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Preliminary results of an investigation on postmortem variations in human skeletal mass of buried bones

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ABSTRACT

Extreme fragmentation can complicate the inventory of human skeletal remains. In such cases, skeletal mass can provide information regarding skeleton completeness and the minimum number of individuals. For that purpose, several references for skeletal mass can be used to establish comparisons and draw inferences regarding those parameters. However, little is known about the feasibility of establishing comparisons between inherently different materials, as is the case of curated reference skeletal collections and human remains recovered from forensic and archaeological settings. The objective of this paper was to investigate the effect of inhumation, weather and heat exposure on the skeletal mass of two different bone types. This was investigated on a sample of 30 human bone fragments (14 trabecular bones and 16 compact bones) that was experimentally buried for two years after being submitted to one of four different heat treatments (left unburned; 500 °C; 900 °C; 1000 °C). Bones were exhumed periodically to assess time-related mass variation. Skeletal mass varied substantially, decreasing and increasing in accordance to the interchanging dry and wet seasons. However, trends were not the same for the two bone types and the four temperature thresholds. The reason for this appears to be related to water absorption and to the differential heat-induced changes in bone microporosity, volume, and composition. Our results suggest that mass comparisons against published references should be performed only after the skeletal remains have been preemptively dried from exogenous water.

1. Introduction

The analysis of skeletal remains in forensic and archaeological settings are sometimes based on their mass, in an attempt to retrieve information regarding the completeness of the assemblage and the minimum number of individuals [1–12]. This option is usually adopted when the remains are extremely fragmented and therefore prevent the application of other inventory methods that depend on the anatomical identification of bones. The mass approach has the advantage of dispensing this anatomical identification procedure and is not greatly affected by fragmentation [8].

Given the potential of skeletal mass for anthropological examinations, several authors have documented it in human skeletons so that references may be available for researchers to compare against practical

cases [6, 13, 14]. The same has been done for burned human skeletons which were obtained in modern crematoria [15–21]. Since assemblages of burned skeletal remains are often very fragmented, researchers tend to rely very frequently on skeletal mass to make inferences [22]. However, these inferences require specific references since mass loss is one of the heat-induced changes affecting bone. Such occurrence has also been broadly investigated in terms of its association to temperature increment. Major mass loss of about 40% until 400–600 °C has been observed [23–26]. This temperature threshold corresponds to the dehydration and organic decomposition stages identified by several authors [25, 27, 28]. Mass seems to stabilize or decrease at a slower rate from that point on [29]. Although percentages were not reported, some authors did report that mass loss becomes again more intense at temperatures higher than 900 °C [23, 25, 26]. However, Ellingham et al.

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Table 1
Mass (g) and relative mass variation according to each bone.

