EFFECT OF MORPHOLOGICAL AND CLINICAL PARAMETERS ON DAMAGE ACCUMULATION IN PORCINE TRABECULAR BONE

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Osteoporosis is a metabolic disease of the bone that undergoes a reduction of its resistance due to the loss of its mass and to the micro-architectural deterioration. It follows that the risk of fracture increases, in other words the osteoporotic bone is weaker than a healthy one.

Despite its primary role in assessing bone strength, bone mineral density (BMD) accounts only for about 70% of fragility fractures, while the remaining 30% could be explained by other parameters, particularly of bone microarchitecture.

Porcine vertebrae are the subject matter of this research. In particular, only the trabecular bone, the inner part of the vertebrae, has been studied because the cortical bone, the compact layer that wraps around the spongy tissue, is less inclined to bone loss and it’s harder, stronger and stiffer. The porcine vertebrae were selected because they have a structure similar to those of humans.

The first aim of this work is to understand what bone morphology has a lower tendency to damage, induced experimentally by a compression test. The second aim is to analyze how an applied load changes the micro-architecture parameters of the bone.

A dual-energy X-ray absorptiometry (DXA) and a micro-computed tomography (μCT) were carried out to evaluate the initial morphology of the bone specimens. Subsequently the samples were compressed to induce damage and to evaluate their mechanical properties. Lastly a DXA and a μCT were performed on the damaged specimens.

From the analysis carried out it is verified that a bone with a low density has a higher tendency to damage than a denser one. The shape of the trabeculae resulted to be an index of predisposition to damage. Moreover, it is clear from the obtained results that the compression test has modified the bone's microarchitecture.

**Keywords:** porcine vertebra; trabecular bone microarchitecture; DXA; micro-CT imaging; damage accumulation; bone strength; bone densitometry.
L'osteoporosi è una malattia metabolica dell'osso il quale subisce una riduzione della resistenza a causa della perdita di massa ossea e del deterioramento microarchitettonico. Ne consegue un aumento del rischio di fratture, in altre parole l’osso osteoporotico è più debole di uno sano.

Nonostante il suo ruolo fondamentale nella valutazione della resistenza ossea, la densità minerale ossea (BMD) spiega solo il 70% delle fratture fragili, mentre il restante 30% potrebbe essere spiegato con altri parametri, in particolare quelli relativi alla microarchitettura ossea.

Le vertebre suine sono l’oggetto di questa ricerca. In particolare, solo l’osso trabecolare, parte interna delle vertebre, è stato studiato in quanto l’osso corticale, strato compatto che avvolge il tessuto spugnoso, è meno incline a una perdita ossea ed è più duro, resistente e rigido. Le vertebre di maiale sono state scelte perché hanno una struttura simile a quelle dell’uomo.

Il primo obiettivo di questo lavoro è quello di capire quale morfologia ossea ha una minore propensione al danneggiamento, indotto sperimentalmente attraverso una prova di compressione. Il secondo obiettivo è quello di analizzare come un carico applicato cambia i parametri di micro-architettura del tessuto osseo.

Una mineralometria ossea computerizzata (MOC) e una microtomografia computerizzata (μCT) sono state svolte per valutare la morfologia iniziale dei provini di osso. In seguito i provini sono stati compressi per indurre un danneggiamento e per valutare inoltre le loro proprietà meccaniche. Da ultimo si sono effettuate nuovamente una MOC e una μCT sui provini danneggiati.

Dalle analisi svolte si è verificato che un osso con una bassa densità risulta più propenso al danneggiamento rispetto ad uno più denso. La forma delle trabecole è risultata essere un indice per la predisposizione al danneggiamento. Inoltre si evince dai risultati ottenuti che la prova di compressione ha modificato la microarchitettura dell’osso.

Questo tesi è stata strutturata in cinque capitoli. Nel primo si fornisce una breve descrizione dell’osso e dell’osteoporosi, motivando inoltre la ragione per cui si è deciso di usare ossa di maiale. Nel secondo si presentano le prove eseguite e i risultati numerici ottenuti. Nel terzo si mostrano alcune analisi preliminare, necessarie per svolgere le analisi, illustrate nel quarto capitolo, che hanno permesso di ottenere i risultati sopracitati. Infine nell’ultimo
capitolo si traggono le conclusioni proponendo i possibili futuri sviluppi del lavoro portato a termine.
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<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2y(h)$</td>
<td>Theoretical variogram [-]</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density [g/cm$^2$]</td>
</tr>
<tr>
<td>BS</td>
<td>Bone surface [mm$^2$]</td>
</tr>
<tr>
<td>BS/BV</td>
<td>Bone surface to bone volume [1/mm]</td>
</tr>
<tr>
<td>BS/TV</td>
<td>Bone surface to total volume [1/mm]</td>
</tr>
<tr>
<td>BV</td>
<td>Bone volume [mm$^3$]</td>
</tr>
<tr>
<td>BV/TV</td>
<td>Bone volume fraction [%]</td>
</tr>
<tr>
<td>D</td>
<td>Damage [-]</td>
</tr>
<tr>
<td>DA</td>
<td>Degree of anisotropy [-]</td>
</tr>
<tr>
<td>E</td>
<td>Residual elastic modulus [MPa]</td>
</tr>
<tr>
<td>$E[X]$</td>
<td>Expected value of variable X</td>
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<td>$E_{PD}$</td>
<td>Perfect-damage modulus [MPa]</td>
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<td>h</td>
<td>Lag vector [pixels]</td>
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<tr>
<td>M</td>
<td>Fabric tensor [-]</td>
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<tr>
<td>$m_i$</td>
<td>Positive eigenvalues of $M$ [-]</td>
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<td>$m_i$</td>
<td>Normalized eigenvectors associated to $m_i$ [-]</td>
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<tr>
<td>MIL</td>
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<tr>
<td>N(h)</td>
<td>Number of pairs separated by lag $h$ [-]</td>
</tr>
<tr>
<td>p</td>
<td>P-value [-]</td>
</tr>
<tr>
<td>r</td>
<td>Pearson correlation coefficient [-]</td>
</tr>
<tr>
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<td>Trabecular number [-]</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>Trabecular spacing [mm]</td>
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<td>Tb.Th</td>
<td>Trabecular thickness [mm]</td>
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<tr>
<td>TBS</td>
<td>Trabecular bone score [-]</td>
</tr>
<tr>
<td>TV</td>
<td>Total volume [mm$^3$]</td>
</tr>
<tr>
<td>U</td>
<td>Dissipation energy [MJ/m$^3$]</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>x</strong></td>
<td>Vector of spatial coordinates [pixels]</td>
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<td>Z(x)</td>
<td>Grey-level function at the coordinate x [-]</td>
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<td>$\varepsilon_p$</td>
<td>Plastic strain [%]</td>
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<td>$\varepsilon_r$</td>
<td>Residual strain [%]</td>
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<tr>
<td>$\varepsilon_t$</td>
<td>Total applied strain [%]</td>
</tr>
<tr>
<td>$\varepsilon_{ul}$</td>
<td>Ultimate strain [%]</td>
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<tr>
<td>$\varepsilon_y$</td>
<td>Yield strain [%]</td>
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<tr>
<td>$\sigma_{ult}$</td>
<td>Ultimate stress [MPa]</td>
</tr>
<tr>
<td>$\sigma_y$</td>
<td>Yield stress [MPa]</td>
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# LIST OF ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Adj MS</td>
<td>Adjusted mean squares</td>
</tr>
<tr>
<td>Adj SS</td>
<td>Adjusted sums of squares</td>
</tr>
<tr>
<td>AN</td>
<td>Animal</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>DF</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>EPD</td>
<td>End plate depth</td>
</tr>
<tr>
<td>EPW</td>
<td>End plate width</td>
</tr>
<tr>
<td>G1%</td>
<td>Group 1%</td>
</tr>
<tr>
<td>G2%</td>
<td>Group 2%</td>
</tr>
<tr>
<td>G3.5%</td>
<td>Group 3.5%</td>
</tr>
<tr>
<td>G5%</td>
<td>Group 5%</td>
</tr>
<tr>
<td>L</td>
<td>Vertebra</td>
</tr>
<tr>
<td>LS-means</td>
<td>Least squares means</td>
</tr>
<tr>
<td>NS</td>
<td>Not significant</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>VBH</td>
<td>Vertebral body height</td>
</tr>
<tr>
<td>μCT</td>
<td>Micro-computed tomography</td>
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CHAPTER 1
GENERAL CHARACTERISTICS OF BONE TISSUE

In this chapter, firstly it is reported a general yet essential description of the bones and of osteoporosis, its pathology. Starting from the classification of bones, particular attention is paid to the lumbar vertebrae, subject matter of this research. Lastly the human vertebrae are compared with the porcine ones, explaining why the pig’s vertebrae have been chosen - as it will be shown, they display similarities to the human ones.

