The G-force awakens: The influence of gravity in bone heat-induced warping and its implications for the estimation of the pre-burning condition of human remains

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Word count of the manuscript: 3192 (included: title, abstract, keywords, full text, references)

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Abstract In forensics, assessing the pre-burning condition is important to determine the circumstances of death. Heat-induced warping (HIW) occurs more frequently on non-dry bone but is not a reliable indicator of the pre-burning condition of human remains. Several factors have a significant effect on warping (e.g., maximum temperature) but others are still unknown. We investigated the effect of Earth’s force of gravity on HIW by experimentally burning 23 human long bones from 4 different unidentified skeletons at different temperatures. Only 56% of the warped bones (n=16) presented HIW compatible with gravity. These bones presented larger masses than the bones presenting warping incompatible with gravity, thus suggesting that bone mass plays a role in HIW. Probably, the intrinsic contractile forces caused by heated collagen are in the origin of warping and such bending may be compatible with gravity depending on bone mass. Possibly, incompatibility occurs when intrinsic bending is counteracted by gravity whenever bone mass produces a stronger force. We therefore conjecture that HIW results from a combination of at least two different forces. HIW is multifactorial and finding a reliable indicator of the pre-burning condition of human remains may be only possible at the microscopic and elemental levels.

Keywords: forensic anthropology; bioarchaeology; taphonomy; heat-induced changes; burned bone.
Introduction

Determining the pre-burning condition of skeletal remains, i.e., if they resulted from a complete body or from a dry and disarticulated skeleton is important to help assessing the circumstances of death. Specifically, it may indicate if the heat event took place during the perimortem stages or merely during the post-mortem stage. The implications are clear. If the body was already partially or completely skeletonized when in contact with the heat source, then the latter was not linked to the cause of death.

Although some examinations allow us to infer the pre-burning condition through colour patterns or fracture biomechanics in burned bone,\textsuperscript{1,2} no entirely reliable inferences can be obtained from homogeneously calcined remains displaying no substantial colour differences or from bones exhibiting no traumas. As a result, since the 1950’s, several researchers focused on the issue of assessing the pre-burning condition of human remains by attempting to determine if it is associated to other specific skeletal features.\textsuperscript{3-13} In particular, warping has been suggested to have the potential to discriminate between burnings of dry bones and fleshed/green bones because it occurs much more frequently in the latter case. However, warping can occur in both cases so its discriminatory power may have been overstated.\textsuperscript{6,7,10-13} Therefore, it is important to understand the origin of warping to truly assess and quantify its possible discriminatory potential.

Gonçalves et al.\textsuperscript{12} suggested that warping depends of bone collagen content which is essential to allow the bone to bend.\textsuperscript{14-16} The rationale here is that that fleshed/green bones are much richer in collagen than dry bones thus explaining why warping seems to occur less frequently on the latter. However, Vassalo et al.\textsuperscript{13} failed to identify collagen content as a very significant variable in this process. Burning dynamics for example, including maximum temperature and duration of burning appeared to have a more important role although a regression model involving these three variables – as well as others such as sex, age at death, bone type and burial period – could not explain all the variability observed on the occurrence of warping.\textsuperscript{13}

It is clear that some variables are still unknown and the quest for them is at the core of this paper. Given that all things are subjected to gravitational forces,\textsuperscript{17} we believe that these may be playing a role in warping. In particular, Earth’s gravity may cause the bone to warp towards the centre of the Earth when submitted to burning. Therefore, our objective is to investigate the effect of the force of gravity on heat-induced warping by experimentally burning human long bones. Expectantly, the results of this experiment will increase our knowledge regarding skeletal changes induced by heat. Only by identifying all variables involved in the occurrence of warping can we hope to establish the true value of this heat-induced feature to determine the pre-burning condition of human remains.

