Mechanical behavior of human bones with different saturation levels

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Abstract: Human bone is one of the most common connective tissue of biological human structure. In   relation to the internal microstructure there are two main types of bone tissue: compact in the cortical zone and spongy or trabecular in the internal zone. The porous structure in general is side for the marrow. Considering the relevant function of that tissue, the porosity is not uniform. Porous diameter increase from the cortical to the centre of bones, as the connections of porous increasing with the thickness of the bone tissue.The presence of serum inside the porous structure of bone tissue produce a different behaviour in bones below loads, and related with the condition of the load is applied. The response of material is different in relation at the level of serum inside the tissue and in relation of the load action direction. In same stress condition the velocity of loading generate different response related with the dimensions of porous and permeability parameters.In this work, three different type of bone tissue are investigated. From Calcaneus, from skull and form rib of human skeletal system. The specimens are subjected at compression test in, displacement control, until they reach the ultimate stress, in dry and wet condition. It is observed that level of serum.A 3 groups (one for each tissue type) of 20 specimens each are tested in dry and wet condition. maximum stress, strain, elastic deformation energy, total deformation energy, are measured. statistical analysis is conducted and qualitative relationship are deducted in reference to the density and specific mechanical characteristics.The tests show compact tissue as skull are more appropriate to perform load action, instead calcaneus work as reticular structure with high deformation levels.A single paragraph of about 200 words maximum. For research articles; abstracts should give a pertinent overview of the work. We strongly encourage authors to use the following style of structured abstracts; but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied; 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

Keywords: porous tissue, bones, skul, rib, resistance, energy

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1. Introduction

"Bone" is an ambiguous term since it can be used to refer to both the organ (individual skeletal segments, such as humerus, vertebrae, etc.) and the tissue (bone tissue). In this article, we will refer to skeletal segments using their names, while the term "bone tissue" or "bone" will be used to refer to the tissue.

There are two types of bone tissue: compact (cortical or compact) and spongy (cancellous, trabecular or spongy). In some research works [3,4,11], cortical and cancellous bone have been

considered to be a single morphological material, characterized by a highly variable porosity or apparent density (mass/total volume, including pores). Other researchers [5,6,23] consider these two types to consist of two different materials. In general, cancellous bone material is much more active metabolically and it is remodeled more often than cortical bone. It can be considered composed of an interconnected network of rods and plates. This structure of rods produces low-density, open cells while a network of the plates gives higher-density, virtually closed cells. Practically, the relative density of cancellous bone varies from 0.05 to 0.7 (hence any bone with a relative density less than 0.7 can be classified as "cancellous" [12].

In contrast, the microstructure of compact bone is composed of regular, cylindrically shaped lamellae, forming closely packed osteons.

Skeletal segments can be classified according to their morphological and structural characteristics. The most common types of bones are three: long, flat and short bones. Other types are classified as irregular, sesamoid, pneumatic and sutural bones. Long bones are found only in the limbs, and were not included in this study. Flat and short bones share a similar structure: briefly, the inner part consists of cancellous bone, which is covered with a layer of compact bone. The spaces between the trabeculae of the cancellous bone contain bone marrow, the hematopoietic organ consisting of a mix of water, filamentous and globular proteins, and cells. The proportions of these components vary depending on the location of the skeletal segment and the age of the subject.

In general, the mechanical properties of a whole bone depend on volume fraction, architecture, and trabecular tissue material properties [2] but itis also important to bear in mind that a crucial role is played by their interaction with a fluid phase. In particular, several studies have demonstrated that some mechanical properties (stiffness, tensile strength, hardness) increase with dehydration, whereas the strain at fracture and energy to fracture decrease [8,9,26,27,31]. Moreover, dehydration also influences the viscoelastic properties of bone. In fact, if compared with wet bone, dry bone shows a reduced anelastic deformation [7], and a much higher relaxation rate [25].

Another parameter that must be considered in this context is which part of skeleton has been studied, because skeletal segments are subjected to different types of stresses, including bending, tension and compression. For this reason, bone properties change in relation to the location and the function that must be performed. Other factors to consider are how specimens are preserved prior to testing (fresh/embalmed/remoistened), the type of test performed (bending/compression/tension), the testing speed (quasi-static/dynamic) and the observation scale. For example, it has been found [6] that the elastic modulus of cortical bone obtained from micro-bending specimens is considerably smaller than that of large tensile specimens tested by others [20] (5.4 against 17.1 GPa). Moreover, a literature review of measured and estimated values of the modulus of trabecular bone material [21,1,30,24,28,29,13,15,18] shows that moduli values range from 1 to 20 GPa.