Set	Bone	°C	Mass (g)	2 months	4 months	6 months	8 months	10 months	12 months	18 months	24 months
Set 1	CC_NI_16 Cuboid	Room	3.4	40.6%	3.5%	7.3%	−4.0%	−17.8%	26.5%	−1.7%	−13.4%
	CC_NI_16 Vertebra 05	Room	2.9	47.9%	−2.0%	14.2%	−6.9%	−23.5%	34.8%	−4.4%	−17.4%
	CC_NI_16 Patella	500 °C	7.0	45.6%	2.6%	7.4%	−1.3%	−30.9%	42.7%	−3.9%	−16.0%
	CC_NI_16 Navicular	500 °C	3.3	49.2%	−0.2%	3.6%	−3.0%	−30.0%	61.5%	−8.8%	−9.6%
	CC_NI_16 Talus	900 °C	8.7	12.3%	1.7%	1.4%	−1.2%	−8.3%	13.3%	−5.6%	−2.7%
	CC_NI_16 Vertebra 06 ^a	900 °C	1.7	28.3%	–	–	–	–	–	–	–
	CC_NI_16 Vertebra 07 ^a	1050 °C	2.7	25.2%	4.1%	5.0%	−5.6%	−12.2%	–	–	–
	CC_NI_16 Tibia 02	Room	38.9	3.9%	3.5%	2.2%	−5.9%	−5.2%	8.3%	−4.1%	−3.3%
	CC_NI_16 Radius 02	Room	9.4	2.1%	1.9%	9.4%	−4.3%	−7.2%	2.8%	2.9%	−5.0%
	CC_NI_16 Femur 02	500 °C	43.9	35.2%	2.0%	1.7%	−0.8%	−25.3%	39.4%	−6.2%	−21.2%
	CC_NI_16 Humerus 01	500 °C	18.3	34.4%	1.1%	3.6%	−0.8%	−25.5%	36.6%	−3.3%	−21.2%
	CC_NI_16 Ulna 01	900 °C	8.5	4.6%	2.4%	2.0%	0.4%	−3.1%	0.4%	1.8%	−1.3%
	CC_NI_16 Fibula 01	900 °C	7.6	3.6%	0.0%	3.8%	0.1%	−4.5%	7.8%	−1.9%	−1.4%
	CC_NI_16 Femur 01	1050 °C	52.5	7.7%	1.6%	1.7%	−1.0%	−5.0%	7.2%	−3.0%	−3.1%
	CC_NI_16 Tibia 01	1050 °C	28.8	6.1%	1.8%	2.3%	−1.5%	−3.7%	5.9%	−3.8%	−1.5%
Set 2	CC_NI_16 Vertebra 04	Room	2.5	–	–	25.0%	–	–	0.4%	–	−20.6%
	CC_NI_17 Cuboid	Room	3.2	–	–	17.1%	–	–	−6.7%	–	−17.9%
	CC_NI_17 Vertebra 05	500 °C	3.5	–	–	35.0%	–	–	−2.5%	–	−22.2%
	CC_NI_17 Vertebra 04	500 °C	3.4	–	–	32.1%	–	–	−3.9%	–	−23.7%
	CC_NI_17 Patella	900 °C	4.7	–	–	18.4%	–	–	6.4%	–	−7.2%
	CC_NI_17 Calcaneus	1050 °C	8.5	–	–	9.7%	–	–	0.6%	–	−10.0%
	CC_NI_17 Talus	1050 °C	7.2	–	–	6.5%	–	–	3.4%	–	−5.2%
	CC_NI_17 Tibia 02	Room	34.7	–	–	7.3%	–	–	−3.7%	–	−4.9%
	CC_NI_17 Radius 01	Room	8.2	–	–	9.0%	–	–	−2.1%	–	−7.5%
	CC_NI_16 Clavicle 02	500 °C	6.0	–	–	25.7%	–	–	−0.6%	–	−27.3%
	CC_NI_17 Femur 02	500 °C	42.4	–	–	35.0%	–	–	−1.8%	–	−25.6%
	CC_NI_16 Radius 01	900 °C	5.6	–	–	3.4%	–	–	−2.9%	–	−1.7%
	CC_NI_17 Ulna 01	900 °C	6.0	–	–	2.9%	–	–	1.0%	–	−3.1%
	CC_NI_16 Humerus 02	1050 °C	16.9	–	–	4.2%	–	–	−1.3%	–	−1.0%
	CC_NI_17 Femur 01	1050 °C	48.1	–	–	8.0%	–	–	−0.3%	–	−5.4%

^a This vertebra presented extremely poor preservation preventing further weighing.

[29] did not observe such trend in their study. The latter is the only research that monitored mass changes longitudinally, i.e., by following changes in the same bone as the heat experiment took place. Therefore, it can be argued that it provided the more reliable data.

