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Calcined bone provides a reliable substrate for strontium isotope ratios as shown by an enrichment experiment

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RATIONALE: Strontium isotopes ($^{87}Sr/^{86}Sr$) are used in archaeological and forensic science as markers of residence or mobility because they reflect the local geological substrate. Currently, tooth enamel is considered to be the most reliable tissue, but it rarely survives heating so that in cremations only calcined bone fragments survive. We set out to test the proposition that calcined bone might prove resistant to diagenesis, given its relatively high crystallinity, as the ability to measure *in vivo* $^{87}Sr/^{86}Sr$ from calcined bone would greatly extend application to places and periods in which cremation was the dominant mortuary practice, or where unburned bone and enamel do not survive.
METHODS: Tooth enamel and calcined bone samples were exposed to a ^{87}Sr-spiked solution for up to 1 year. Samples were removed after various intervals, and attempts were made to remove the contamination using acetic acid washes and ultrasonication. $^{87}Sr/^{86}Sr$ was measured before and after pre-treatment on a Nu Plasma multi-collector induced coupled plasma mass spectrometer using NBS987 as a standard.
RESULTS: The strontium isotopic ratios of all samples immersed in the spiked solution were strongly modified showing that significant amounts of strontium had been adsorbed or incorporated. After pre-treatment the enamel samples still contained significant amounts of ^{87}Sr-enriched contamination while the calcined bone fragments did not.
CONCLUSIONS: The results of the artificial enrichment experiment demonstrate that calcined bone is more resistant to post-mortem exchange than tooth enamel, and that *in vivo* strontium isotopic ratios are retained in calcined bone.

Two isotopes of strontium, ^{86}Sr and ^{87}Sr, are widely used in mobility studies of humans and fauna. Strontium-87 is the product of the radioactive decay of rubidium-87 (^{87}Rb), so strontium isotope ratios ($^{87}Sr/^{86}Sr$) vary between different types of bedrock, depending on its age and on the initial Rb/Sr ratio.[1] The older and more Rb-enriched the bedrock, the more enriched it is in ^{87}Sr. $^{87}Sr/^{86}Sr$ values higher than 0.710 and up to as high as 0.9 may be observed in some granites, for instance in the Mourne Mountains in Northern Ireland[2] and in South Africa.[3] Younger geological formations generally have values below 0.706, and those with very low initial Rb/Sr ratios, such as basalt, typically have values of 0.703–0.704.[4] Modern ocean water has a $^{87}Sr/^{86}Sr$ ratio of 0.7092,[5] an important value as it is imparted to world-wide precipitation and to geologically recent marine calcareous deposits.

Strontium isotopes can be measured in bone and teeth to track the place of origin of animals and humans. Calcified tissues contain trace amounts of strontium that substitute for calcium in bioapatite.[6,7] Strontium in biological systems ultimately derives from a combination of *in situ* weathering of bedrock during pedogenesis, and input from precipitation and air-borne particles (e.g. Saharan dust), local geology and groundwater, entering the foodchain initially through uptake by plants and hence into the tissues of consumers. Therefore, $^{87}Sr/^{86}Sr$ values provide a reflection of the location in which an individual lived during the time of tissue formation. However, when studying archaeological remains that have been in contact with soil for centuries and millennia, an important issue to consider is the exchange and/or intake of strontium by bone and teeth from the burial environment.[8–10]

Attempts to remove post-depositional strontium have relied largely on leaching with solutions of buffered or non-buffered acetic acid.[11–13] It has been shown that successive leaching with acetic acid leaves up to 80% non-biogenic strontium in bone compared with less than 5% in tooth enamel.[14] Tooth enamel has been shown to be more resistant to post-depositional strontium absorption, due largely to its higher crystallinity[a] and stability, than bone and tooth dentine, which are both far more reactive. In bone, apatite crystallites are heavily substituted and therefore extremely tiny and distorted.[15] Because of these problems, currently

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[a]Crystallinity denotes size and perfection of crystals.

only tooth enamel is considered to provide reliable results with archaeological material, while bone and tooth dentine may incorporate variable amounts of strontium in solution from the soil in which they were buried.[e.g. 10,13]

Measurements on tooth enamel relate to the time during which the tooth crown formed, and so present an indication of dietary intake during infancy through to early adolescence, depending on the tooth measured. They cannot, however, inform on residence in adulthood. Bone on the other hand continues to remodel, and so it provides information relating to the last decade or more of adult life.[16–18] An important exception to this is the inner part of the petrous bone, which forms during late gestation to infancy, and does not remodel thereafter.[19,20]