1.1 Human lumbar vertebrae

The lumbar vertebra will be described in this paragraph, being subject of study in this thesis. In particular, its description is preceded by a general introduction about the human bones and the vertebral column, because the lumbar vertebrae are the bones that make up the third part of the spine.

1.1.1 General description of bones

Bones are organs of different shape and volume, of whitish or yellowish color and of solid consistency, characterized by high mechanical strength. Bones support and protect the various organs of the body, produce red and white blood cells, store minerals, provide structure and support for the body and enable mobility. It can be stated that from 25 years up to 30 years of age bones are 203, but this number undergoes changes according to age; the number of bones is higher in young people, where some bones are split into various pieces which will later merge.
into a single bone; the number of bones may decrease in older people due to the union of bones which are normally independent.

From the point of view of their general conformation, bones are divided into three groups: long bones, flat bones and short bones. Long bones are those in which one of the three dimensions, length, exceeds the other two (width, thickness). Long bones are subjected to most of the load during daily activities and they are crucial for skeletal mobility. Most of them are situated in the limbs and are divided into a body and two extremities. The body, called diaphysis, is mostly prismatic and triangular, sometimes irregularly cylindrical. The ends, called epiphysis, are generally more voluminous of the body, having one or more smooth surfaces to facilitate articulation with the adjacent bones. The long bones include the femora, tibiae, and fibulae of the legs; the humeri, radii, and ulnae of the arms.

*Figure 1.1 – Examples of the three different types of bones*
Flat bones are those in which the width and length prevail on the thickness. Their principal function is either that of extensive protection or the provision of broad surfaces for muscular attachment. There are flat bones in the skull and in the thoracic cage (sternum and ribs).

Short bones have their three dimensions, length, width and thickness, almost equal. Their primary function is to provide support and stability with little to no movement. Examples of these bones include the tarsals in the foot, the carpals in the hand and the vertebrae.

The bone tissue can be classified into cortical bone tissue and trabecular bone tissue. The cortical bone tissue consists of a compact layer apparently homogeneous. The trabecular bone tissue is formed by interconnections of trabeculae quite similar to a sponge. In long bones (Figure 1.2.a) the epiphyses are made almost exclusively of trabecular tissue, only the external layer is composed by a thin layer of cortical tissue. The diaphysis is basically composed by cortical tissue, which reaches its maximum thickness in the middle part of the bone, occupying however only the peripheral part; in the center it is located a longitudinal cavity in which the bone marrow is present. The bone marrow is a pulpy substance, which is found in all the cavities of the bone tissue, that is, both in the central canal of the long bones and in the cavities of the spongy tissue. Its function is to produce, together with other organs, the blood cells and the immune system cells. The flat bones (Figure 1.2.b) are constituted by two layers of compact tissue that cover the two faces of the bone, enclosing between them a layer of spongy tissue. In correspondence of the bone margins, the two laminae of cortical tissue merge, so that the spongy tissue is wrapped around by a continuous layer of compact tissue. The short bones (Figure 1.2.c) are, in their inner part, similar to the epiphyses of the long bones because they are composed by trabecular tissue, surrounded throughout by a thin layer of compact tissue.

Figure 1.2 – Internal conformation of long bone (a), flat bone (b) and short bone (c)

The bone tissue has its typical physical characteristic in the presence of inorganic substance combined with the organic substance. The organic substance is basically represented by proteins (osteoid and glycoprotein). The inorganic substance, that in the adult
accounts for about 60-70% of the total mass of the bone tissue, is represented by calcium phosphate (86%), calcium carbonate (12%), magnesium phosphate (1.5%), fluoride calcium (0.5%) and iron oxide (traces).

### 1.1.2 Structure of vertebral column

The vertebral column, also known as spine, consists of 33 vertebrae. Individual vertebrae are named according to their region and position. From top to bottom, the vertebrae are:

- **Cervical**: 7 vertebrae (C1 – C7)
- **Thoracic**: 12 vertebrae (T1 – T12)
- **Lumbar**: 5 vertebrae (L1 – L5)
- **Sacrum**: 5 (fused) vertebrae (S1 – S5)
- **Coccyx**: 4 (fused) vertebrae (Co1 – Co4)

The vertebral column provides support to the body, protection to the spinal cord and it allows, thanks to its joints, to move the head, to bend the body and extend it in the opposite direction and to rotate it.

![Overview of the distinct parts of the vertebral column](image)

*Figure 1.3 – Overview of the distinct parts of the vertebral column*

The adult vertebral column presents four anteroposterior curvatures: thoracic and sacral, both concave anteriorly (thoracic and sacral kyphosis), and cervical and lumbar, both concave posteriorly (cervical and lumbar lordosis). The transition between a curvature and another is
gradual with the exception of the one between lumbar lordosis and sacral kyphosis that is sudden and corresponds to the articulation between the fifth lumbar vertebra and the sacrum. In front view the spine has no curve, otherwise the person would be suffering from scoliosis.

Inside the spine is the spinal canal that is formed by the overlap of the vertebral holes, called vertebral foramen, in the individual vertebrae. The canal includes in its inside the spinal cord covered by the meninges.

### 1.1.3 Overview of human vertebrae

Vertebrae, regardless of the region they belong to, have common features. Vertebrae are short bones, mainly made by trabecular bone tissue covered by a thin layer of compact bone tissue. In the vertebrae it can be recognized a body and an arch that together delimit the vertebral foramen. The body is the voluminous part and it has a roughly cylindrical shape, with a central portion made of trabecular bone tissue and a peripheral ring made of cortical bone tissue.

The lumbar vertebrae are described in detail because they are subject of study in this thesis. The body of these vertebrae is big compared with the other ones and its size increases from L1 to L5. Moreover the body has a wedge shape, being thicker ventrally that dorsally.

![Figure 1.4 – Lumbar vertebra, superior view](image)
1.2 Bone disease: osteoporosis

Osteoporosis is a metabolic bone disease defined as a “disease characterized by low bone mass and micro architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk” [3]. Fractures are the clinical manifestations of osteoporosis, as bone strength reflects the integration of bone “quantity”. The most typical are fractures of the hip, spine and wrist, but almost all bones are susceptible. Each year in Italy around 100,000 wrist osteoporotic fractures and 65,000 femur osteoporotic fractures occur.

Bone mass increases during childhood and adolescence, peaks in the fourth decade of life and declines progressively thereafter. Adult females have less bone than men at all ages and are subjected to a bone loss during the first 5 years following menopause. Aging-associated losses approximate at 1% per year, but women in the postmenopause undergo an annual loss of 3% to 5%.

We can distinguish between two types of osteoporosis: primary osteoporosis and secondary osteoporosis. Primary osteoporosis is the more common form and is due to the typical age-related loss of bone from skeleton. Secondary osteoporosis has the same symptoms as primary osteoporosis, but it occurs as a result of having certain medical conditions, such as hyperthyroidism or leukemia.

Osteopenia is a condition in which bone mineral density is lower than normal, but is not as severe as osteoporosis. Osteopenia is not the condition which necessarily precedes osteoporosis. It is also a sign of normal aging, in contrast to osteoporosis which is present in pathological aging.