In the present study, three skeletal segments with different biomechanical characteristics, parietal bone, rib bone and calcaneus, were used. The parietal bone is part of a rigid vessel, the skull, which must provide protection against external forces (shock or trauma); the rib bone is a part of an elastic enclosure, the rib cage, which must be able to expand and retract during breathing. Rib bones must also be able to withstand external forces (shock or trauma). Calcaneus, or heel bone, is a part of the rear portion of the foot, or tarsus, constituted by a cluster of bones which must support the weight of the body and transmit it to the rest of the foot. In particular, the calcaneus must support the weight of the body during stasis, as well as withstand forces (shock or trauma) while walking.

A study on the mechanical properties of embalmed skull compact bone [17] demonstrated that skull bone shows a static elastic modulus on the average of 103.42 MPa. In another paper [10], the average ultimate compressive strength of human parietal bone was found to be close to 0.15 MPa, i.e. an order of magnitude less. This means that minor changes in the sampling area (without considering condition, age of donor and fluid percentage) lead to great differences in the detected mechanical properties.

Regarding the calcaneus and a series of past studies, it is worthwhile to mention one article [32], in which fresh-frozen cadaver specimens and embalmed cadaver specimens were compared with cyclic loading (preload 20 N, load was increased every 100 cycles by 100 N from 1,000 to 2,500 N, 0.5

mm/s) and load to failure (0.5 mm/s) testing, where different specimen groups showed different stability and different displacement characteristics in the primary loading direction during cyclic loading.

To the best of our knowledge, no previous studies are available about the compressive properties of rib bones, probably because the mechanical stresses to which they are subjected are mainly flexural.

However, in thelatter case, a significant relationship has been found between the age and elastic modulus of the ribs [19]. In such a study, two age groups, i.e., between the ages of 10 and 15 and between the ages of 16 and 22, were analyzed, and statistically significant differences were obtained for Young's modulus (p = 0.0001) amounting to, respectively, 2.79 ± 1.34 GPa for the first group and 7.44 ± 2.85 GPa for the second group. These results show a significant impact of age on the mechanical properties of ribs.

Aim of this paper is to compare the mechanical characteristics of three kind of human bones (calcaneus, skull and rib) with and without the presence of human serum.

In order to achieve this goal, several dry specimens from a same skeleton have been subjected to ultimate compressive test. In the second series of the specimen obtained from the same skeleton, have been saturated by human serum and the compressive tests have been carried out.

Results show that the fluid phase strongly influences the ultimate stress of the bones. The ductility of the wet bones shows a significant increase as well as the energy of fracture. It is to be remarked that each kind of bone show a very specific behaviour depending of the architectural structure of bones.

2. Material and methods

In order to study how the fluid phase affects the mechanical behavior of three types of skeletal segments, two main sets were considered: the first consisting of fully dried bone specimens and the other composed of specimens remoistened with human serum. A comparison between the fully dried and remoistened specimens is reported below. Furthermore, considering that mechanical tests on biological materials, such as bones, rarely show a consistent behavior, a comparison between fully dried and remoistened specimens was carried out on adjacent samples (i.e. obtained from consecutive zones of bone tissue).

2.1. Sample characteristics

Samples from a single human skeleton were obtained from a local anatomic donation program. The bones originated from a middle-age male donor. Three different skeletal segments were chosen: parietal bone, rib and calcaneus.