When bone is heated its crystallinity increases dramatically, reaching levels surpassing even that of tooth enamel in fully calcined bone (white).[21] In principle, then, this raises the possibility that calcined bone might retain its original *in vivo* strontium isotope composition, but, to date, only two studies of which we are aware include strontium isotope analyses of calcined bone. In the first it was shown that no modification of the strontium isotope ratio occurred before and after heating bone fragments without fuel in a furnace.[22] This experiment, however, did not consider the possibility of the uptake of strontium from fuel wood or the burial environment. The second study compared the strontium isotopic ratios of tooth enamel and calcined petrous bone from a cremated individual which, unusually, had retained its enamel. These tissues gave similar results suggesting that the calcined petrous bone provides a reliable *in vivo* strontium isotope signal.[23] As noted in that study the otic capsule (the portion of the petrous bone that was sampled) is composed of uniquely dense bone, whereas most calcined fragments would originate from less dense, ordinary bone. Both studies, then, hint at the potential for reliable $^{87}Sr/^{86}Sr$ measurements on calcined bone, but both leave several unanswered questions. Is there any exchange of strontium between calcined bone and its surrounding environment, either during the burning process itself (as has been demonstrated for carbon[e.g. 24–26]), or during a subsequent exchange in the burial environment?

We set out to address this gap. In the present study, fragments of calcined animal bone and unburned tooth enamel were placed in a highly ^{87}Sr-enriched solution for different amounts of time and the isotopic ratios were then measured before and after treatment with acetic acid and ultrasonication.

EXPERIMENTAL

Design

No strontium isotopic fractionation should be expected during the heating of bone as the relative atomic mass difference between the two strontium isotopes is low and observable fractionation during biochemical transformations is implausible. Furthermore, the melting point of strontium carbonate is around 1500 °C, much higher than the temperatures reached during cremation and so it is unlikely to volatilize from bone apatite either. However, it is more likely that there is an intake and/or exchange of strontium between bone apatite and its surrounding environment once buried. Nevertheless, because of the higher crystallinity of calcined bone apatite, it is hypothesised here that any such alteration will occur to a much lower extent than in unburned bone. Since enamel is considered to provide results reflecting the original strontium isotope composition because of its higher crystallinity and higher resistance to diagenetic changes, cremated bone apatite should likewise provide reliable results.

Nevertheless, crystallinity is not the only factor that has to be taken into account; porosity also should be considered. Tooth enamel is compact and has a low porosity while both calcined and unburned bone are much more porous. Many cavities are left unfilled after cremation due to the combustion and disappearance of collagen. This leads to a higher contact surface between bone apatite and its surrounding environment, which could potentially increase the strontium intake and/or exchange.

To test the reliability of the strontium isotope results obtained from calcined bone, several experiments were designed. Although it is recognised that the possibility of exchange of strontium in the fuel with bone during burning is remote, experiments were carried out as a check. One fragment of cow tibia was heated in a laboratory muffle furnace and another was burned on an open wood fire of known isotope composition. If the strontium isotopic ratios are identical (within measurement error) for both fragments, we can infer that no exchange occurred between bone and wood during heating. The ceramic walls of the muffle furnace could contain traces of strontium that could be incorporated in the samples but even if some strontium is incorporated into the sample from the furnace, the variation would be negligible as calcined bone contains significant amounts of strontium.[27]

To study the potential exchange/intake of strontium from the surrounding depositional environment of buried calcined bone, several fragments of calcined cow tibia (the same bone as above) were immersed in an ^{87}Sr-enriched solution and left to rest at room temperature for up to 12 months. This '^{87}Sr-spiked' source used in the experiment is more enriched than any existing in the natural world by many orders of magnitude, and so it can be thought of as greatly speeding up the process of artificial contamination. In parallel, horse tooth enamel fragments were placed in the same solution. The samples were also monitored using infrared spectroscopy to observe any compositional or structural modifications due to the immersion of calcined bone in the ^{87}Sr-enriched solution. A new pre-treatment protocol aiming at removing strontium contamination from cremated bone using acetic acid and ultrasonication was developed.

We are aware that immersing pieces of calcined bone in an ^{87}Sr-enriched solution for a maximum of 12 months is not representative of real burial conditions lasting centuries or millennia. However, several field and experimental studies have found that uptake of strontium in unburned bone is rapid.[e.g. 10,28] The use of enamel as a comparison allows us to argue that if the behaviour of calcined bone in this 'artificial' environment is similar to that of tooth enamel, calcined bone should be considered to be at least as reliable as tooth enamel for strontium isotope determinations.

Samples

A modern horse tooth was selected for the immersion experiments. The dentine was mechanically removed using a micro-drill and enamel flakes were collected. The calcined bone fragments were obtained by burning a cow tibia on an

outdoor wood fire. In both cases, fragments were collected for the immersion experiments without crushing. For the IR measurements by Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance (FTIR-ATR) mode, calcined bone powder from the same cow tibia heated in a muffle furnace was used. Aliquots of powder were also immersed in the enriched solution.

Enriched solution

An ^{87}Sr-enriched solution was made using strontium carbonate of certified composition (reference: MSR87C; Euriso-top, Saint-Aubin, France). This carbonate contained mainly ^{87}Sr (89.7%) and only a small amount of ^{86}Sr (0.98%), with a strontium isotopic ratio of 91.53061. A solution was made using milliQ water (Merck Millipore, division of Merck KGaA, Darmstadt, Germany) and 11 mg/L of this strontium carbonate, homogenized by ultrasonication. Several fragments of calcined bone and tooth enamel of ca 100 mg were placed into tubes and immersed in 10 mL of solution for 15 days, 1, 3, 6, 9 and 12 months.