Dual energy X-ray absorptiometry (DXA) is a diagnostic tool for osteoporosis or osteopenia, able to determine the extent of bone loss and to intervene with appropriate treatments to prevent fractures. DXA examinations have three major roles, namely the diagnosis of osteoporosis, the assessment of patients’ risk of fracture and monitoring response to treatment. The DXA scan is able to measure the bone mineral density (BMD) of the scanned bone and a software then calculates the trabecular bone score (TBS) from the DXA images; TBS gives information on the bone micro-architecture, and two other parameters useful for the diagnosis of osteoporosis: T-score and Z-score. T-score and Z-score are statistical indexes computed in order to compare the measured BMD with the mean value of a healthy population. T-score is used in patients over the age of 30 and it is calculated by taking the difference between the patient’s measured BMD and the mean BMD in healthy young adults, matched for gender and ethnic group, and expressing the difference relative to the young adult population standard deviation (SD):

\[
T - \text{score} = \frac{\text{Measured BMD} - \text{Young adult mean BMD}}{\text{Young adult population SD}}
\]

Eq. (1.1)
Z-score is used to compare the patient’s BMD with the mean BMD of population with the same age, gender and ethnic of the patient:

\[ Z - \text{score} = \frac{\text{Measured BMD} - \text{Age matched mean BMD}}{\text{Age matched population SD}} \]  
\[ \text{Eq. (1.2)} \]

Z-score is used in patients under the age of 30, because it must be kept in mind that children have less bone mass than fully developed adults.

In the following table are reported the World Health Organization (WHO) definitions of osteopenia and osteoporosis, used to interpret spine DXA scan results.

<table>
<thead>
<tr>
<th>Normal bone density</th>
<th>T-score -1.0 or above</th>
<th>BMD not more than 1.0 SDs below young adult mean</th>
</tr>
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<tr>
<td>Osteopenia</td>
<td>T-score between -1.0 and -2.5</td>
<td>BMD between 1.0 and 2.5 SDs below young adult mean</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>T-score -2.5 or below</td>
<td>BMD 2.5 or more SDs below young adult mean</td>
</tr>
</tbody>
</table>

TBS is not a direct measurement of bone microarchitecture but it is related to the bone’s morphological characteristics. The definition of TBS is reported in paragraph 2.2. An elevated TBS appears to represent a strong, fracture-resistant microarchitecture, while a low TBS reflects weak, fracture-prone microarchitecture. As such, there is evidence that TBS can differentiate between two 3D microarchitectures that exhibit the same bone density, but different trabecular characteristics. For this reason, the measurement of BMD is supported by the use of TBS in better predicting fracture risk. Figure 1.5 (adapted from “A new bone structure assessment technique enhances identification of fracture risk”, by Medimaps Group, 2016) shows the microstructural difference between two lumbar vertebrae with similar BMD and different TBS.

BMD = 0.969 g/cm²; TBS = 1.457  
BMD = 0.967 g/cm²; TBS = 1.130  

Figure 1.5 – Illustration of a good (left) and poor (right) microstructures with similar BMD
Treatments for osteoporosis typically include education on nutrition, exercise (if there are no fractures) and medications. Firstly, education regarding the appropriate calcium and Vitamin D intake, as well as overall nutrition, is necessary. As appropriate, exercise and fitness are also important to help maintain bone density and reduce the risk of falls. There are a number of medications to treat osteoporosis and help reduce the risk of fractures. In general, these medications work by helping to strengthen the bones and prevent further bone loss.

1.3 Comparison of the complete human and porcine spine

In Italy the donation of the human cadaver for scientific purpose is a legal practice but practically unknown because citizens are not informed of the possibility of being able to donate their body. Therefore, the availability of fresh frozen human cadaver material is very limited, with the inevitable consequence of an increase in its cost. For these reasons the samples tested in this study are extracted from the lumbar section of vertebral columns excised from six young (one and a half years old) pigs provided from a local butcher. Among all animals, it has been chosen to use the porcine vertebrae, because the porcine spine is said to be the most representative model for the human spine.

The aim of this study is not intended to obtain knowledge regarding the health and welfare of animals, but the results emerging from this study are also indicative for a better understanding of the properties of human bones. This analogy between the porcine bones with those of humans has been verified from the dimensional point of view by two studies ([2] and [5]), whose main results are presented below.

The porcine spines have 7 cervical, 15 thoracic, and 6 lumbar vertebrae, in comparison to the human spines which had, respectively, 7, 12, and 5. In [2] it was found that the mean total spine length does not differ between the human and porcine spines. Therefore, the porcine spine minus the 4 extra vertebrae is shorter. The human spine shows more lumbar lordosis, measured as the angle between the upper end-plate of L1 and the lower end-plate of L5 or L6, respectively. Regarding the principal dimensions of the lumbar vertebrae, the porcine lumbar vertebrae results to have comparable vertebral body height (VBH), but lower end plate width (EPW) and depth (EPD). The conclusion of this study is that, taking scaling differences into account, it is believed that the porcine spine can be a representative anatomical model for the human spine in specific research questions. A limitation, however, of this study were the difference in age between the human specimens (varying from 55 to 84 years at time of death) and the porcine ones (4-month-old).

The study [5] confirms that the porcine vertebrae present lower end plate width and depth, but, contrary to what was found in [2], the vertebral body height results greater, because
the pigs used in this work were older (between 18 and 24 months old). In fact it has been found in [17] that spinal growth speed in pigs is high, from 120 mm/month between the 1st and 2nd months to 80 mm/month between the 3rd and 4th months. The spine (from T1 to L5) grew up from 300 mm (at 4 weeks of age) to 450–500 mm (at 4 months of age).

![Figure 1.6 – Anatomical dimensions of lumbar vertebra](image)

It is also interesting to discuss the similarities and the differences from a morphological point of view. The direction of the main trabecular struts in porcine lumbar vertebrae were parallel to the longitudinal axis of the spine, which is similar to that of human lumbar vertebrae. Pigs have a higher vertebral bone volume fraction, as demonstrated in [24] in which it was obtained that the thickness and the number of struts in the human femoral trabecular bone are very similar to the pig lumbar trabecular bone.

From a mechanical point of view, the typical load-deformation response was similar to that of trabecular bone in human spines. The mechanical properties are different, in fact in [24] it was found that the strength of porcine trabecular bone is greater. This is a direct consequence of the difference in porosity.

As conclusion, it can be stated that the ideal model for human spine does not exist. All models selected for spine research involve a compromise and the nature of these differences must be recognized and taken into account in both experimental designs and data interpretation.
In this study the results obtained with regard to the mechanical and morphological characteristics, measured for the main aim of this thesis, were also used to verify the analogy between human bones and porcine ones, confirming what was reported in other studies.
In the following chapter are reported the modalities of the samples preparation and the tests carried out in the following described order. Six different vertebral columns were provided from a local butcher. The full vertebrae were scanned with a dual-energy X-ray absorptiometry (DXA), measuring the density and the “quality” of the full vertebra. Forty porcine trabecular specimens were extracted from the lumbar vertebrae. A first DXA scanning were performed on the samples, followed by a micro-computed tomography (μCT) in order to compute the most relevant architectural parameters. Then monotonic compression tests were carried out to induce a mechanical damage in the samples. Lastly the damaged samples were scanned using DXA and μCT. Figure 2.1 shows the various stages of work taken place.

The results of the experimental tests are presented at the end of this chapter and compared with the ones obtained in other similar studies. In particular, the similarities and the dissimilarities between the properties of the porcine bone and of the human one are highlighted.

### 2.1 Sample preparation

Forty porcine trabecular specimens, extracted from the body of the lumbar vertebrae of six different animals, were included in this study. The animals were one and a half years old and were provided from a local butcher. Samples were drilled using a drilling device (WÜRTH®) along with the anatomical direction of the vertebral column. The coring device had the inside diameter of 16 mm and length of 40 mm. Subsequently, the samples were transferred to a lathing machine (OPTIMUM®) to reduce them to cylinders with the diameter of about 13.85 mm and height of 30 mm. During the drilling and milling, specimens were kept wet with water.
Afterward, as demonstrated in [13], to reduce the edge effects and eliminate the local damage effects, the end of the bone samples were glued (3M Scotch-WeldTM EPXTM Adhesive DP490) in custom-made aluminum endcaps. The endcaps had an inside diameter of 14 mm, outside diameter of 20 mm, height of 15 mm and they covered 3 mm of the specimens on each side (Figure 2.2).