The human skull bones have a well-defined shell of compact bone separated by a core of spongy cancellous bone. The thickness of the spongy core increases toward the center of the bone away from the sutures. The thickness and initial curvature of the specimens could not be assessed, but care was taken to extract specimens with the least evident curvature. With regards to the rib bone, it should be noted that the specimens were obtained from a single rib (the sixth), from the head to the costo-chondral joint. As for the sampling criteria used for the parietal bone, the specimens obtained were sectioned from adjacent areas. Finally, samples of calcaneus were obtained from the trabecular network of a single bone, avoiding, as much as possible, areas near the compact bone. The same sampling criteria were used in this case.
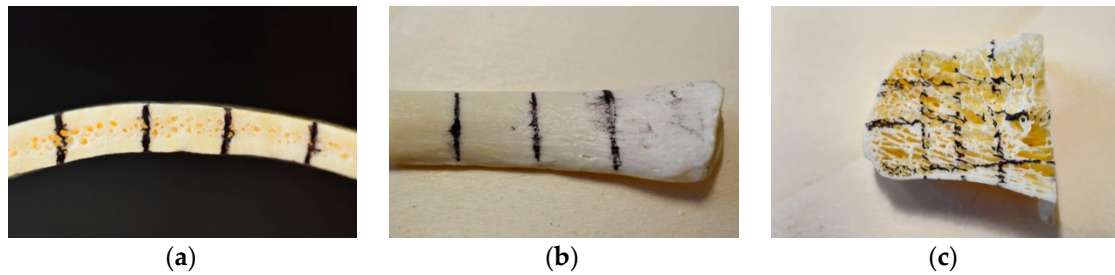
Figure 1. Sampling zones for each bone: (**a**)skull,(**b**) rib and (**c**) calcaneus.

All material was thoroughly examined in order to ensure there was no deterioration due to pathological conditions. Subsequently, specimens were harvested from each region using a low-speed diamond blade saw. All harvesting was performed by one person and standard visual guidelines were used. The specimens from the skull and calcaneus were extracted as cuboid sections (8-11 mm/side), while the rib samples were obtained cutting the whole bone into identical portions (i.e. the distance along the main axis of rib is the same, and in the range of 9-11 mm). The cross section of rib samples were considered as oval, in order to simplify calculations.

2.2. Relative density

Before mechanical testing, all specimens were dehydrated by drying them for 24 h in an oven at 103 °C, in order to remove remaining moisture. A total of sixty specimens were subjected to drying (20 specimens from each skull, rib and calcaneus) and subsequently divided into two groups:

- the first group (thirty specimens, ten for each bone) was weighed immediately after drying, and the volume of each specimen was evaluated to obtain an average value of the relative density ($\rho_{\text{fullydried}}$) of fully dried specimens. The specimens were then subjected to a compressive test, with the aim of characterizing the mechanical compressive properties of dry bones;

- the second group (also thirty specimens, ten for each bone) was weighed after drying and the relative density was calculated (ρ^*). Shortly after, these specimens were immersed in human serum until weight stabilization. This procedure was performed in order to evaluate the weight gain and the relative density of remoistened specimens ($\rho_{\text{remoistened}}$). At the end, compressive tests were carried out, evaluating the mechanical properties of remoistened bones.

The measure of dentity is operated applying the Archimedes Method.

Error! Reference source not found. reports the mean values and the standard deviation of the fully dried specimens in it is reported the mean of relative density (and standard deviation) for the second set of specimens (i.e. dry and remoistened with human serum). It is important to note that a comparison could be made only for the specimens in the second group.

Comparing values obtained for specimens of the second group, it is clear that immersion in human serum (ρ =1.022g/ml) produced an increase in bulk density. In particular, an increase of 9.8 %, 10.1 % and 37.9 % were observed in specimens from skull, rib and calcaneus, respectively.

Table 1. Relative density of fully, dried and dried and remoistened specimens.

Source	Relative densities (g/cm³)					
	$\rho_{\text{fullydried}}$		ρ^*		$\rho_{\text{remoistened}}$	
	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Skull	1,394	0,146	1,225	0,148	1,341	0,109
Rib	0,849	0,036	0,807	0,051	0,889	0,049
Calcaneus	0,231	0,082	0,253	0,068	0,335	0,026

2.3. Compressive test

Uniaxial compression tests were performed using a Zwick-Roell Universal Testing Machine (UTM) equipped with a load cell of 5 kN. A total of forty specimens (twenty from each skull, rib and calcaneus) were tested in compressive configurations. Two main conditions (i.e. dried or remoistened with human serum) were investigated. In particular, tests on cubic (skull and calcaneus) specimens and on elliptical cylinder (rib) specimens were performed, at a displacement speed of 1.5 mm/min.