The sample-to-solution ratio was chosen to ensure that the maximum variation in isotopic ratio would be around 30% between uncontaminated and immersed sample. The strontium concentration of the calcined bone used here is around 150 ppm, and in 100 mg of calcined bone there is approximately 0.015 mg of strontium. In 10 mL of solution there is 0.011 mg of strontium carbonate or ca 0.0065 mg of strontium. This facilitates the observation of the isotopic variations and avoids the complete overwriting of the original signal by the enriched solution.

Sample pre-treatments

The most common pre-treatments for isotopic analyses of enamel are the mechanical removal of the external layers of enamel following the questionable idea that diagenesis should be restricted to the outermost layers,[e.g. 29] or successive acid leaching on crushed sample.[e.g. 14] Sillen and colleagues,[3] for example, used buffered 0.1 M acetic acid on crushed samples and ultrasonication for 1 min and repeated this twenty times. Mechanical procedures are not possible on calcined bone as they cause the sample to disintegrate, while consecutive acid leaching on crushed calcined bone sample leads to complete loss. Here, we based the pre-treatment protocol on the procedure using acetic acid. We use 1 M acetic acid (as used for radiocarbon dating of calcined bone[30]) and ultrasonication on uncrushed samples but omitted the subsequent leaching steps.

After immersion, the tooth enamel and calcined bone samples were rinsed three times with milliQ water for 10 min in an ultrasonication bath. The samples were then divided into two 50 mg fractions. One fraction was treated with 1 M acetic acid (1 mL per 10 mg of sample) for 3 min in an ultrasonic bath, followed by three rinses with milliQ water and 10 min ultrasonication. The other fragments did not undergo any further treatment. All samples were then dried in a freeze-dryer overnight. The limit of 3 min in the ultrasonication bath was chosen in order to limit the sample loss observed when treating calcined bone fragments. Three sub-samples of enamel were also ultrasonicated for 30 min in acetic acid.

Strontium isotopic composition: sample preparation and MC-ICP-MS (Multi-Collector Inductively Coupled Plasma Mass Spectrometry) analyses

The entire acid digestion process and subsequent Sr purification were carried out under a class 100 laminar flow hood in a class 1000 clean room (Université Libre de Bruxelles, Brussels, Belgium, hereafter ULB). About 50 mg of uncrushed sample was dissolved in closed ®Savillex containers (LabAS, Brussels, Belgium) overnight using 14 M HNO_3 on a heating plate at 110°C. Once dissolved the samples were dried and left to cool to room temperature, before being redissolved in 2 M HNO_3 and ultrasonicated for 20 min. Strontium was extracted by column chromatography using successive acid elution on a Sr-Spec resin following a similar protocol to that described in Míková and Denková.[31] The sample was charged onto the column with 4×0.5 mL of 2 M HNO_3. The column was then rinsed with 2×0.5 mL 2 M HNO_3, 6×0.5 mL 7 M HNO_3 and 1×0.5 mL 3 M HNO_3. Finally, the column was eluted and the strontium collected with 4×0.5 mL 0.05 M HNO_3.

The purified strontium samples were then evaporated, and dried residues were dissolved in 100 µL of concentrated HNO_3, evaporated and finally dissolved in 1.5 mL of 0.05 M HNO_3. Strontium isotope compositions were measured on a Nu Plasma MC-ICP mass spectrometer (Nu015 from Nu Instruments, Wrexham, UK) at ULB. Particular attention was paid to the purity of the Ar gas used inside the spectrometer in order to avoid any interference (from Kr for instance) on Sr isotope masses. The Sr isotopes were measured by static multi-collection. Each analysis consisted of 60 ratio measurements (3 blocks of 20 cycles), resulting in a data collection duration for each individual sample of 12–13 min. All the Sr isotopes (84, 86, 87, 88) were measured, while the masses 85 (Rb) and 83 (Kr) were simultaneously monitored, allowing for interference corrections on masses 84, 86 (Kr) and 87 (Rb). The Sr isotopic ratios were automatically normalized to $^{86}Sr/^{88}Sr = 0.1194$ using an exponential law except for the spike samples for which the internal normalization does not apply. During the course of this study, repeated measurements of the NBS987 standard yielded $^{87}Sr/^{86}Sr = 0.710214 \pm 40$ (2SD for 15 analyses), which is, for our purposes, sufficiently consistent with the mean value of 0.710252 ± 13 obtained by TIMS (Thermal Ionization Mass Spectrometry).[32] All the sample measurements were normalized using a standard bracketing method with the recommended value of $^{87}Sr/^{86}Sr = 0.710248$.[32] For each sample a 2σ error (absolute error value of the individual sample analysis – internal error) was calculated. In order to control the reproducibility, one sample was measured in duplicate and another in quadruplicate (see Table 3).