Material and Methods

This pilot study was carried out on a sample composed of 23 dry long bones, thus showing no visible soft tissues, of four unidentified adult skeletons (Table 1) from the cemetery of Capuchos (Santarém) and therefore have the same origin of the skeletons composing the 21st Century Identified Skeletal Collection.\textsuperscript{18} These remains belong to unclaimed skeletons that were donated to the University of Coimbra. Only dry bones were used since access to large samples
of fresh long bones is hard to obtain. Nonetheless, dry bones are suitable for the specific purpose of this paper, since no attempt at estimating the pre-burning condition of the remains was carried out. Instead, the research focus was the potential effect of the gravity force on warping. Long bones were selected because warping tends to be more frequent on such bones mainly composed of compact bone.\textsuperscript{11} Since the sample was composed of unidentified skeletons, precise sex and age at death were unknown but the anthropological examination indicated that all individuals were elderly adults, two of them were males (CC_NI_31 and CC_NI_32) and the others two were females (CC_NI_33 and CC_NI_34).

Table 1. Composition of the sample of long bones in function of each skeleton (CC_NI) and each maximum temperature (°C).

<table>
<thead>
<tr>
<th></th>
<th>CC_NI_31</th>
<th>CC_NI_32</th>
<th>CC_NI_33</th>
<th>CC_NI_34</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Clavicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humerus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius</td>
<td>1000 (P)</td>
<td>800 (M)</td>
<td>900 (P)</td>
<td></td>
</tr>
<tr>
<td>Ulna</td>
<td>900 (M)</td>
<td>800 (L)</td>
<td>1000 (A)</td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td>900 (P)</td>
<td></td>
<td>900 (A)</td>
<td>1000 (A)</td>
</tr>
<tr>
<td>Tibia</td>
<td>1000 (P)</td>
<td>1000 (A)</td>
<td>800 (P)</td>
<td></td>
</tr>
<tr>
<td>Fibula</td>
<td></td>
<td>800 (M)</td>
<td></td>
<td>900 (A)</td>
</tr>
</tbody>
</table>

Key: S: bone placed on its superior surface; I: bone placed on its inferior surface; P: bone placed on its posterior surface; A: bone placed on its anterior surface; M: bone placed on its mesial surface; L: bone placed on its lateral surface

Bones presented no visible relevant pathologies and post-depositional taphonomic alterations that could theoretically interfere with heat-induced warping such as osteoporosis, bone bending or fractures on the diaphyses, both antemortem and perimortem. Bones were then submitted to controlled burning in a three-phased electric muffle Barracha K-3, up to different maximum temperatures (800, 900, 1000° C) for 120 min. These maximum temperatures were chosen to make sure that burning conditions were met to favour warping. Exposure to higher temperatures is more frequently associated to bone warping.\textsuperscript{13} Probably, the 800° C temperature would be enough to assure that bones presented warping. However, the samples were also used in other parallel investigations thus explaining why three temperatures were taken into consideration for this paper. Bones were placed inside the muffle according to random orientations and elevations. The latter was achieved by elevating one or both ends to random heights up to 7 cm to intentionally favour the direction of warping according to gravity since the gap between the bone and the basis gave it room to deform (Figure 1). Once burned, the diaphyses of long bones were analysed macroscopically to check for warping and if its
occurrence and direction were influenced by the gravity force. If warping manifested itself downwards, according to gravitational force pulling towards the centre of the Earth, it was scored as being compatible with gravity. If warping manifested itself any other direction, it was scored as being incompatible with gravity. The type of bone (clavicle, humerus, radius, ulna, femur, tibia, and fibula) was also recorded to assess if this had a possible effect on the occurrence of heat-induced warping. However, no inferential statistics were carried out in this paper due to the small sample size.

![Figure 1](image.jpg)

**Figure 1.** Position of some of the bones in the muffle before burning. Gravity-compatible warping was intentionally favored by elevating one or both ends of each long bone.