Although anthropological inferences can be made taking skeletal mass as a variable, the efficacy of methods based on this parameter may be strongly affected by post-mortem bone mass loss, especially in cases involving the burial of the skeleton. Although the effect of inhumation has been recurrently investigated [e.g. 30–33], little is known about the quantified effect of inhumation on the mass of both unburned and burned human skeletons. Obviously, it is relatively easy to deduce that the loss of water and organic matter leads to the loss of mass. For instance, during their investigation regarding the association between temperature increment and mass loss, Enzo et al., [34] observed a much smaller reduction (17%) in bones burned up to 900 °C than other authors who generally obtained values higher than 30% [23–26]. That may have occurred because Enzo et al. [34] used archaeological bone instead of the modern animal bone used by the other authors. In this last case, an important loss of water and organic matter probably occurred leading to such contrasting results. However, mass variation in each bone is probably not the result of dehydration and organic decomposition alone. Predictably, other variables may have an effect on mass. We hypothesize that water assimilation during wet seasons leads to mass increase so weather must have a major impact on skeletal mass even when the remains are buried. To our knowledge, no data resulting from controlled experiments on human bones have been obtained so far regarding this matter.

The impact of inhumation on skeletal mass variation may prevent: i) comparisons with published mass references [15–21]; ii) the application of mass-based methods such as the ones that aim at reconstructing the living body mass or stature [17, 35, 36]; and iii) the estimation of the skeletal total mass based on single bones [37, 38]. As a result, the objective of this research was to document and investigate the effect of inhumation and weather conditions on skeletal mass of both unburned

and burned human bones. To accomplish this goal, bones were experimentally burned and buried for two years. During that time period, periodical exhumations were carried out to assess skeletal mass.

2. Material and methods

Sampling was performed on two unclaimed human skeletons (CC_NI_16 and CC_NI_17) of undocumented sex and age at death that were donated to the University of Coimbra. Their provenance is the cemetery of Capuchos (Santarém, Portugal) so they have the same place of origin of the documented skeletons from the 21st Century Identified Skeletal Collection housed at the University of Coimbra [39]. These skeletons have been inhumed at the cemetery for an unknown period of time which was nonetheless larger than three years. As mentioned above, the sex of each skeleton was unknown but via an anthropological examination, we estimated them as probable females through the DSP tool [40].

The total sample was composed of 30 bone fragments. Since we wanted to investigate the two types of bone structure, 14 samples comprised bones mostly composed of trabecular bone (calcanei, tali, cuboids, naviculars, and vertebrae) while the remaining 16 samples included bones mostly composed of cortical bone (clavicles, humeri, radii, ulnae, femora, tibiae, and fibulae). For simplification, these two groups will be designated as “cortical bones” and “trabecular bones”, respectively. Each one of these two groups were subsequently divided into four sub-groups representing four distinct heat exposures: i) room temperature; ii) 500 °C; iii) 900 °C; and iv) 1050 °C. Although the original project envisaged the inclusion of four different specimens in each sub-group, only three were ultimately included in the 900 °C and 1050 °C sub-groups. This was due to inconsistencies regarding two specimens burned at these maximum temperatures. By looking at their infrared profile within the framework of a side project, we realized that the samples still presented considerable organic content, which we deem impossible at such burning intensities. It is therefore possible that



Fig. 1. Section of femoral diaphysis from individual CC_NI_17 (left); thoracic vertebra with removed neural arch from individual CC_NI_16 (right).

some samples (including others not used in this paper) have been switched during the exhumations/re-inhumation operations. We therefore have no confidence in the results they provided and decided to remove them from the study which is now based on 30 rather than 32 samples. The same happened partially for the CC_NI_16 vertebra 07. In this case, only the last three observations seem to be compromised. The distribution of samples is given in Table 1.

Prior to burning and burial, the epiphyses of cortical bones were sectioned off while the neural arches of vertebrae were also removed (Fig. 1). This was done to ensure that these bones mostly comprised cortical or trabecular bone, respectively. No such preparation was deemed necessary for the remaining representatives of trabecular bones. The unburned samples were weighed prior to inhumation. The burned samples were weighed both prior and after the burning with a digital weight scale Kern EW3000-2M (error = 0.1 g). Experimental burnings were then carried out by using an electric muffle furnace (Barracha, K-3 three-phased). The desired temperature thresholds were attained after two hours and the samples were then allowed to naturally cool down to room temperature before retrieving them. The furnace temperature was measured with a type K probe (negative: nickel-aluminum, positive: nickel-chrome) following norm IEC 60584-2. This means that the expected standard error for the temperatures thresholds investigated in this research were the following: i) 500 °C = ± 3.8; ii) 900 °C = ± 6.8; and iii) 1050 °C = ± 7.9. Finally, flower pots were used for the burials. Each one comprised 8 delimited squares accordingly labelled with the identification of each sample (Fig. 2). This experiment recreated relatively shallow burials. The distance of bones from the surface randomly varied from 1 to 25 cm. A soil substrate with a pH of 4.0–4.5 was used.