FTIR

Powdered immersed samples were analyzed by FTIR-ATR using a Cary 640 FTIR instrument (Agilent Technologies, Stockport, UK) with a GladiATR accessory (Pike Technologies, Madison, WI, USA). Each sample was measured three times, applying enough pressure onto the diamond crystal to have a minimum absorbance of 0.06 for the highest peak. The background was subtracted and a baseline correction was carried out using Agilent Resolution Pro software. The spectra

were normalized and the three spectra of each sample were averaged. The infrared splitting factor (IRSF) was calculated following Weiner and Bar-Yosef.[33]

RESULTS

IR analyses

The IR spectra obtained for uncontaminated enamel and calcined bone (Fig. 1) show that the carbonate band is higher in enamel than in calcined bone. This to be expected as substituted carbonates decrease significantly in heated bone, while hydroxyl groups increase.[34] At the same time the crystallinity increases, as reflected in the infrared splitting factor (IRSF), an indicator of crystal order in the phosphate domain. The calculated IRSF is lower in enamel (3.30) than in calcined bone (5.45). The IR spectra of the calcined samples exposed to the ^{87}Sr-enriched solution (Figs. 2(a) and 2(b)) indicate the appearance and increasing intensity with time of a band at ca 3690 cm^{-1}. No variations in the IRSF or the OH band at 3580 cm^{-1} are observed with time.

Strontium isotope analyses – Intake of strontium from the fuel

When the strontium isotope compositions measured for a cow tibia fraction calcined in a muffle furnace are compared with those obtained for another fraction of the same bone burned on a wood pyre of different strontium isotope composition (Table 1), no significant difference is observed. This implies that there is no detectable exchange of strontium with the fuel.

Strontium isotope analyses – Exchanges with the burial environment

Isotope ratios were measured on the samples immersed in the ^{87}Sr-enriched solution before and after treatment with acetic acid and ultrasonication (Table 2). The percentage variations between uncontaminated and immersed samples (Table 3) are calculated as follows:

$$\Delta^{87}Sr/^{86}Sr(\%) = \frac{\text{soaked} - \text{unsoaked}}{\text{enriched solution} - \text{unsoaked}}$$

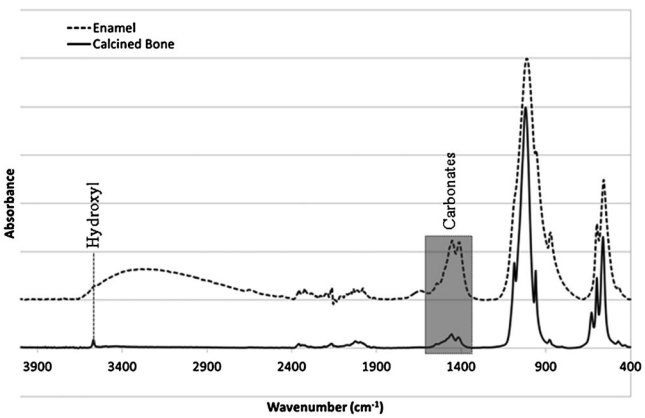
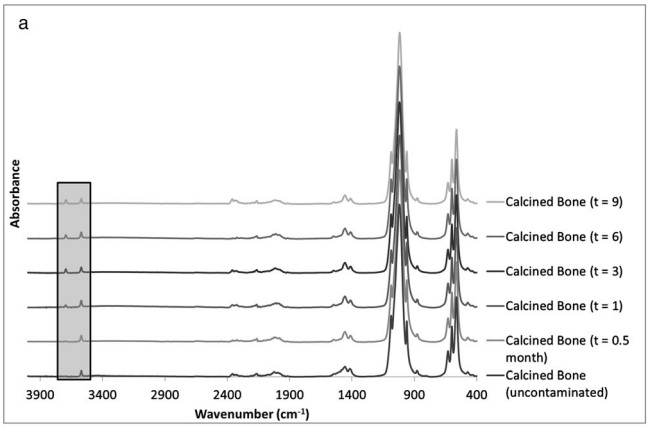


Figure 1. IR spectra of enamel and calcined bone highlighting the absorbance peaks specific to hydroxyl groups and carbonates.