## Results

Heat-induced warping compatible with gravity was observed on 9 bones (1 radius, 3 femora, and 5 tibiae) while 7 bones (1 radius, 3 ulnae, and 3 fibulae) presented warping incompatible with gravity (Table 2). The three clavicles used in this pilot study presented no visible warping while no reliable inferences about this heat-induced change could be made for the remaining four bones (2 humeri, 1 radius, and 1 ulna). Although warping was observable, these four bones fractured during heating and were therefore displaced from their original position. As a result, we were unable to assess if warping was compatible with gravity or not. Given that only 16 warped bones were evaluated, only about 56% of the bones presented heat-induced warping compatible with the influence of Earth’s gravity force. Bone’s orientation appeared to have no effect on warping. Both gravity-compatible and gravity-incompatible warping were observed for all types of orientation. The only exception was the lateral orientation for which only gravity-incompatible warping was recorded.

## Discussion

Although bones were positioned in the muffle to intentionally favour warping according to the gravitational force, many of them presented warping incompatible with gravity. When a force is exerted on an object, it is dependent on the mass of that object, as well as, in this case, on the Earth’s gravity force \((\text{force} = \text{mass} \times \text{acceleration}; \text{weight} = \text{mass} \times \text{gravity})\). Therefore,
when a bone bends, it should do it towards the centre of the Earth due to its weight. Since that was not the case for several bones in our sample, results seem to suggest that the gravity force is not a factor for heat-induced warping. However, we believe that it is and that another force must have counteracted the gravitational force in some cases.

Since gravity was predictably always the same, the force exerted on bones was dependent of bone mass. This means that bones subjected to weaker gravitational forces may have been unable to warp towards the Earth’s centre and instead warp under the influence of a second force. An interesting trend regarding the differences between gravity-compatible warped bones and gravity-incompatible warped bones was found. The first group was composed of heavier bones such as the femur and tibia (although one radius was also included) while the second group was composed of lighter bones such as the radius, ulna, and fibula. The quotient of the division between the mass and the maximum length of each bone was calculated to give information about the mass in function of each mm, and confirms that conclusion (Table 2). Bones presenting gravity-compatible warping had a mean quotient of 0.52 g per mm while bones presenting gravity-incompatible warping had a mean quotient of 0.12 g per mm. The resulting quotients strongly suggest an association between bone mass and its effect on warping. In lighter bones, weight - as a result of gravity - was apparently not strong enough to counteract a second force that participated in warping. This second force may be intrinsic to the bone and probably is either the contraction force or the contractile force of heated collagen.\(^{14}\) Gonçalves et al.\(^{11}\) proposed that this force had a role in heat-induced warping. Our results partly confirm that thesis but also support the idea that warping is the product of different kinds of forces. As a side note, quotients are probably not the most exact way of assessing proportional mass since the calculation of mass in function of volume would be more reliable. However, we only detected this possible relationship between mass and warping after the experiment was completed thus preventing us from assessing bone volume before the burning. Instead, we had to rely on parameters such as mass and length of the unburned bones, which had fortunately been recorded.

**Table 2.** Quotient of mass/maximum length of each bone and type of warping (GC = gravity-compatible; GI = gravity incompatible). Only bones displaying warping and allowing reliable inferences regarding type of warping are here included.

<table>
<thead>
<tr>
<th>Bone</th>
<th>Mass (g)</th>
<th>Maximum length (mm)</th>
<th>g/mm</th>
<th>Type of warping</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC_NI_31 left radius</td>
<td>44.55</td>
<td>233</td>
<td>0.19</td>
<td>GI</td>
</tr>
<tr>
<td>CC_NI_31 right radius</td>
<td>46.60</td>
<td>232</td>
<td>0.20</td>
<td>GC</td>
</tr>
<tr>
<td>CC_NI_31 left femur</td>
<td>352.21</td>
<td>434</td>
<td>0.81</td>
<td>GC</td>
</tr>
<tr>
<td>CC_NI_31 right tibia</td>
<td>217.27</td>
<td>362</td>
<td>0.60</td>
<td>GC</td>
</tr>
<tr>
<td>CC_NI_32 left ulna</td>
<td>55.67</td>
<td>275</td>
<td>0.20</td>
<td>GI</td>
</tr>
<tr>
<td>CC_NI_32 right ulna</td>
<td>52.62</td>
<td>281</td>
<td>0.19</td>
<td>GI</td>
</tr>
<tr>
<td>CC_NI_32 right femur</td>
<td>355.71</td>
<td>472</td>
<td>0.75</td>
<td>GC</td>
</tr>
</tbody>
</table>
The major conclusion of our results is that the contractile force of collagen may sometimes be countered by gravitational forces, especially in heavier bones. This may help explaining why bones with larger masses were more prone to present warping compatible with the Earth’s gravity force. In addition, it may help explaining why bones burned with soft tissues tend to warp more frequently than bones burned without soft tissues. The interaction of larger mass with larger collagen content and/or with the buffer effect of soft tissues that allow for more constant and warping-friendly temperatures\(^{20}\) may lead to fleshed bones being more prone to warping. Nonetheless, we should not forget that the right radius from CC_NI_31 was quite light and yet presented gravity-compatible warping. This can either mean that the intrinsic force and the gravity forces followed the same direction or that another unknown mechanism is at play here and that our hypothesis is not a valid one.