Exhumations were performed following two different time intervals. A set of 15 samples (two for each temperature and eight for each bone type) was exhumed bi-monthly for a year and every six months during the second year. The second set of 15 samples was exhumed every six months during the first year and then at the end of the second year. These sets will be from now on referred to as Set 1 and Set 2, respectively. The two different observation strategies resulted from the fact



Fig. 2. Example of flower pot with delimited squares for the burial of bones. These were afterwards covered with soil substrate. Smaller fragments refer to burned teeth which had originally been included in this research, but their rapid post-depositional destruction prevented any mass measurements.

that the data here presented are merely the result of one fraction of the entire research project which includes additional samples and is planned to last for at least 10 years. This plan includes several interval times of observation (bi-annually; once every 5 years; and once at the end of the 10 years). A set of fifteen samples was nonetheless exhumed every two months during the first year so that a better resolution of eventual skeletal mass variations could be obtained. Also, for Set 2, we decided to make an extra exhumation after the first six months to see if the data were in compliance with those from Set 1. The data from Set 2 was used to check if they were coherent with the data obtained from Set 1.

Data regarding the weather conditions in Coimbra from October of 2015 to October of 2017 were collected at the website of the *Instituto Português do Mar e da Atmosfera* (www.ipma.pt) which is the governmental agency providing official climatic data. These were the dates of the beginning and end of this experiment, respectively. The total rain precipitation and the mean maximum monthly temperature were recorded from the climatological bulletins provided by the agency.

3. Results

The bi-monthly, bi-annual and annual post-depositional mass variation of each bone class is given in Table 1 and Figs. 3-4. In Set 1, trabecular bones and cortical bones presented distinct patterns. In the first case, both the Trabecular_UNB and Trabecular_500 presented a substantial increase in mass during the first two months of wet season I. Classes Trabecular_900 and Trabecular_1050 also presented an increase during that period but not as large. From then on, mass was kept relatively stable until the end of wet season I and then tendentially decreased until the end of dry season I. The second year of the experiment benefitted only of bi-annual exhumations but, generally, a trend similar to the first year occurred. In the case of cortical bones, the mass of Cortical_UNB, Cortical_900 and Cortical_1050 presented little variation during the full extent of this research, regardless of the type of weather. In contrast, the mass of Cortical_500 presented a trend similar to the one observed for the trabecular bones. The results of Set 2 basically replicated those observed for Set 1.

4. Discussion

The observed results of this experiment confirmed our assumption that skeletal mass in contexts of inhumation presents important variation over time – with intermittent increases and reductions – and that it is dependent of weather conditions. In summary, wet environments lead to mass increases and dry environments lead to mass reductions. Other factors may have had an effect in mass variation but were not assessed here. For instance, acidic soils may increase bone surface deterioration and decomposition [30, 41, 42] thus leading predictably to mass loss.