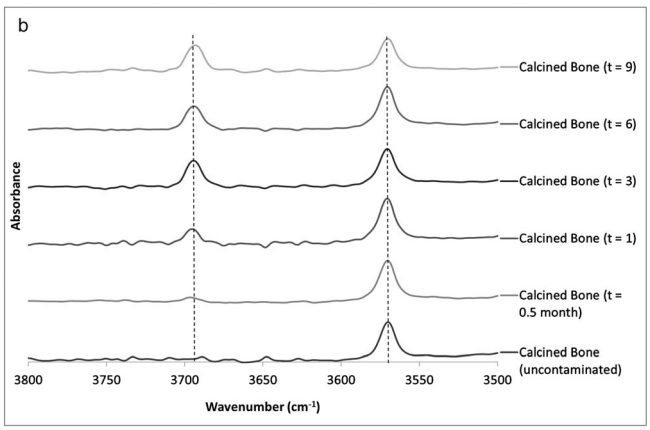


Figure 2. (a) IR spectra of uncontaminated calcined bone and calcined bone immersed in strontium-enriched solution for 0.5, 1, 3, 6 and 9 months; the grey shaded area highlights the appearance of a peak at ca 3690 cm^{-1}. (b) Zoom on the IR spectra of uncontaminated calcined bone and calcined bone immersed in strontium-enriched solution for 0.5, 1, 3, 6 and 9 months; this shows the appearance of a peak at ca 3690 cm^{-1} in calcined bone immersed in the enriched solution.

Table 1. Strontium isotopic ratio for calcined cow tibia heated under different conditions

Sample	^{87}Sr/^{86}Sr (±2σ*)
Muffle furnace	0.710306 ± 11
	0.710323 ± 14
	0.710291 ± 16
	0.710303 ± 10
Outdoor wood pyre	0.710321 ± 8
	0.710394 ± 10
Wood used for outdoor pyre	0.708519 ± 7

*2σ has been calculated following the equation: 2 × mean of the 60 ratio measurements × standard error

SIMULATION

The results presented above are not representative of the conditions to be expected in a burial environment comprised of soil and water. In the experiment, the burial environment is

Table 2. Strontium isotopic ratio for horse enamel and calcined cow bone immersed in the enriched solution

	Time (months)						
$^{87}Sr/^{86}Sr$ (±2σ*)	0	0.5	1	3	6	9	12
Untreated enamel	0.70877 ± 1	7.25861 ± 102	11.86877 ± 128	12.25365 ± 127	18.11921 ± 366	14.42634 ± 230	16.48228 ± 649
Treated enamel (3 min)		5.75578 ± 52	9.10461 ± 80	6.18321 ± 46	12.92154 ± 192	10.73395 ± 86	13.88819 ± 342
Treated enamel (30 min)		5.56857 ± 23	4.03884 ± 23	3.66933 ± 13	n/a	n/a	n/a
Untreated calcined bone	0.71036 ± 1	6.12693 ± 31	7.38861 ± 50	5.71230 ± 25	8.15062 ± 71	5.38431 ± 29	8.69744 ± 252
Treated calcined bone		0.89179 ± 1	0.96404 ± 1	0.84011 ± 1	1.21357 ± 2	0.82947 ± 2	2.58847 ± 82

*2σ has been calculated following the equation: 2 × mean of the 60 ratio measurements × standard error

replaced by a spiked solution giving $^{87}Sr/^{86}Sr$ higher than 90. Most soils would be characterized by $^{87}Sr/^{86}Sr$ values between 0.70 and 0.75.[4] Considering the most extreme case of an individual having an original isotopic ratio of 0.70 buried in a soil having a value of 0.75 and taking into account the isotopic changes measured experimentally (Table 3), the effects of strontium exchange between sample and soil have been simulated (Table 4) using the following equation:

$$\text{Simulated } {}^{87}Sr/{}^{86}Sr = 0.70 + \%_{\text{calculated (Table 3)}} * (0.75 - 0.70)$$

The simulated variations in enamel, after 3 min treatment with acetic acid and ultrasonication, are still significant and shifts of up to 0.00672 are observed, while for treated calcined bone, the largest offset is 0.00103. This is more than six times less than that seen in enamel.

DISCUSSION

The changes in $^{87}Sr/^{86}Sr$ with immersion time as well as the differences between untreated and treated samples (Fig. 3) show a rapid change in isotope composition during the first month of immersion, after which the increase is slower. The variation is higher in enamel (up to 19%) than in calcined bone (up to 8.2%). After treatment with acetic acid, the isotopic variation is negligible in calcined bone (≤2.1%) but remains high in enamel (between 5 and 15%). When ultrasonicated for 30 min, the variation in tooth enamel falls to between 3 and 6%, still higher than in treated calcined bone.

The exogenous strontium could be either adsorbed onto the surface of the samples or incorporated into the crystal structure. The initial intake in the first month is so rapid that it suggests adsorption of strontium onto the surface of the bone fragment followed (possibly) by its slower adsorption/incorporation onto the crystallites present deeper into the bone fragment. The rapid adsorption at the surface of bone crystals is similar to that described in medical experiments.[28] Since after pre-treatment with acetic acid and ultrasonication the amount of exogenous strontium is minimal in calcined bone, it is most likely to have been only adsorbed and not incorporated into the crystal structure. However, in the case of enamel, a significant amount of exogenous strontium remains after treatment with acetic acid suggesting that some exogenous strontium was incorporated into the crystal structure. This is not altogether surprising,

Table 3. Percentage variations between uncontaminated and immersed samples for horse enamel and calcined cow bone