One curious outcome of our research was that all clavicles presented no heat-induced warping. Vassalo et al.\(^{13}\) also found that the occurrence of warping was much less frequent in clavicles than in other long bones. It was already known that bones mainly composed of compact bone are more affected by heat-induced warping than bones composed mostly of trabecular bone.\(^{11}\) This may be due to differences between the collagenous network of lamellar bone of the two types of bone structure. Although both present the same collagen structural organization based on the coexistence of ordered and disordered fibril arrays, the former is aligned with the long axis of the trabecula in trabecular bone while, in contrast, being aligned with the anatomical axis of the long bone in compact bone.\(^{21}\) As a result, trabecular and compact bone probably behave differently to mechanical stress caused by forces originating from the heating of collagen. However, such hypothesis does not explain why clavicles seem to display warping much less frequently than other long bones since all are structurally similar. The clear difference in shape between the clavicle and the other long bones leads us to hypothesize that bone shape may influence the occurrence of warping as well. Therefore, shape as a factor for heat-induced warping deserves future investigation. Other possible factors were either too superficially investigated or not addressed in this paper. For example, bone’s orientation did not seem to have an effect on warping. Even though we exclusively observed gravity-incompatible
warping on bones placed on their lateral surface, the sample (n = 2) was too small to allow for solid inferences.

This study was performed on a sample of dry bones and the burnings were experimentally controlled through the use of an electric muffle. Such conditions were useful to fulfil the objective of this paper, which was to assess the influence of the gravitational force on heat-induced warping. However, it would be convenient to assess if our results are replicated on a sample that includes soft tissues and exposed to different types of burnings since the latter may possibly lead to different outcomes. Also, our results were obtained on older adults and it is worth wondering how applicable they are to skeletons from younger individuals. Due to a negative correlation between age and skeletal mass, especially in females, different results could theoretically be obtained. Since younger people have heavier bones, collagen’s contractile force may be unable to counteract gravity’s force as often as in older people. Further research about this topic is also advisable.

The occurrence of heat-induced warping is multifactorial. Several factors have been proposed until now and comprise collagen content, burning dynamics including temperature and duration, age at death, sex, and bone type. The present investigation proposes other two factors, shape and weight, that allow us to better understand warping but add to the complexity of attempting to determine the pre-burning condition of human remains. This complexity explains why it has been so difficult to find one reliable feature to estimate this parameter on completely skeletonized and completely calcined remains. Probably, a method based on macroscopic bone features is impossible to find because mastering such complex multifactorial package may be impractical. The solution for determining the pre-burning condition of human remains possibly lies elsewhere, at the microscopic and elemental level of bone.

Acknowledgments

The authors thank Calil Makhoul for his aid with the experimental burnings. They also thank the reviewers for their valuable comments on a previous version of the paper.

Funding

This research was funded by the Fundação para a Ciência e Tecnologia (SFRH/BPD/84268/2012; SFRH/BPD/110710/2015; PTDC/IVC-ANT/1201/2014).

References