Samples from different bone types did not show the same behaviour. Trabecular bones tended to be much more susceptible to mass variations than cortical bones. This is not surprising due to clear

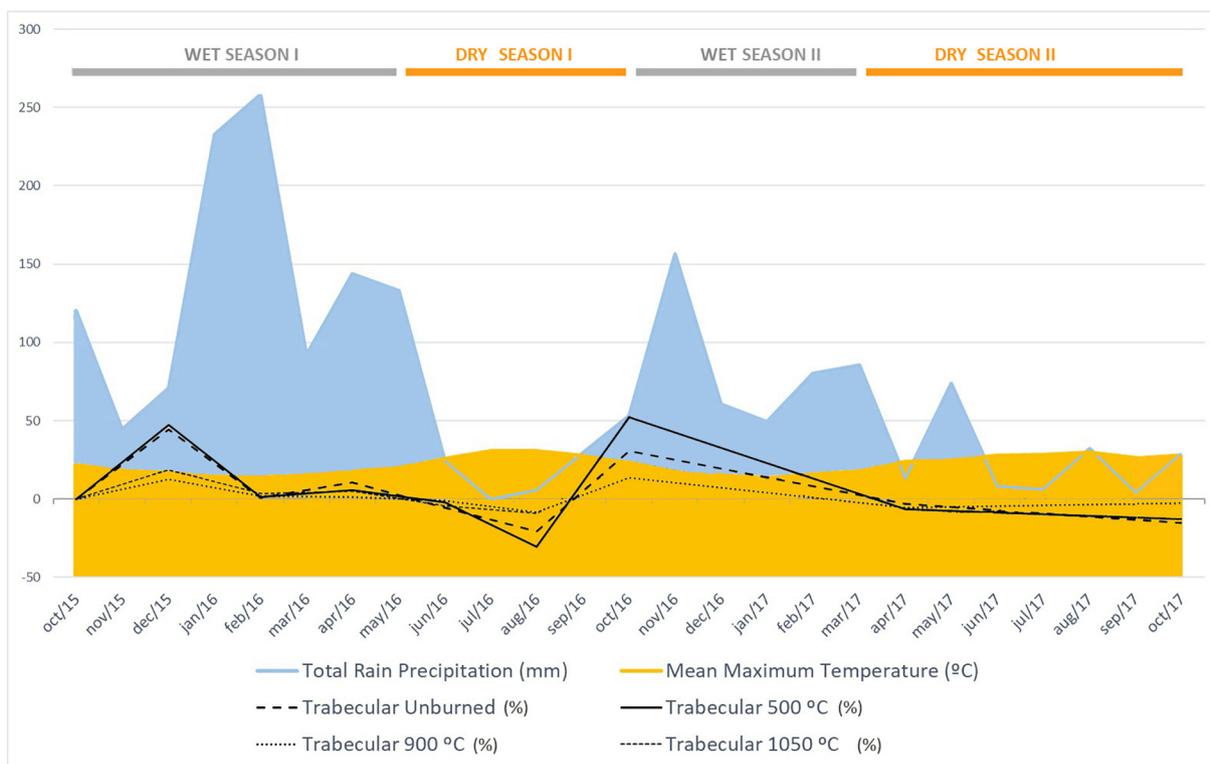


Fig. 3. Bi-monthly post-depositional mass variation of bones mostly composed of trabecular bone, monthly total rain precipitation, monthly mean maximum temperature and predominant type of weather. The values of unburned trabecular bones as well as trabecular bones experimentally burned at 500 °C, 900 °C and 1050 °C are given. In the case of bones unburned and burned at 500 °C, the average of two bones was used. For the other two temperatures, information refers only to one bone. At 1050 °C, the information refers only to the first 10 months of the experiment.