	Months					
$\Delta^{87}Sr/^{86}Sr$ (%)	0.5	1	3	6	9	12
Untreated enamel	7.2	12.3	12.7	19.2	15.1	17.4
Treated enamel (3 min)	5.6	9.2	6.0	13.4	11.0	14.5
Treated enamel (30 min)	5.4	3.7	3.3	n/a	n/a	n/a
Untreated calcined bone	6.0	7.4	5.5	8.2	5.1	8.8
Treated calcined bone	0.2	0.3	0.1	0.6	0.1	2.1

Table 4. Simulation of the strontium isotopic ratio for enamel and calcined bone in 'natural' condition

	Months						
$^{87}Sr/^{86}Sr$	0	0.5	1	3	6	9	12
Untreated enamel	0.70000	0.70361	0.70614	0.70636	0.70958	0.70755	0.70868
Treated enamel (3 min)		0.70278	0.70462	0.70301	0.70672	0.70552	0.70726
Untreated calcined bone		0.70298	0.70368	0.70275	0.70410	0.70257	0.70440
Treated calcined bone		0.70010	0.70014	0.70007	0.70028	0.70007	0.70103

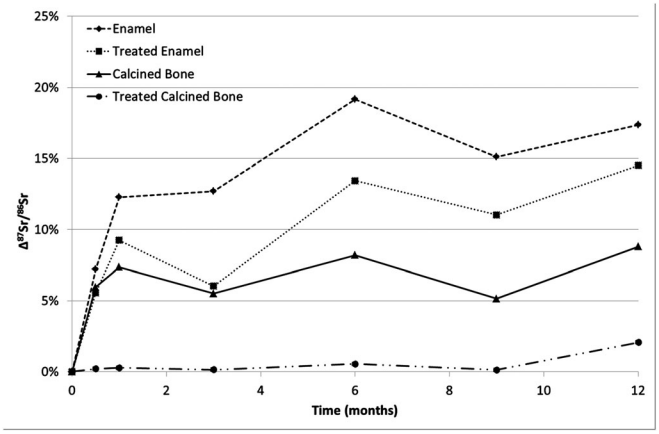


Figure 3. Variation in the strontium isotopic ratio of horse enamel and calcined cow bone between uncontaminated and immersed in the enriched solution.

given that the IR spectra of calcined bone and enamel show that calcined bone has a much higher IRSF, suggesting higher crystallinity. This result suggests that crystallinity of the calcified tissue plays a more important role in post-burial alterations than porosity. Porosity may be important for the initial passage of strontium ions into the interstices of calcined bone, but the data suggest that enhanced crystallinity prevents or greatly inhibits its incorporation into the crystal structure. The results indicate that, even under the extreme conditions in which our experiments were carried out, calcined bone is almost impervious to the enriched, exogenous strontium and that the impact of external strontium on the *in vivo* strontium isotopic ratio is minimal once calcined bone fragments have been properly pre-treated.

The reasons for the appearance of a peak at ca 3690 cm^{-1} in the IR spectra of the samples immersed in the enriched solution are still unclear. It could be that this peak is characteristic of hydroxyl groups in strontium-rich calcium apatites. However, this peak was not observed in strontium-rich hydroxyapatite crystals.[35]

In summary, the results for the experiments demonstrate that there is no significant change in the $^{87}Sr/^{86}Sr$ composition of bone during heating. This result is consistent with previous research.[22,23] It also appears that calcined bone is resistant to post-burial exchanges and retains its original *in vivo* strontium isotope ratio. Furthermore, it is clear that calcined bone is less affected than enamel by the presence of strontium in the burial environment, which is the consequence of calcined bone being more crystalline than enamel and so more resistant to strontium incorporation into its mineral structure. Once treated with acetic acid and ultrasonication, the isotopic variation observed is minimal in calcined bone while still significant in enamel.

ARCHAEOLOGICAL IMPLICATIONS

The results presented here will greatly expand the use of Sr isotopes in the archaeological study of mobility of humans to times and places where cremation is the main funeral rite or where the soils are so acidic that only calcined bone fragments survive. This is the case, for example, across much of Ireland, Scotland and Scandinavia. Moreover, cremation became the dominant mortuary rite over the course of the European Bronze Age, so that the studies of human mobility that are having an increasing impact on Neolithic archaeology[e.g. 36,37] have not been possible for many later periods. Archaeological applications to a number of Irish Neolithic sites and the English Late Neolithic site of Stonehenge are currently under way. The ability to obtain $^{87}Sr/^{86}Sr$ results from calcined bone will also enable the study of the provenance and habitats of animals found at sites where only burned bone survives. Forensic science can also benefit from these results since it is now possible to investigate provenance for individuals who were burned in accidental or intentional fires.

