger than bone carbonate and thus cannot explain the young ages obtained.

The young ages measured in sulfixed samples raises the question of contamination of the CO_2 gas during the sulfix treatment, perhaps through incomplete degassing of the sulfix prior to reaction with CO_2. This hypothesis is supported by the fact that large age differences (up to 6000 yr) were obtained for duplicates of calcined samples, while repeats of unburned (non-sulfixed) bones gave reproducible ages (Table 3). This hypothesis is in contrast with the small amount of contamination (<1 µg modern C) calculated based on IAEA-C1 marble ages measured with and without sulfix (Table 4). This apparent contradiction could be resolved if the pollution originated from the diagenetic evolution of the CN_2^{2-} group during fossilization. Cyanamide ions are in A-site, substituting for OH, and are easily accessible (Habelitz et al. 2001). Micro-organisms are able to break C-N bonds and this could promote carbon isotope exchange. Further work is needed to fully grasp the origin of this contamination.

Table 4 ^{14}C activity of Carrara Marble IAEA-C1 prepared with and without sulfix.

Reference	Code SacA-	pMC
C1 consensus age (average, *n* = 36)[a]		0.000 ± 0.020
C1 without sulfix (average, *n* = 86)		0.096 ± 0.026
C1 with sulfix 1	9891	0.154 ± 0.011
C1 with sulfix 2	9892	0.187 ± 0.013
C1 with sulfix 3	9893	0.139 ± 0.011
C1 with sulfix, averaged		0.160 ± 0.025

[a]Rozanski et al. (1992).

^{14}C laboratories developed various strategies to purify calcined bone samples. These techniques include sulfix (Lanting et al. 2001, although not mentioned in the paper, Aerts-Bijma, personal communication), or combustion in the presence of CuO and Ag (Hüls et al. 2010), sometimes followed by freezing on $KMnO_4$ (Van Strydonck et al. 2010). We are currently working on implementing our own purification technique at the MNHN lab, in order to avoid the use of sulfix. But these procedures are labor intensive and time consuming and should be avoided as much as possible. Prescreening with FTIR and $\delta^{13}C$ analysis prior to off-line acid digestion could help sorting samples that need pretreatment from those that can be graphitized directly.

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