The measures engineering stress and strain, denoted in σ and ε, respectively, are determined with the measured load and axial deformation using the original specimen cross-sectional area A_0 and length l_0 as follows:

$$\sigma = N/A_0 \tag{1}$$

$$\varepsilon = \Delta l/l_0 \tag{2}$$

Prior to presenting the data and the graphs obtained from calculations, it is to specify which engineering magnitudes have been considered. In Figure 1 is reported a stress-strain graph, in particular, this is obtained by averaging the curves obtained from data of wet ribs, with the only aim of highlighting the mechanical characteristics considered.
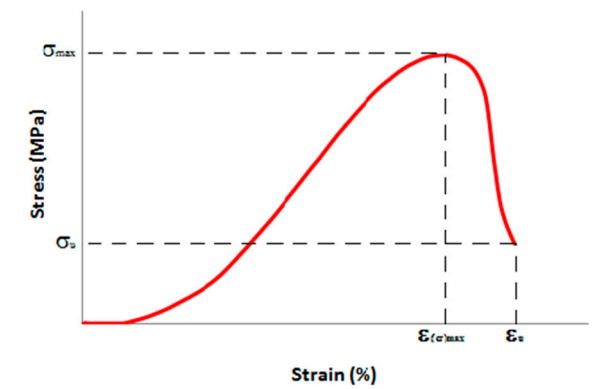

Figure 1. Generic stress-strain graph in which are highlighted the mechanical parameters considered

From the curves, as shown in Figure 1, ultimate stress (ultimate force/cross-sectional area of test cube/cylinder specimen) σ_u (MPa), the corresponding ultimate strain ε_u(%), maximum stress σ_{max} (MPa) and the corresponding strain $\varepsilon_{(\sigma)max}$ (%) have been evaluated. Moreover, the Young modulus E (GPa) and energy absorption at failure U(Jm^{-3}) were calculated. In particular, the Young modulus was determined by the linear portion of the stress-strain curve. Finally the energy absorbed was measured considering the area below the curve at maximum stress and at ultimate stress, as reported below, respectively:

$$U, (\sigma)_{max} = \int_0^{\epsilon_{(\sigma)max}} \sigma d\epsilon \tag{3}$$

$$U, ultimate = \int_0^{\epsilon_u} \sigma d\epsilon \tag{4}$$

For specimens with serum, in order to avoid the loss of serum during the test, the specimens were hydrated throughout the testing process by keeping them wrapped in a parafilm sheet, a semi-transparent, flexible thermoplastic material, since it is highly waterproof and resistant.

3. Results

In this section the results of compression tests performed on the above specified specimens are reported. In fig. 3, 4 and 5 the average curve in terms of stress-strain are reported for wet and dry conditions for rib, skull and calcaneus, respectively.

In table 3, the mechanical properties for the three different kind of bones (dry and wet) in terms of maximum stress (σ_{max}) and corresponding strain $\varepsilon_{(\sigma)max}$), ultimate stress ($\sigma_u$) and corresponding ultimate strain (ε_u) are reported., the energy absorbed at maximum stress and at failure (i.e. at ultimate stress) are reported.

3.1 Consideration about mechanical behaviour of the dry skull

The transverse section of the human skull bone shows thin plates of periosteum-covered compact bone on the outside with endosteum-covered spongy bone on the inside. This architecture provides a very stiff, lightweight structure which is capable of carrying external loads (such as impact and crush). The cortical part of the bones provide bending and shear strength to the whole structure, whereas the inner core provides space for intercranial channels as well as a lightweight, energy absorbing cancellous bone core, much like engineered sandwich panels (Figure 2) as in architectural building skins or in the aircraft wing structures (Leestma, 2015).
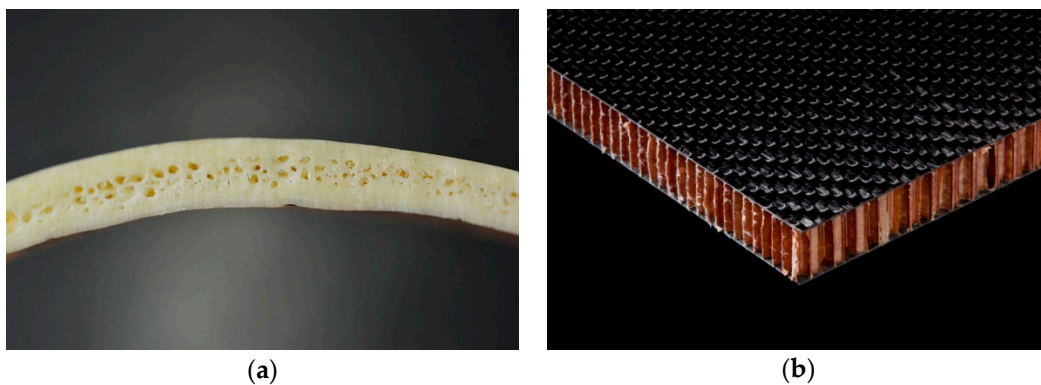

(**a**)   (**b**)

Figure 2. Comparison between a sectioned skull bone (a) and a common sandwich panel (b)

In addition, it is to consider that the curved sandwich structure of human skull bone leads to a distribution of load across the bone, carrying the load by the bone to its margins, where the load is shared and transmitted to the other skull bones through the cranial sutures.