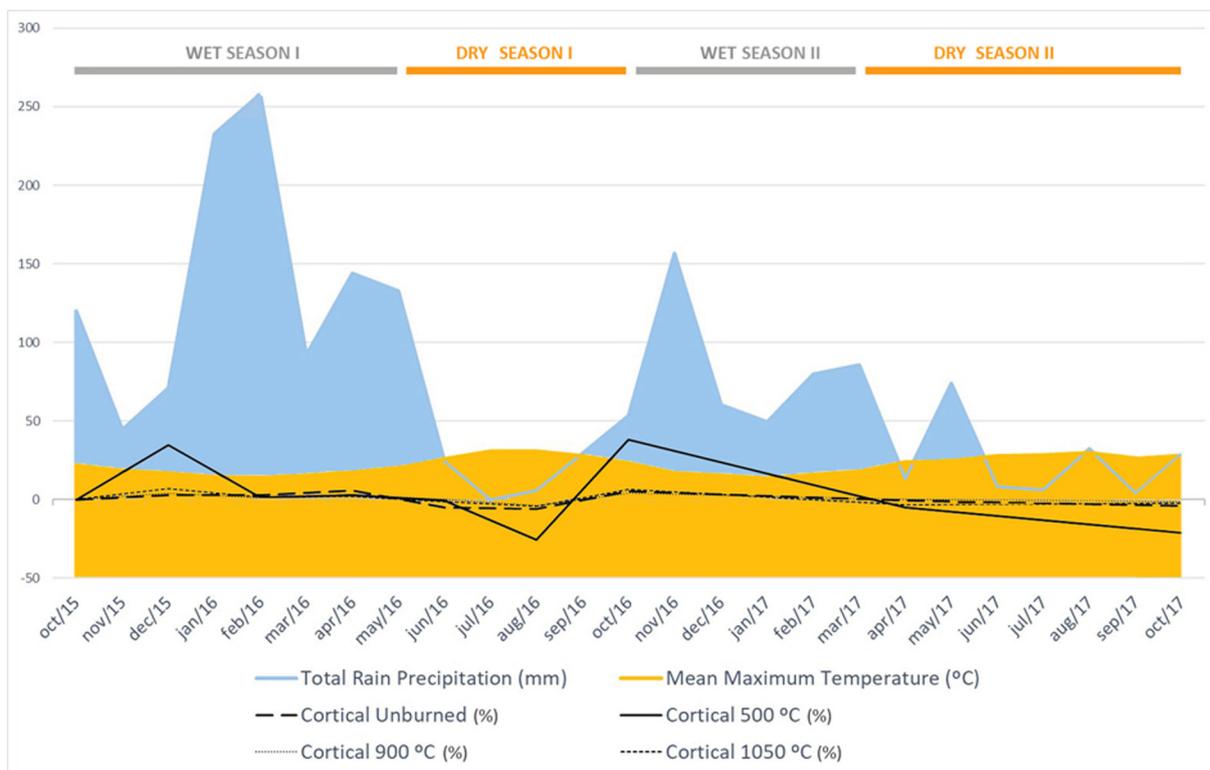


Fig. 4. Bi-monthly post-depositional mass variation of bones mostly composed of cortical bone, monthly total rain precipitation, monthly mean maximum temperature and predominant type of weather. The values of unburned cortical bones as well as cortical bones experimentally burned at 500 °C, 900 °C and 1050 °C are given. The averages of two bones are provided for each temperature category.

architectural differences between the two bone types. Due to its typical structure, trabecular bone is theoretically more prone to assimilate water and soil residues than cortical bones so this result was expected at first. However, the data obtained for the Cortical_500 seem to contradict this postulate. Its mass variation replicates more closely the trend observed for trabecular bones than the trend observed for other cortical bones both unburned and burned at 900 °C and 1050 °C.

This observation may be related with the fact that at 500 °C, water and a large fraction of organics have been removed from the bone [28], leaving room for exogenous materials to occupy it. However, if that is the case, it does not explain why such phenomenon was not observed in bones burned at 900 °C and 1050 °C as well. We believe that part of the explanation may be linked to the bone microstructure. Heat increment leads to important changes in the microporosity of bone [43, 44]. Bones burned at 500 °C and up to 700–800 °C present enlarged pores probably due to the pyrolysis of collagen [43, 44]. This increase may reach 30% according to Nielsen-Marsh and Hedges [45]. The loss of organic matter has been recurrently associated to the increase of bone porosity [45–47]. At temperatures equal or higher than 900 °C, pores decrease again in size and become less numerous [43], this most probably being the result of the fusion stage leading to the coalescence of pores during the melting of the inorganic phase [28, 43]. Given this, the larger porosity of our cortical samples burned at 500 °C, when compared to the porosity present in unburned cortical bones and cortical bones burned at higher temperatures may be the cause for the striking mass variation differences that were observed. Samples with smaller and fewer pores must have been less prone to harbour exogenous materials that would add to the mass of each bone.