To have perfectly parallel surfaces, both ends of bone specimens were smoothened using a circular blade saw (abrasive cutting instrument HITECH EUROPE). Bone samples, as well as the aluminum tubes, were defatted using acetone before gluing. A custom-made alignment tool was used to keep the bone and the endcaps aligned with the direction of axial loading. The first part of the assembling was the filling of the endcap’s hole with the glue. Then the sample was provisionally attached to the first endcap and it was inserted between the lower part of the alignment tool (Figure 2.3). Four M3 screws were used in order to join the two parts of the alignment device and to avoid any misalignments between the endcap and the sample.
The upper part of the alignment tool (Figure 2.4) was joined to the lower one with two M3 screws. The center hole in the upper part was used for a M2 screw that, once it was tightened, pushed the sample against the endcap, achieving a perfect aligned assembly.
After a few minutes, during which the glue started to dry, the alignment tool was disassembled and it was then used for another sample. The same procedure has been followed for the assembly of the second endcap.

Specimens were kept frozen at -18°C and were rehydrated in saline solution (NaCl 0.9%) at 4°C for 12 h before mechanical testing.

The following table reports the number of samples extracted from the vertebrae of each animal’s vertebral column.

Table 2.1 – Number of samples extracted from the vertebrae of each animal's vertebral column

<table>
<thead>
<tr>
<th>Animal</th>
<th>Lumbar vertebrae</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
</tr>
<tr>
<td>AN1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AN2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>AN3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>AN4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AN5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AN6</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

2.2 DXA scans

Porcine lumbar spine has been scanned with a dual-energy X-ray absorptiometry (DXA) system installed at the Bone Metabolic Unit of the Nuclear Medicine of the Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico.

The DXA system produces a two-dimensional data output using an X-ray source. The X-ray source is mounted beneath the patient and generates a narrow, tightly collimated, fan shaped beam of X-rays. The energy of X-ray beams that are passed through bones is absorbed, and what is not absorbed is detected. The more dense the bones, the more energy is absorbed, and the less energy detected. The radiation energy per pixel is detected and converted into a Bone Mineral Density (BMD).

Trabecular Bone Score (TBS) is a texture index that evaluates pixel gray-level variations in the lumbar spine DXA image, providing an indirect but highly correlated evaluation of trabecular microarchitecture. In order to understand more in detail how the trabecular bone score is computed and what information it provides, the following example are presented. A healthy patient has a well-structured trabecular bone. This implies that his trabecular structure is dense, with thick and well-grouped trabeculae. If this structure is
projected onto a plane, the resulting image contains a large number of pixel variations with small amplitudes. On the contrary, an osteoporotic patient has an altered trabecular bone. This signifies that his trabecular structure is porous, with less thin trabeculae. Projecting this structure onto a plane, the image obtained contains a low number of pixel value variations, but the amplitudes of these variations are high.

![Diagram of 3D structures and DXA images of healthy and osteoporotic bone](image)

*Figure 2.5 – 3D structures and DXA images of healthy and osteoporotic bone*

A variogram of the trabecular bone projected image is a method able to differentiates these two types of structures, therefore it is capable of providing useful information regarding 3-dimensional structure. In probabilistic notation, the variogram is defined as the sum of the squared grey-level differences between pixels at a specific distance

\[
2\gamma(h) = E \left[ (Z(x + h) - Z(x))^2 \right] = \frac{1}{N(h)} \sum_{N(h)} \left[ (Z(x + h) - Z(x))^2 \right]
\]

*Eq. (2.1)*
where $2\gamma(h)$ is the variogram, $\mathbf{x}$ is the vector of spatial coordinates, $h$ is the lag distance, $Z(\mathbf{x})$ is the grey-level function at the coordinate $\mathbf{x}$ and $N(h)$ represents the number of pairs separated by lag $h$. TBS is calculated as the slope of the log-log transformation of this variogram. A steep variogram slope with a high TBS value is associated with better bone structure, while low TBS values indicate worse bone structure.

To measure BMD, it has been used APEX software installed on the previously mentioned system whereas TBS has been calculated automatically by software provided by Media Maps and installed on the same machine.

Before the sample preparation a lumbar scan has been performed for each spine. The scan has been analyzed manually to remove the residual ribs left from the butcher. Trabecular bone score and bone mineral density have been calculated from the first to the fourth lumbar vertebra. The fifth and the sixth vertebrae have not been scanned because the DXA system is able to scan only the first four vertebrae.

After the sample preparation, all the samples have been scanned a first time before the mechanical test and a second time after it for the measurement of the TBS and BMD. Both time four specimens have been scanned simultaneously.

### 2.3 Image analysis

Micro-computed tomography (µCT) is an X-ray transmission image technique. X-rays are emitted from an X-ray generator, travel through a sample, and are recorded by a detector. The sample is then rotated by a fraction of a degree and another projection image is taken at the new position. This procedure is repeated until the sample has rotated 360 degrees, producing a series of projection images. The projection images are then processed using a computer software to show the internal structure of the object nondestructively. This series of images are typically called reconstructed images or cross sections.

Micro-computed tomography (µCT) images of trabecular specimens were collected using an x-view scanning equipment (North Star Imaging Inc.) with a spatial resolution of 25.6 $\mu$m and 26.3 $\mu$m for the first and the second scans respectively. The parameters of the scanning were fixed at 60 kV and 150 $\mu$A. Simultaneously, three specimens were placed in the CT equipment and the total imaging time was 110 minutes. Specimens were submerged in saline solution during the scanning to simulate the physiological conditions of the body during the scanning process.

The x-view CT software has performed image reconstruction. Image analysis has been done in MATLAB® and in ImageJ using BoneJ which is a plugin for bone image analysis. It provides tools for trabecular geometry and whole bone shape analysis.
Before the computation of the morphological parameters, noises were removed from the images using a Gaussian blur filter ($\sigma = 1.5$). Consequently, the images were converted to gray-level 8-bit images. Otsu local thresholding method [19] has been used for the segmentation of the images, which resulted in binary images with the voxel value of 1, for the bone, and 0, for the empty spaces.

![Figure 2.6 – (Left) Input μCT image. (Right) Binary filtered image](image)

The computation of the morphological parameters has been made possible thanks to the implementation of a MATLAB® code (reported in A.1) created specifically for this study. The code is able to compute the external boundary of the sample's cross section for each slice and a binary mask, used to change the black external background to white (Figure 2.7). The principal implemented steps, executed for each slice, are listed below with the name of the MATLAB® functions putted in bracket.

1. The binary image is converted into a matrix whose cells represent the pixels of the source image (im2double). The elements of the matrix are 1s, if the corresponding pixels are white (bone), and 0s otherwise.
2. All connected components that have fewer than 30 pixels are removed from the binary image (bwareaopen).
3. The position of the white pixels is computed and saved in a n-by-2 matrix, containing the x position (first column) and the y position (second column) of the white pixels; n is the number of white pixels of the binary image (find).
4. The external boundary and the cross section of the sample's slice are calculated from the n-by-2 matrix (boundary).
5. A binary mask image that fits the cross section is created and used to change from black to white the external background of the original binary image that includes all the connected components that have fewer than 30 pixels (roipoly). It is intended to emphasize that the mask is being applied to the original image and not to the image obtained with the method explained at the point 2, in order to keep into account in the upcoming analysis the isolated particles inside the trabecular bone.

6. The white pixels of the original binary image are counted inside the boundary for the computation of the bone volume fraction (\(\text{sum}\)).

These steps are executed for each slice of all porcine samples. This code has been implemented in particular for those samples that do not have a perfect circular cross section. Otherwise, the computation of all morphological parameters can be performed in ImageJ using the bonej plugin.