It is important to note that $^{87}Sr/^{86}Sr$ studies on calcined bone differ in an important respect from those on enamel. In the latter, the sequential development of the dentition and the lack of any subsequent changes in the chemical structure of the tooth crown enable a 'life history' approach. Thus, in humans, measurements of enamel on the first molar will reflect residence during infancy, while the third molar records residency during later childhood and early adolescence.[38] Very high-resolution life histories become possible with microsampling techniques. Bone, by contrast, undergoes remodelling once formed, although the rates at which this occurs once skeletal maturity has been attained are still unclear.[16–18] Thus, $^{87}Sr/^{86}Sr$ measurements will reflect a long-term average. However, where recovery of cremated material from distinct individuals is good, it should be possible to follow a similar life history approach by analyzing calcined dentine, since dentine undergoes minimal changes once formed in mid through to late childhood and, in the case of the roots of the third molar, late adolescence.[39] It may also be possible to obtain a signal reflecting residence during infancy, by measuring the optic capsule of the petrous bone, which forms in the first 2 years of life and, unlike the rest of the skeleton, does not appear to remodel subsequently.[20,23] The potential of this life history approach is currently being investigated.

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CONCLUSIONS

This study clearly demonstrates that calcined bone provides at least as reliable strontium isotope results as tooth enamel. This finding opens the door to the use of calcined bone in mobility studies in both archaeological and forensic contexts. Furthermore, the results provide additional information about the process of adsorption/incorporation of strontium. While porosity allows strontium to penetrate within the sample and be adsorbed onto the surface of crystallites within the sample, it appears that the high crystallinity of these crystallites prevents the strontium from being incorporated into the crystallites themselves, showing that crystallinity plays a major role in the susceptibility of a sample to post-burial alterations while porosity does not.

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REFERENCES

[1] G. Faure, T. Powell. *Strontium Isotope Geology*. Springer, New York, **1972**.
[2] I. G. Meighan, A. G. McCormick, D. Gibson, J. A. Gamble, I. J. Graham. Rb-Sr isotopic determinations and the timing of Tertiary central complex magmatism in NE Ireland. *Geological Society, London, Special Publications* **1988**, *39*, 349.
[3] A. Sillen, G. Hall, S. Richardson, R. Armstrong. $^{87}Sr/^{86}Sr$ ratios in modern and fossil food-webs of the Sterkfontein Valley: implications for early hominid habitat preferences. *Geochim. Cosmochim. Acta* **1998**, *62*, 2463.
[4] GEOROC – Geochemistry of Rocks of the Oceans and Continents. Available: http://georoc.mpch-mainz.gwdg.de/georoc/ (accessed 08.07.2014).
[5] J. Hess, M. L. Bender, J. G. Schilling. Evolution of the ratio of strontium-87 to strontium-86 in seawater from Cretaceous to present. *Science* **1986**, *231*, 979.
[6] R. Z. LeGeros, O. R. Trautz, E. Klein, J. P. LeGeros. Two types of carbonate substitution in the apatite structure. *Cell. Mol. Life Sci.* **1969**, *25*, 5.
[7] B. Wopenka, J. D. Pasteris. A mineralogical perspective on the apatite in bone. *Mater. Sci. Eng. C* **2005**, *25*, 131.
[8] A. Sillen, in *The Chemistry of Prehistoric Human Bone*, (Ed: T. D. Price). Cambridge University Press, Cambridge, **1989**, p. 211.
[9] J. C. Sealy, N. J. van der Merwe, A. Sillen, F. J. Kruger, H. W. Krueger. $^{87}Sr/^{86}Sr$ as a dietary indicator in modern and archaeological bone. *J. Archaeol. Sci.* **1991**, *18*, 399.
[10] N. Tuross, A. K. Behrensmeyer, E. D. Eanes. Strontium increases and crystallinity changes in taphonomic and archaeological bone. *J. Archaeol. Sci.* **1989**, *16*, 661.
[11] A. Sillen. Biogenic and diagenetic Sr/Ca in Plio-Pleistocene fossils of the Omo Shungura Formation. *Paleobiology* **1986**, *12*, 311.
[12] A. Sillen, R. Z. LeGeros. Solubility profiles of synthetic apatites and of modern and fossil bones. *J. Archaeol. Sci.* **1991**, *18*, 385.
[13] P. Budd, J. Montgomery, B. Barreiro, G. C. Thomas. Differential diagenesis of strontium in archaeological human dental tissues. *Appl. Geochem.* **2000**, *15*, 687.
[14] K. A. Hoppe, P. L. Koch, T. T. Furutani. Assessing the preservation of biogenic strontium in fossil bones and tooth enamel. *Int. J. Osteoarchaeol.* **2003**, *13*, 20.
[15] R. Z. LeGeros. *Calcium Phosphates in Oral Biology and Medicine*. Karger, Paris, **1991**.
[16] D. W. Dempster, in *Dynamics of Bone and Cartilage Metabolism*, (Eds: M. J. Seibel, S. P. Robins, J. P. Bilezikian). Academic Press, San Diego, **1999**, p. 261.
[17] R. E. M. Hedges, J. G. Clement, C. D. L. Thomas, T. C. O'Connell. Collagen turnover in the adult femoral mid-shaft: modeled from anthropogenic radiocarbon tracer measurements. *Am. J. Phys. Anthropol.* **2007**, *133*, 808.
[18] S. P. Robins, S. A. New. Markers of bone turnover in relation to bone health. *Proc. Nutr. Soc.* **1997**, *56*, 903.
[19] N. Jeffery, F. Spoor. Prenatal growth and development of the modern human labyrinth. *J. Anat.* **2004**, *204*, 71.
[20] M. L. S. Jørkov, J. Heinemeier, N. Lynnerup. The petrous bone – A new sampling site for identifying early dietary patterns in stable isotopic studies. *Am. J. Phys. Anthropol.* **2009**, *139*, 199.
[21] M. C. Stiner, S. L. Kuhn, S. Weiner, O. Bar-Yosef. Differential burning, recrystallization, and fragmentation of archaeological bone. *J. Archaeol. Sci.* **1995**, *22*, 223.
[22] M. Harbeck, R. Schleuder, J. Schneider, I. Wiechmann, W. W. Schmahl, G. Grupe. Research potential and limitations of trace analyses of cremated remains. *Forensic Sci. Int.* **2011**, *240*, 191.
[23] L. Harvig, K. M. Frei, T. D. Price, N. Lynnerup. Strontium isotope signals in cremated petrous portions as indicator for childhood origin. *PLoS ONE* **2014**, *9*, e101603.
[24] C. M. Hüls, H. Erlenkeuser, M.-J. Nadeau, P. M. Grootes, N. Andersen. Experimental study on the origin of cremated bone apatite carbon. *Radiocarbon* **2010**, *52*, 587.
[25] A. Zazzo, J.-F. Saliege, M. Lebon, S. Lepetz, C. Moreau. Radiocarbon dating of calcined bones: insights from combustion experiments under natural conditions. *Radiocarbon* **2012**, *54*, 1.
[26] C. Snoeck, F. Brock, R. J. Schulting. Carbon exchanges between bone apatite and fuels during cremation: impact on radiocarbon dates. *Radiocarbon* **2014**, *56*, 591.
[27] G. Grupe, S. Hummel. Trace element studies on experimentally cremated bone. I. Alteration of the chemical composition at high temperatures. *J. Archaeol. Sci.* **1991**, *18*, 177.
[28] S. G. Dahl, P. Allain, P. J. Marie, Y. Mauras, G. Boivin, P. Ammann, Y. Tsouderos, P. D. Delmas, C. Christiansen. Incorporation and distribution of strontium in bone. *Bone* **2001**, *28*, 446.
[29] J. A. Evans, C. A. Chenery, A. P. Fitzpatrick. Bronze Age childhood migration of individuals near Stonehenge, revealed by strontium and oxygen isotope tooth enamel analysis. *Archaeometry* **2006**, *48*, 309.
[30] F. Brock, T. Higham, P. Ditchfield, C. Bronk Ramsey. Current pretreatment methods for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (ORAU). *Radiocarbon* **2010**, *52*, 103.
[31] J. Míková, P. Denková. Modified chromatographic separation scheme for Sr and Nd isotope analysis in geological silicate samples. *J. Geosci.* **2007**, *52*, 221.
[32] D. Weis, B. Kieffer, C. Maerschalk, J. Barling, J. de Jong, G. A. Williams, D. Hanano, W. Pretorius, N. Mattielli, J. S. Scoates, G. Goolaerts, R. M. Friedman, J. B. Mahoney. High-precision isotopic characterization of USGS reference materials by TIMS and MC-ICP-MS. *Geochem., Geophy., Geosystems* **2006**, *7*, Q08006.