In view of these consideration, it is clear that this bone can store an high amount of energy and this is evident considering Figure 3, in that is shown the average compressive stress-strain curve for specimens tested in this work. Hence, this astonishing structure, thanks to its architecture and curvature, allows to protect the entire skull against any kind of shock or trauma.
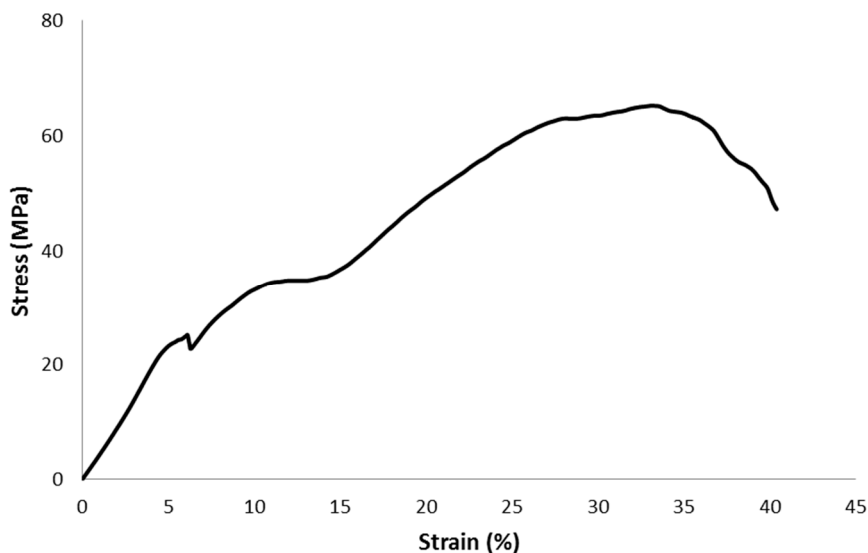
Figure 3. Average compressive stress-strain curve for skull specimens in dry state.

3.2. Consideration about the mechanical behaviour of the dry calcaneus

In the morphology of a human heel bone (i.e. calcaneus), it is immediately recognizable that it can be assimilated to an open-cell foam (in Figure 4 a qualitative comparison between structure of human heel bone and an open cell foam is reported). In fact, open-cell foams shows a complex microstructure that consists of a series of interconnected network of ligaments that form along the edges of randomly packed cells that evolve during the foaming process (Jang, Kyriakides, Kraynik, 2010).

(**a**)   (**b**)
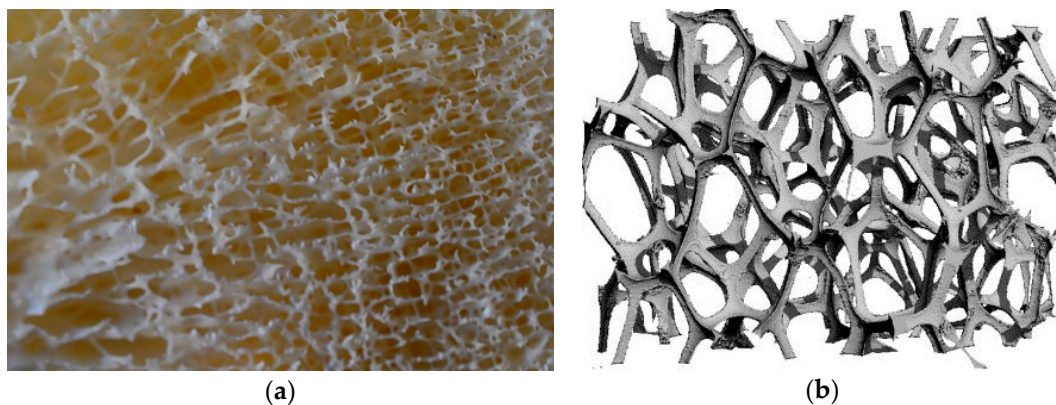
Figure 4. Comparison between a sectioned heel bone (a) and a common open cell foam (b).