![Diagram of the principal outputs obtained from the implemented MATLAB® code](image)

The morphological parameters analyzed in this study are listed and described below. The **Bone Volume Fraction** (BV/TV) was defined as the total number of voxels with value of 1, that represents the bone volume (BV), to the total number of voxels, that is the total volume (TV), in ROI in the binary image. A voxel has a volume equal to the spatial resolution of the scanning equipment to the power of 3.

The **Trabecular Thickness** (Tb.Th) is determined as an average of the local thickness at each voxel representing the bone. Local thickness for a point in solid is defined as the diameter of a sphere which encloses the point and is entirely bounded within the solid surfaces [10]. In an equivalent way, the **Trabecular Spacing** (Tb.Sp) is an average of the local thickness at each voxel representing non-bone and it indicates the mean distance between trabeculae.
The **Bone Surface** (BS) was defined as the inside surfaces of the bone materials and it was calculated constructing a triangular surface mesh by marching cubes and calculates the bone surface area (BS) as the sum of the areas of the triangles making up the mesh.

**Structure Model Index** (SMI) is an estimation of the plate-rod characteristic of the foam’s structure. For an ideal plate the SMI value is 0 and for a rod structure the SMI value is 3. For a structure with both plates and rods of equal thickness, the value is between 0 and 3, depending on the volume ratio of rods to plates. SMI was calculated from the binary images based on the method proposed in [11].

The main direction of the microstructures of the bones was measured by the Mean Intercept Length (MIL) method [26]. The principle of the MIL method is to count the number of intersections between a family of equidistant, parallel lines and the bone/empty interface as the function of the 3D orientation of the family of lines. The MIL value for a particular 3D orientation of the family of lines is the total line length within the image over the number of intercepts. It has been shown that an ellipsoid can approximate MIL in three-dimensions [9] and lead to the definition of a positive definite second-order fabric tensor that characterizes the degree of anisotropy of the bone microstructures. Moreover, based on the general theory developed by [4], fabric tensor, which is the inverse of MIL tensor, has been applied to measure the local structural anisotropy. The fabric tensor is defined as its spectral decomposition:

\[
M = \sum_{i=1}^{3} m_i M_i = \sum_{i=1}^{3} m_i (m_i \otimes m_i)
\]

*Eq. (2.2)*

where \(m_i\) is the positive eigenvalues and the \(m_i\) is the corresponding normalized eigenvectors. \(M\) is normalized to \(\text{tr}(M) = 3\), which ensures that fabric is independent of volume fraction. The three eigenvectors of \(M\) represent the principal axes of material symmetry, which also correspond to the main orientations of the trabeculae. The **Degree of Anisotropy** (DA) can be defined as

\[
DA = 1 - \frac{\min(m_i)}{\max(m_i)}
\]

*Eq. (2.3)*

DA describes how the structural elements are oriented. DA value of 0 means total isotropy and 1 means total anisotropy.

Bone volume fraction (BV/TV) has been computed with the MATLAB® code described previously and the other morphological parameters have been computed using ImageJ with the bonej plugin. The evaluation of trabecular spacing (Tb.Sp) has been carried out with the binary image with the white background.

The Figure 2.8 represents the 3D of two samples (animal 1 – vertebra L2 and animal 6 – vertebra L1) and it reports the principal morphological properties of the specimens.
**EXPERIMENTAL TESTS**

**ANIMAL 1 – VERTEBRA L2**

BV/TV = 44.6%; Tb.Th = 0.25 mm; Tb.Sp = 0.13 mm; SMI = 0.34; DA = 0.64

**ANIMAL 6 – VERTEBRA L1**

BV/TV = 41.9%; Tb.Th = 0.29 mm; Tb.Sp = 0.45 mm; SMI = 1.82; DA = 0.58

*Figure 2.8 – 3D view of two samples with their morphological properties*

### 2.4 Damage test

Monotonic compression tests were carried out on an MTS machine (Alliance, RF/150) with a load cell of 150 kN (class 1 ISO 7500-1). Prior to mechanical testing, the specimens were rehydrated in water with 0.9% sodium chloride. The specimens were loaded in displacement control along the central cylindrical axis. All tests were performed at room temperature. The axial strain was measured using an 8 mm extensometer gauge (MTS 632.26F-20) attached to the sample. The quasi-static test was performed at a strain rate of 0.0002 s⁻¹ (constant stroke rate of 0.05 mm/s). The loading protocol contained three preconditioning compression cycles up to 0.1% axial strain in order to improve reproducibility. After that, the specimens were loaded and unloaded three times until certain strain level, to obtain damage and residual plastic strain.

*Figure 2.9 shows the testing protocol of a specimen loaded until strain level of 5% axial strain.*
2.4 Damage test

Figure 2.9 – Testing protocol with monotonic loading until 5% axial strain

Forty specimens were randomly divided into four groups of 10 samples, each set loaded with a different strain value. In particular:

- Group 1%, called G1%; the specimens have been loaded to 1% of strain, close to the yield strain;
- Group 2%, called G2%, the specimens have been loaded to 2% of strain, near to the ultimate strain;
- Group 3.5%, called G3.5%, the specimens have been loaded to 3.5% of strain;
- Group 5%, called G5%, the specimens have been loaded to 5% of strain;

All the tests were conducted at room temperature. Data were acquired at a sampling rate of 20 Hz. The recording included time, stroke, force, and axial strain.

The normal stress was defined as the ratio of the axial force to the minimum cross section, computed with a MATLAB® code analyzing the images obtained from μCT scans.

The computation of the Initial Elastic Modulus ($E_0$) required a partition of the loading path in 0.2% strain intervals defined for each point. A regression line has been computed for each interval. The initial elastic modulus was defined from the regression line with the greater slope. With this moving regression method, also used in other studies as [28], $E_0$ was obtained from the stiffest section of the loading part.

The Yield Stress ($\sigma_y$) and Yield Strain ($\epsilon_y$) were obtained from the intersection between the loading curve and the regression line used for the evaluation of $E_0$ shifted by 0.2% strain.

The Ultimate Stress ($\sigma_{ult}$) was defined as the maximum stress in the loading curve and its corresponding strain defines the Ultimate Strain ($\epsilon_{ult}$). For same samples, it was not possible to define $\sigma_{ult}$ and $\epsilon_{ult}$ because the damage test ended before the loading curve reached the ultimate point.

The Residual Strain ($\epsilon_r$) was obtained from the point of zero stress at the end of the last unloading cycle.
The **Plastic Strain** \( (\varepsilon_p) \) has been calculated as defined in [15] from the total applied strain \( (\varepsilon_t) \), the perfect-damage \( (E_{PD}) \) and initial elastic \( (E_0) \) moduli. \( E_{PD} \) is defined as the slope of a straight line drawn from the origin of the stress-strain curve to the initial point of the unloading, corresponding to the total applied strain \( (\varepsilon_t) \). The plastic strain is computed as

\[
\varepsilon_p = \varepsilon_t \cdot \left(1 - \frac{E_{PD}}{E_0}\right)
\]

*Eq. (2.4)*

The **Residual Elastic Modulus** \( (E) \) was calculated, equivalently it has been done for \( E_0 \), from the steepest part of the last loading cycle.

The **Dissipation Energy** \( (U) \) was defined as the area under the stress-strain curve till the loading strain.

The **Damage** \( (D) \) was defined as decay in modulus:

\[
D = \frac{E_0 - E}{E_0}
\]

*Eq. (2.5)*

The mechanical damage, which is related to microdamage accumulation in the bone tissue, was quantified by calculating the percentage reduction of the elastic modulus \( (D) \), because the modulus degradation \( (D) \) is a direct measure of mechanical damage [6].

The computation of the mechanical parameters has been done implementing for each of them MATLAB® codes. The main codes are reported in Appendix A.

*Figure 2.10 – Output parameters*
2.5 Results of the experimental tests

In this section, it is reported the results of the experimental tests carried out before and after the mechanical test. Since a whole chapter was devoted to the analysis of the results of tests carried out after the damage and since in this paragraph it is intended to compare the porcine bones with human ones, in this section only the results of tests carried out before compression are discussed.