The above hypothesis fittingly explains the observations made on the sample of cortical bones. However, it does not fit adequately to the scenario observed on the sample of trabecular bones. Although the mass variation trends of trabecular bones burned at 500 °C, 900 °C and 1050 °C were somewhat similar, albeit much more intense, to the one seen for cortical bones heated at the same temperatures, a different scenario was observed for the unburned bones. In contrast, the mass variation of unburned trabecular bone was similar to the trend observed for samples burned at 500 °C. The reason for this difference between unburned trabecular and cortical bones is difficult to pinpoint. Such a major mass increase in trabecular bone could be related with the known hydrophilic properties of collagen [46]. Unburned bones with preserved collagen possibly trapped water more efficiently than burned bones whose collagen had been pyrolysed. However, this hypothesis does not explain the meaningless mass variation recorded for the cortical unburned bones which, supposedly, also comprised well preserved collagen.

The explanation possibly lies elsewhere, more specifically on the macroscopic heat-induced changes of bone. When subjected to heat, skeletal elements suffer changes in volume. At temperatures above 700 °C, during the fusion stage, bones shrink considerably [28, 48–53]. That also occurred on length, breadth and height bone measurements for our trabecular and cortical samples burned at high temperatures (900 °C and 1000 °C) that respectively shrunk 15.8% and 19.8% on average. Possibly, shrinkage was not as relevant in the case of cortical bones because we were able to remove any macroscopic exogenous material from the medullary canal before weighing. However, that was not possible for trabecular bones. Any water or dirt harboured inside the bones was not directly accessible. Given that trabecular unburned bones and trabecular bones burned at 500 °C experienced no volume shrinkage or small average volume shrinkage (4.8%) respectively, this means that both maintained most of their original capacity to harbour exogenous materials. In contrast, bones heated at higher temperatures lost an important fraction of their original capacity, turning them denser, and thus reducing the amount of exogenous materials that could be harboured inside them. This occurrence, in combination with the microporosity hypothesis, may help explaining the diverse results obtained for the different bone types and burning treatment. It should be

noted that our discussion regarding bone type differences is based on results obtained on a relatively small sample and may therefore lack representativeness. The explanation hypotheses we propose here should ideally be further tested on larger samples.

This research demonstrates that the application of methods based on mass for the analysis of skeletal human remains is not straightforward. Post-depositional mass increases and reductions occur continuously, at least for a prolonged amount of time, and this has a clear impact on the reliability of such methods which include comparisons with published references for both burned and unburned human skeletons [6, 13–21] and regression approaches to predict skeletal mass or living parameters [17, 35–38]. By association, this conclusion may predictably be extended to methods aimed at non-human assemblages such as the “weight method” which refers to the use of faunal skeletal mass to estimate the potential meat yield (for a review, check Barrett [54]).

Additionally, it became clear that differential mass variation affects different parts of the skeleton. For example, although unburned cortical bones did not seem to be substantially affected, the opposite was observed for unburned trabecular bones. The case is even more complex for burned bones because the intensity of burning, or more specifically, heat-induced changes in microporosity and bone volume, seem also to be influential factors in post-mortem skeletal mass variation. Bones burned at different temperatures revealed quite contrasting behaviours. However, it should be noted that these conclusions were obtained on a small sample and our results may deviate from future experiments. Even so, the data obtained from the samples subjected to bi-annual exhumations (Set 2) corroborate the data obtained from the bi-monthly exhumations thus reinforcing the observations made on Set 1. It is not possible from this experiment alone to infer if skeletal mass variation can continue to occur after longer periods of time than the one here investigated although we think it to be very likely. In conclusion, the application of skeletal mass-based methods may become more reliable if researchers make sure that both the published references and the practical case studies are implemented in well preserved bones that are pre-emptively dried at the lab.

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