Obviously, a load applied on that structure produces a series of collapse in the ligaments, this term does not relates to the fibrous connective tissue that connects bones to other bones but is referred to the thin rods that form the whole structure of the bone/foam. In that process, it can be mentioned that cell size, anisotropy, ligament (or rod) length distribution highly influence the response in terms of mechanical behaviour. What is certain is that the bone as well as any open-cells foam shows a stress-strain curve characterized by three main zone. At the beginning, when the compression is applied, the load detected by the cell begins to increase in a linear manner with respect to the displacement. The material becomes deformed in a linear elastic range (this corresponds to a bending of the inner ligaments/ribs), in that the energy can be recovered by unloading.
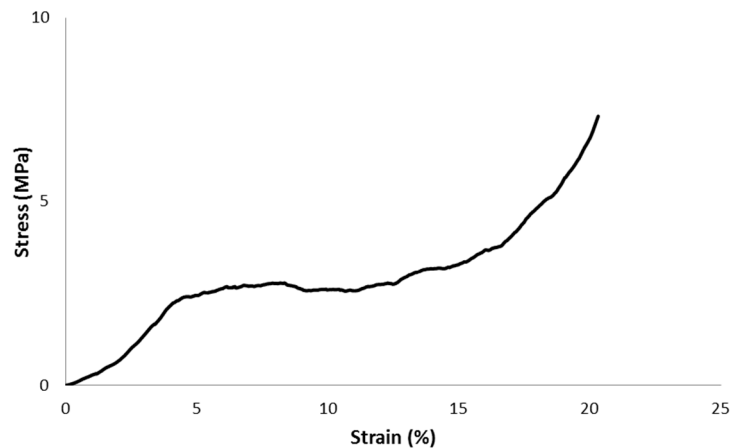
Figure 5. Average compressive stress-strain curve for calcaneus specimens in dry state.

After this elastic phase, the load remains almost constant and the deformation continues to grow (plateau zone), storing a high amount of energy. For the case of calcaneus, this is due to the fact that it must support the weight of the body during stasis, as well as withstand forces (shock or trauma) while walking.

Finally, the curve exhibits a rapid growth of the stress, as a direct consequence of the complete compaction of the cells. In that phase, the response to compression is that of the solid matrix, without any void. The deformation returns to be homogeneous and there remains no trace of those localized.

3.3 Consideration about the mechanical behaviour of the dry rib

The first function of the rib cage is to protect the vital organs that lie within the protective enclave of the ribs but rib cage also helps breathing by the function of the intercostal muscles lifting and lowering the rib cage, aiding inhalation and exhalation. For these reason, it is clear that every single rib must be strong but also able to dissipate the energy caused by an impact or shock.

Through the observation of the cross section of a human rib (Figure 6), in that is recognizable the outer stiffer bone enclosing a complex trabecular structure, appears clear that this bone provide for the duplex function discussed above.

Figure 6. Cross section of a human rib.

Moreover, the average compressive stress-strain curve of a series of dry rib specimens shows that mechanical behaviour of human dry rib can be considered as a mixture of those obtained for skull and calcaneus (Figure 8) and, although the whole response to compressive stress is dominated

by the trabecular compliance, it can be expected to be stiffer than pure trabecular bone because of the presence of stiffer cortical bone, as it is, in fact.
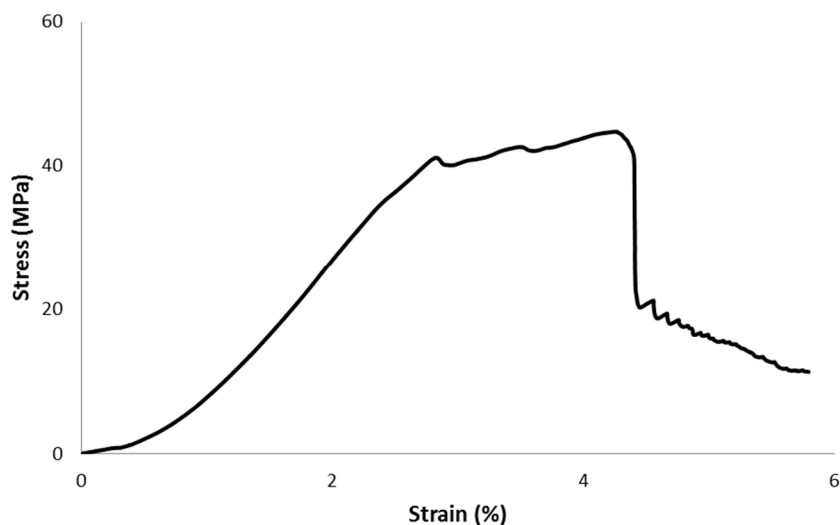

Figure 7. Average compressive stress-strain curve for rib specimens in dry state.