The data obtained after the compression test are presented divided into the four mechanical groups as it is unknown a priori whether the induced damage affects on the final measurement of the morphological parameters. This possible influence will be studied deeply in Chapter 4.

The morphometric properties measured before and after the compression test are sorted in the Table 2.2 and Table 2.3 respectively. The results are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Morphometric properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone volume fraction (BV/TV) [%]</td>
<td>42.40 ± 4.88</td>
</tr>
<tr>
<td>Trabecular Thickness (Tb.Th) [mm]</td>
<td>0.27 ± 0.14</td>
</tr>
<tr>
<td>Trabecular Spacing (Tb.Sp) [mm]</td>
<td>0.44 ± 0.17</td>
</tr>
<tr>
<td>Bone surface to bone volume (BS/BV) [1/mm]</td>
<td>10.36 ± 1.20</td>
</tr>
<tr>
<td>Bone surface to total volume (BS/TV) [1/mm]</td>
<td>4.35 ± 0.35</td>
</tr>
<tr>
<td>Degree of Anisotropy (DA) [-]</td>
<td>0.55 ± 0.09</td>
</tr>
<tr>
<td>Structure Model Index (SMI) [-]</td>
<td>1.18 ± 0.52</td>
</tr>
</tbody>
</table>

Morphology parameters measured before the compression test in this study are in the same range of reported value in the literatures, as [24].

It is interesting to observe that the BV/TV of the porcine bone is much higher if compared with the human one, whose bone volume fraction studied in [8] is 28.8% ± 4.6%. It can be stated that the struts, whose interconnections generate the structure of the trabecular geometry, have a mean thickness (Tb.Th) of 0.27 mm and the void can be ideally filled with spheres whose mean diameter (Tb.Sp) of 0.44 mm. In [8] Tb.Th is 0.23 ± 0.02 mm and Tb.Sp is 0.54 ± 0.13 mm. The greater value of Tb.Sp in [8] justifies a lower BV/TV compared the value obtained in this study. It can be noticed that the DA is different from 1, so the vertebra exhibit an anisotropic behavior, as it was found for the human vertebra [7]. The SMI mean value indicates that the structure of the trabecular bone is composed by both plates and rods.
EXPERIMENTAL TESTS

Table 2.3 – Morphometric properties measured after the damage

<table>
<thead>
<tr>
<th>Morphometric properties</th>
<th>G1%</th>
<th>G2%</th>
<th>G3.5%</th>
<th>G5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV [%]</td>
<td>37.30 ± 7.27</td>
<td>31.98 ± 3.53</td>
<td>33.16 ± 4.36</td>
<td>35.82 ± 4.25</td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.29 ± 0.05</td>
<td>0.25 ± 0.01</td>
<td>0.26 ± 0.04</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>Tb.Sp [mm]</td>
<td>0.48 ± 0.06</td>
<td>0.50 ± 0.06</td>
<td>0.48 ± 0.03</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>BS/BV [1/ mm]</td>
<td>9.20 ± 1.82</td>
<td>10.67 ± 0.86</td>
<td>10.34 ± 1.61</td>
<td>9.62 ± 1.32</td>
</tr>
<tr>
<td>BS/TV [1/mm]</td>
<td>3.32 ± 0.23</td>
<td>3.39 ± 0.25</td>
<td>3.37 ± 0.18</td>
<td>3.40 ± 0.24</td>
</tr>
<tr>
<td>DA [-]</td>
<td>0.55 ± 0.07</td>
<td>0.61 ± 0.03</td>
<td>0.63 ± 0.06</td>
<td>0.59 ± 0.07</td>
</tr>
<tr>
<td>SMI [-]</td>
<td>0.78 ± 0.78</td>
<td>1.03 ± 0.38</td>
<td>0.96 ± 0.51</td>
<td>0.41 ± 0.66</td>
</tr>
</tbody>
</table>

The clinical parameters are presented in the Table 2.4 and in the Table 2.5 as mean ± standard deviation. It is reported the BMD and TBS of the full vertebra and of the trabecular specimen before damage in the first table and the BMD and TBS of the trabecular specimen after damage in the second table.

Table 2.4 – Clinical parameter of full vertebra and of trabecular samples before damage

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Full vertebra</th>
<th>Before damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Mineral Density (BMD) [g/cm²]</td>
<td>1.16 ± 0.12</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>Trabecular Bone Score (TBS) [-]</td>
<td>1.58 ± 0.08</td>
<td>1.18 ± 0.13</td>
</tr>
</tbody>
</table>

The clinical results change passing from the full vertebra to the individual samples of trabecular bone. This difference is because the full vertebrae are made by the trabecular bone and by cortical bone, which increase the overall density. Furthermore, the presence of cortical bone contributes to an increasing of the TBS due to its feature to be well-structured osseous tissue.

Table 2.5 – Clinical parameter of trabecular samples after damage

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>G1%</th>
<th>G2%</th>
<th>G3.5%</th>
<th>G5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD [g/cm²]</td>
<td>0.421 ± 0.071</td>
<td>0.370 ± 0.047</td>
<td>0.377 ± 0.054</td>
<td>0.383 ± 0.045</td>
</tr>
<tr>
<td>TBS [-]</td>
<td>0.807 ± 0.221</td>
<td>0.472 ± 0.192</td>
<td>0.501 ± 0.135</td>
<td>0.536 ± 0.188</td>
</tr>
</tbody>
</table>

The BMD of the human lumbar spine are reported in the Table 2.6, whose data are taken from a study made by the National Health and Nutrition Examination Survey (NHANES) [18].
Results of the experimental tests considering all the races and the ethnicities. It can be noticed that BMD of the full vertebra, obtained from this study, has a mean value that is slightly higher compared with the human’s values. The TBS measured on human lumbar vertebrae assume values that depend on many factors, such as age, sex and ethnicity, but a rule of thumb is used to evaluate the human bone quality:

- \( TBS > 1.350 \) → normal bone
- \( 1.200 < TBS < 1.350 \) → partially degraded bone
- \( TBS < 1.200 \) → degraded bone

This rule must be applied on the full vertebrae, therefore on bone tissue composed by cortical bone and trabecular bone.

**Table 2.6 – Lumbar spine bone mineral density (g/cm\(^2\)) of persons aged 8 years and over**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sample size</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Sample size</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 – 11 years</td>
<td>1421</td>
<td>0.685</td>
<td>0.075</td>
<td>1465</td>
<td>0.730</td>
<td>0.106</td>
</tr>
<tr>
<td>12 - 15 years</td>
<td>2264</td>
<td>0.845</td>
<td>0.147</td>
<td>2318</td>
<td>0.962</td>
<td>0.135</td>
</tr>
<tr>
<td>16 – 19 years</td>
<td>2309</td>
<td>1.047</td>
<td>0.137</td>
<td>2027</td>
<td>1.050</td>
<td>0.123</td>
</tr>
<tr>
<td>20 – 29 years</td>
<td>1530</td>
<td>1.064</td>
<td>0.143</td>
<td>1378</td>
<td>1.074</td>
<td>0.123</td>
</tr>
<tr>
<td>30 – 39 years</td>
<td>1450</td>
<td>1.050</td>
<td>0.151</td>
<td>1372</td>
<td>1.080</td>
<td>0.133</td>
</tr>
<tr>
<td>40 – 49 years</td>
<td>1549</td>
<td>1.042</td>
<td>0.156</td>
<td>1570</td>
<td>1.066</td>
<td>0.150</td>
</tr>
<tr>
<td>50 – 59 years</td>
<td>1187</td>
<td>1.034</td>
<td>0.167</td>
<td>1200</td>
<td>1.014</td>
<td>0.159</td>
</tr>
<tr>
<td>60 – 69 years</td>
<td>1350</td>
<td>1.023</td>
<td>0.187</td>
<td>1419</td>
<td>0.978</td>
<td>0.170</td>
</tr>
<tr>
<td>70 – 79 years</td>
<td>825</td>
<td>1.008</td>
<td>0.199</td>
<td>750</td>
<td>0.952</td>
<td>0.173</td>
</tr>
<tr>
<td>80 years and over</td>
<td>501</td>
<td>0.992</td>
<td>0.229</td>
<td>594</td>
<td>0.924</td>
<td>0.175</td>
</tr>
</tbody>
</table>

The mechanical properties obtained in this study are reported in the Table 2.7 and in the Table 2.8 as mean ± standard deviation. Two specimens were removed from the data due to the slippage of the extensometer during testing. These specimens have been tested in G1%.