[33] S. Weiner, O. Bar-Yosef. States of preservation of bones from prehistoric sites in the Near East: A survey. *J. Archaeol. Sci.* **1990**, *17*, 187.
[34] C. Snoeck, J. A. Lee-Thorp, R. J. Schulting. From bone to ash: Compositional and structural changes in burned modern and archaeological bone. *Palaeogeogr., Palaeoclimatol,, Palaeoecol.* **2014**, *416*, 55.
[35] Z. Y. Li, W. M. Lam, C. Yang, B. Xu, G. X. Ni, S. A. Abbah, K. M. C. Cheung, K. D. K. Luk, W. W. Lu. Chemical composition, crystal size and lattice structural changes after incorporation of strontium into biomimetic apatite. *Biomaterials* **2007**, *28*, 1452.
[36] R. A. Bentley, L. Chikhi, T. D. Price. The Neolithic transition in Europe: comparing broad scale genetic and local scale isotopic evidence. *Antiquity* **2003**, *77*, 63.
[37] R. A. Bentley. Mobility and the diversity of early Neolithic lives: Isotopic evidence from skeletons. *J. Anthropol. Archaeol.* **2013**, *32*, 303.
[38] D. H. Ubelaker, in *Age Markers in the Human Skeleton*, (Ed: M. Y. Iscan), Charles C. Thomas, Springfield, Illinois, **1989**, p. 55.
[39] A. Nanci. *Ten Cate's Oral Histology: Development, Structure and Function*. Mosby, St Louis, MO, **2003**.