4   Comparison

After a first phase in that the mechanical behaviour of three skeletal segments have been clarified, in this section a comparison between dry and remoistening with human serum specimens is reported. Considering **Error! Reference source not found.** in which the average curves of human skull bone (dry and remoistened) are reported, the effect of the remoistening is evident in terms of improvement of maximum stress and, consequently, energy stored (the curve is higher and then the absorbed energy). The ultimate strain is approximately the same for the dry and wet specimens and the fluid does not affect that characteristic.
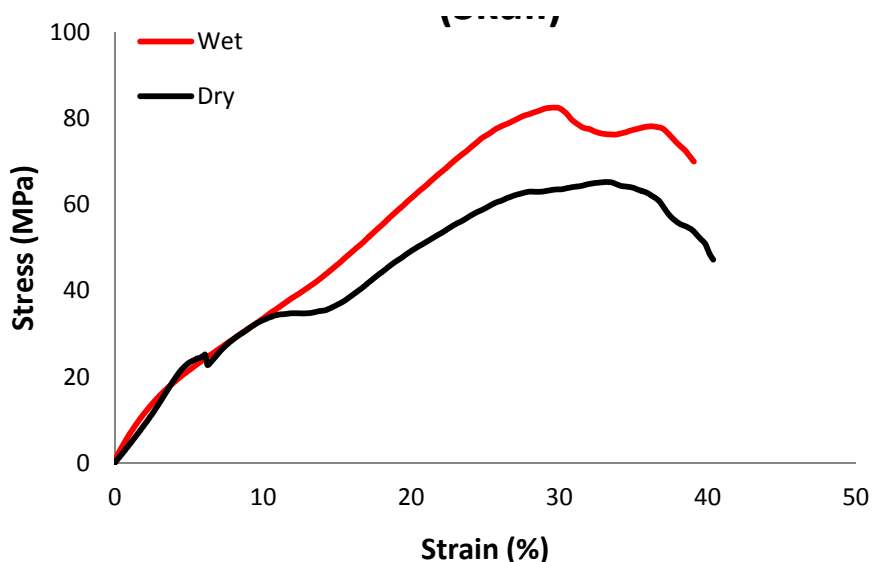

Figure 8. Average stress-strain curves for skull specimens for the different conditions (dry and wet).

Absolutely different is the effect caused by fluid considering human heel bone. In fact, as shown in **Error! Reference source not found.**, specimens of calcaneus embedded in human serum exhibit a higher ultimate compressive strain and a plateau phase more evident than those found for dry specimens.
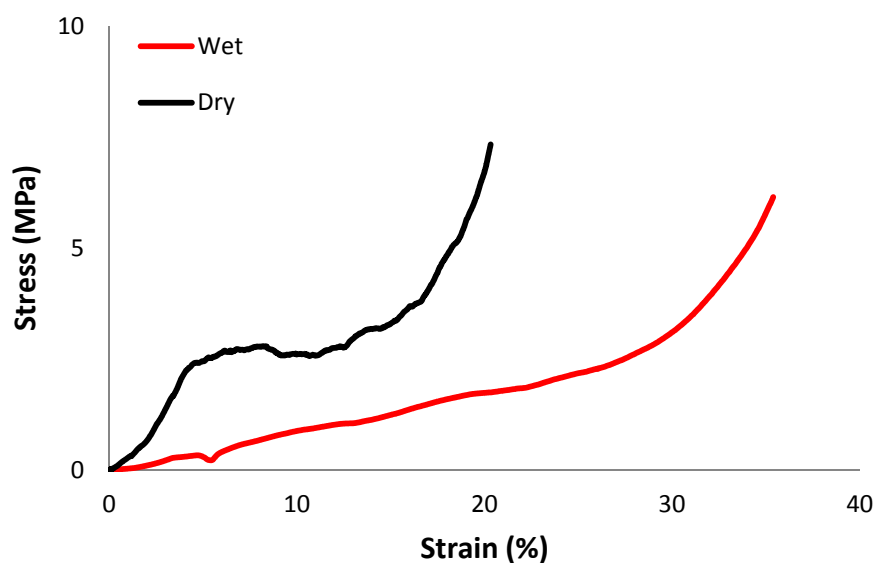
Figure 9. Average stress-strain curves for calcaneus specimens for the different conditions (dry and wet).