**Table 2.7 – Mechanical properties**

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>( E_0 ) [MPa]</th>
<th>1614.34 ± 458.54</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial elastic modulus ( (E_0) ) [MPa]</td>
<td>12.48 ± 3.21</td>
<td></td>
</tr>
<tr>
<td>Yield stress ( (\sigma_y) ) [MPa]</td>
<td>1.00 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Yield strain ( (\varepsilon_y) ) [%]</td>
<td>14.80 ± 2.73</td>
<td></td>
</tr>
<tr>
<td>Ultimate strain ( (\varepsilon_{ult}) ) [%]</td>
<td>1.68 ± 0.49</td>
<td></td>
</tr>
</tbody>
</table>
EXPERIMENTAL TESTS

Table 2.8 – Mechanical properties in the four loading conditions

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>G1%</th>
<th>G2%</th>
<th>G3.5%</th>
<th>G5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual Elastic Modulus (E) [MPa]</td>
<td>1674.04 ±</td>
<td>845.28 ±</td>
<td>672.08 ±</td>
<td>383.76 ±</td>
</tr>
<tr>
<td>Damage (D) [-]</td>
<td>0.14 ± 0.10</td>
<td>0.45 ± 0.14</td>
<td>0.59 ± 0.08</td>
<td>0.74 ± 0.08</td>
</tr>
<tr>
<td>Residual strain (εr) [%]</td>
<td>0.09 ± 0.07</td>
<td>0.50 ± 0.23</td>
<td>1.58 ± 0.39</td>
<td>2.67 ± 0.50</td>
</tr>
<tr>
<td>Plastic strain (εp) [%]</td>
<td>0.20 ± 0.14</td>
<td>1.18 ± 0.37</td>
<td>2.67 ± 0.19</td>
<td>4.27 ± 0.31</td>
</tr>
<tr>
<td>Dissipation energy (U) [MJ/m³]</td>
<td>0.07 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.40 ± 0.07</td>
<td>0.58 ± 0.12</td>
</tr>
</tbody>
</table>

Mechanical properties determined from the porcine samples are in the same range of the values reported in the literatures, as [24] and [23].

It is interesting to compare the mechanical properties of the porcine bone with the human ones. In the following table, the results of other studies on human trabecular bones are reported.

Table 2.9 – Mechanical properties of human trabecular bone

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>Rincón et al., 2008</th>
<th>Homminga et al., 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial elastic modulus (E₀) [MPa]</td>
<td>597.9 ± 401.6</td>
<td>635 ± 265</td>
</tr>
<tr>
<td>Yield stress (σ₀) [MPa]</td>
<td>8.98 ± 7.57</td>
<td>6.7 ± 2.7</td>
</tr>
<tr>
<td>Yield strain (ε₀) [%]</td>
<td>1.52 ± 0.36</td>
<td>1.1 ± 2.1</td>
</tr>
<tr>
<td>Ultimate stress (σult) [MPa]</td>
<td>10.16 ± 8.92</td>
<td>-</td>
</tr>
<tr>
<td>Ultimate strain (εult) [%]</td>
<td>2.31 ± 0.77</td>
<td>-</td>
</tr>
<tr>
<td>Age [year]</td>
<td>73.5 ± 16.8</td>
<td>80 ± 12</td>
</tr>
</tbody>
</table>

It is not aim of this study to perform a statistical analysis on the difference between the human and the porcine trabecular bone. The Table 2.9 helps the reader to have an idea of the mechanical properties’ values of the human trabecular bones. The human trabecular bone has a lower stiffness and a lower strength. This may be due to the age of the human cadavers from which the specimens were extracted. In fact for Rincón et al., 2008 the mean age was 73.5 and a standard deviation of 16.8 and for Homminga et al., 2002 it was 80 and 12 (mean and standard deviation respectively). Instead the animals used in this study were one and a half years old, therefore their age results to be considerably lower with respect to those of the two aforementioned studies, considering that the pigs that are well cared for live an average lifespan of 15 to 20 years.

The following figures represent the stress-strain curve of the specimens divided in the four groups.
Results of the experimental tests

Figure 2.11 – Load stress-strain curve for Group 1%

Figure 2.12 – Load stress-strain curve for Group 2%

Figure 2.13 – Load stress-strain curve for Group 3.5%
Figure 2.14 – Load stress-strain curve for Group 5%
CHAPTER 3
PRELIMINARY STATISTICAL ANALYSIS

In this chapter the data previously presented is analyzed firstly performing an Analysis of Variance (ANOVA) using MATLAB® in order to understand if the vertebra and the animal are significant factors and, if so, a Tukey’s HSD test has been adopted to find which treatments are significantly different. A correlation analysis has been used to study the relationship between clinical, morphological and mechanical parameters. The results obtained allow to understand first of all that the DXA scan provide useful information on the morphology of the trabecular bone, but in less detailed manner than a μCT. In addition, the mechanical properties of bone, as it might be expected, depend on its microarchitecture.

The analysis described in this chapter are performed using only the data obtained before the mechanical test, in order to not weigh the statistical analysis down.

3.1 Dependency on the vertebra and on the animal

An Analysis of Variance (ANOVA) test is carried out by using MATLAB® in order to understand if the vertebra (designated L1 to L6) and the animals (AN1 to AN6) are significant factors. The significance level has been set to 0.05. A Bartlett’s test and an Anderson–Darling’s test have been carried out to check the homoscedasticity and the normality distribution of the standardized residuals, respectively. A Box-Cox transformation has been adopted when the normality hypothesis and the homogeneous variance hypothesis are refused. It has been also verified if there are any outlier in the analysis, checking that the standardized residuals belong to the interval [-3,+3].

The Table 3.1 reports the p-values from the ANOVA test. The parameters which may also depend on the loading condition have been analyzed with a three-way ANOVA and the resulting p-values are sorted in the Table 3.2.
Table 3.1 – Table of p-values from two-way ANOVA with vertebra and animal as factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>p-values Vertebra</th>
<th>p-values Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density (BMD) – Full vertebra</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Bone mineral density (BMD) – Before damage</td>
<td>0.489</td>
<td>0.005</td>
</tr>
<tr>
<td>Trabecular bone score (TBS) – Full vertebra</td>
<td>0.158</td>
<td>0.000</td>
</tr>
<tr>
<td>Trabecular bone score (TBS) – Before damage</td>
<td>0.811</td>
<td>0.397</td>
</tr>
<tr>
<td>Initial elastic modulus (E₀)</td>
<td>0.782</td>
<td>0.036</td>
</tr>
<tr>
<td>Yield strain (εᵥ)</td>
<td>0.899</td>
<td>0.643</td>
</tr>
<tr>
<td>Yield stress (σᵥ)</td>
<td>0.883</td>
<td>0.034</td>
</tr>
<tr>
<td>Ultimate strain (εₚₚ)</td>
<td>0.525</td>
<td>0.685</td>
</tr>
<tr>
<td>Ultimate stress (σₚₚ)</td>
<td>0.315</td>
<td>0.016</td>
</tr>
<tr>
<td>Bone volume fraction (BV/TV)*</td>
<td>0.350</td>
<td>0.111</td>
</tr>
<tr>
<td>Trabecular thickness (Tb.Th)*</td>
<td>0.951</td>
<td>0.390</td>
</tr>
<tr>
<td>Trabecular spacing (Tb.Sp)*</td>
<td>0.385</td>
<td>0.076</td>
</tr>
<tr>
<td>Bone surface to bone volume (BSBV)</td>
<td>0.754</td>
<td>0.239</td>
</tr>
<tr>
<td>Bone surface to total volume (BSTV)</td>
<td>0.466</td>
<td>0.053</td>
</tr>
<tr>
<td>Degree of anisotropy (DA)</td>
<td>0.330</td>
<td>0.902</td>
</tr>
<tr>
<td>Structure model index (SMI)</td>
<td>0.002</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* transformed data due to a rejection of the normality assumption