Furthermore, the effect of the fluid phase are more evident in the case of human rib bone. All mechanical features are improved (ultimate stress, ultimate strain and amount of energy absorbed) and the general behaviour is different from those obtained for the specimens in dry state.
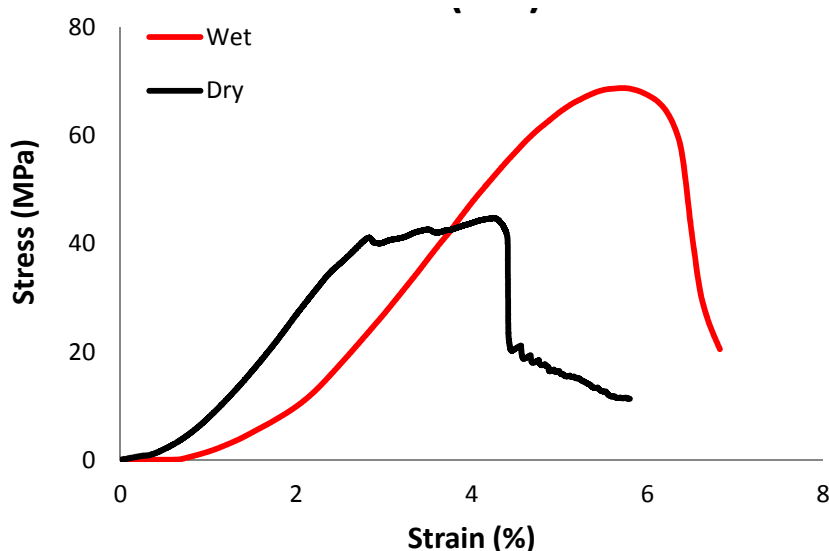

Figure 10. Average stress-strain curves for rib specimens for the different conditions (dry and wet).

To better understand how fluid phase affects the mechanical behaviour of these three skeletal segment (that show different features in terms of morphology and function), all these consideration regarding the mechanical properties are reported in the **Error! Reference source not found.**.

Mechanical properties	Units	Rib		Skull		Calcaneus	
		Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Maximum Stress (σ_{max})	[MPa]	44,73	68,75	65,20	82,49	7,33	7,23
Maximum Strain ($\varepsilon_{(\sigma)max}$)	[%]	4,25	5,71	33,24	29,68	20,35	36,45
Ultimate Stress (ε_{u})	[MPa]	11,43	22,40	50,75	72,86	*	*

Ultimate Strain (σ_u)	[%]	5,80	6,82	40,39	39,08	*	*
Energy absorption at maximum stress	[kJm-3]	27,41	58,46	767,67	1007,32	83,74	111,70
Energy absorption at ultimate stress	[kJm-3]	32,73	69,77	953,38	1359,68	*	*

Table 2. Mechanical properties (average) of the properties measured on dry and wet specimens.

From the stress-strain curves and from the synthetic results reported in table 3, some consideration may be withdrawn:

For all the specimens the mechanical properties strongly depend on the presence or absence of the serum;

The total energy absorption at failure increase for the presence of the serum from 34% (calcaneus) up to 115% for the rib;

The ultimate stress for the skull and rib increases, from dry to wet of 53% and 26%, respectively and remain almost equal for the calcaneus. This is why the calcaneus behaves like a foam. As it may be observed, the typical behavior of cellular materials is due to the fact that, when the load increase, a densification of the foam is observed, that is all the structure of the calcaneus collapse and the voids practically disappear.

5. Conclusions

Experimental test performed on three different skeletal segments with different mechanical characteristics in presence of human serum or not. The three skeletal segments under study was skull, rib and calcaneus. Each of them have been tested in dry conditions and then remoistened with human serum. The average stress-strain curves in the two different conditions allow us to conclude that maximum stress, maximum strain as well as the energy absorption at maximum stress and at ultimate strain strongly depend of the presence of the serum.

Because each of three selected skeleton segments have different characteristic (sponge for calcaneus and compact for skull and rib), the increase of the mechanical characteristics is different for the three skeleton segments.

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