Table 3.2 – Table of p-values from three-way ANOVA with vertebra, animal and loading condition (group) as factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>p-values Vertebra</th>
<th>p-values Animal</th>
<th>p-values Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage (D)</td>
<td>0.608</td>
<td>0.097</td>
<td>0.000</td>
</tr>
<tr>
<td>Residual strain (εᵥ)</td>
<td>0.479</td>
<td>0.538</td>
<td>0.000</td>
</tr>
<tr>
<td>Plastic strain (εₚₚ)</td>
<td>0.375</td>
<td>0.656</td>
<td>0.000</td>
</tr>
<tr>
<td>Dissipation energy (U)</td>
<td>0.671</td>
<td>0.106</td>
<td>0.000</td>
</tr>
</tbody>
</table>

All the assumptions for ANOVA have been checked with the previously mentioned tests and the data of three parameters (BV/TV, Tb.Th, Tb.Sp) has been transformed due to a rejection of the normality assumption.

The animal and the vertebra are significant factors for BMD of the full vertebra (before the sample preparation), instead the BMD before the damage depends only on the animal. Therefore, it can be concluded that from the point of view of the density, trabecular bone is similar between the different vertebrae that differ only in the cortical bone. Similar conclusions can be drawn for the TBS that is different between the animal if it is measured on the full vertebra. This indicates that, as one might expect, once again the cortical bone significantly
affects the measurement of clinical parameters. The initial elastic modulus, the yield stress and the strength result having a slight dependency on the animal. Both the vertebra and the animal are significant factors for the structure model index.

The damage, the residual strain, the plastic strain and the dissipation energy do not depend on the vertebra or on the animal, but, as expected, they depend on the loading conditions.

A Tukey’s HSD test (pairwise comparison with a 95% family confidence level) were performed for those parameters that present a dependency on the vertebra and/or on the animal. In order to present the results, reported in the following tables, it has been assigned for each level a letter; those levels that do not share a letter are significantly different. Furthermore this method allows to order the treatment from that with greater mean (A) to the one with lower mean (C). The interaction terms are not significant for all parameters analyzed, therefore the post-ANOVA has been performed separately on the vertebra and on the animal.

*Table 3.3* presents the post-ANOVA results of the comparison among the animals. Even if the ANOVA p-values associated to $E_0$ indicates that there are differences between the levels, the multiple comparison output indicates the contrary. In the literatures it is stated to generally trust the results of the Tukey’s test and it is accepted this suggestion. It can be noticed that Animal 3 presents a low BMD of the full vertebra compared with the others, but the samples extracted from its spine result to be more dense compared the others samples’ spines before the damage. It is considered that this is due to a low density of the cortical bone of that spine. Apart from this anomaly, it can be observed from a qualitative and a approximate point of view that the animals that present the highest value of BMD result to be the strongest. Furthermore it can be seen also that there is a inverse correlation between the strength and the structure model index, suggesting that a trabecular structure made by plates is better than one made by rods. These considerations are confirmed with a correlation analysis that is going to be discussed in the following paragraph.

*Table 3.3 – Table of post-ANOVA results with animal as factor and of parameters’ mean values associated to each animal*

<table>
<thead>
<tr>
<th>BMD [g/cm²]</th>
<th>AN1</th>
<th>AN2</th>
<th>AN3</th>
<th>AN4</th>
<th>AN5</th>
<th>AN6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full vertebra</td>
<td>A</td>
<td>A</td>
<td>C</td>
<td>B</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>1.274</td>
<td>1.325</td>
<td>1.058</td>
<td>1.118</td>
<td>1.149</td>
<td>1.014</td>
<td></td>
</tr>
<tr>
<td>A/B</td>
<td>A</td>
<td>A</td>
<td>A/B</td>
<td>A/B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>0.427</td>
<td>0.473</td>
<td>0.445</td>
<td>0.421</td>
<td>0.399</td>
<td>0.351</td>
<td></td>
</tr>
<tr>
<td>Before damage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBS [-]</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Full vertebra</td>
<td>1.661</td>
<td>1.664</td>
<td>1.581</td>
<td>1.570</td>
<td>1.556</td>
<td>1.449</td>
</tr>
</tbody>
</table>
3 PRELIMINARY STATISTICAL ANALYSIS

Table 3.3 – Table of post-ANOVA results with animal as factor and of parameters’ mean values associated to each animal

<table>
<thead>
<tr>
<th></th>
<th>AN1</th>
<th>AN2</th>
<th>AN3</th>
<th>AN4</th>
<th>AN5</th>
<th>AN6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial elastic modulus [MPa]</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>1836</td>
<td>1805</td>
<td>1527</td>
<td>1881</td>
<td>1395</td>
<td>1151</td>
</tr>
<tr>
<td>Yield stress [MPa]</td>
<td>A</td>
<td>A/B</td>
<td>A/B</td>
<td>A/B</td>
<td>A/B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>14.6</td>
<td>14.0</td>
<td>10.7</td>
<td>13.3</td>
<td>12.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Ultimate stress [MPa]</td>
<td>A</td>
<td>A</td>
<td>A/B</td>
<td>A</td>
<td>A/B</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>16.9</td>
<td>16.1</td>
<td>12.1</td>
<td>15.8</td>
<td>14.9</td>
<td>11.8</td>
</tr>
<tr>
<td>Structure model index [-]</td>
<td>C</td>
<td>B/C</td>
<td>B/C</td>
<td>B/C</td>
<td>A/B</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>0.704</td>
<td>1.003</td>
<td>1.157</td>
<td>1.133</td>
<td>1.449</td>
<td>1.816</td>
</tr>
</tbody>
</table>

Table 3.4 reports the results of the Tukey’s test. As stated in the paragraph 2.2 DXA scans, only the first four vertebrae (L1 – L4) have been scanned, this is the reason why the cells referred to the vertebrae L5 and L6 are empty. It can be observed that moving toward the end of the vertebral column, the vertebrae are slightly less dense with an increasing of the SMI. As stated previously, looking at these results higher BMD leads to a lower SMI.

Table 3.4 – Table of post-ANOVA results with vertebra as factor and of parameters’ mean values associated to each vertebra

<table>
<thead>
<tr>
<th>BMD [g/cm²]</th>
<th>Full vertebra</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>L5</th>
<th>L6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.184</td>
<td>1.201</td>
<td>1.143</td>
<td>1.097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structure model index [-]</td>
<td>C</td>
<td>B/C</td>
<td>A/B</td>
<td>A/B</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.865</td>
<td>0.984</td>
<td>1.054</td>
<td>1.230</td>
<td>1.484</td>
<td>1.810</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Correlation analysis

Correlation analysis have been performed by using MATLAB® in order to understand how the different parameters in examination are related each other. The Pearson’s coefficients are shown in tables as results of the analysis and highlighted with different colors, as indicated in Table 3.5.
3.2 Correlation analysis

Table 3.5 – Explanatory table of the correlation’s strength

<table>
<thead>
<tr>
<th>Color</th>
<th>Correlation</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>weak</td>
<td>0.00 ≤</td>
<td>r</td>
</tr>
<tr>
<td>moderate</td>
<td>0.50 &lt;</td>
<td>r</td>
</tr>
<tr>
<td>strong</td>
<td>0.75 ≤</td>
<td>r</td>
</tr>
<tr>
<td>NS</td>
<td>not significant correlation</td>
<td></td>
</tr>
</tbody>
</table>

In this paragraph, firstly the interrelationship between the parameters belonging to the same family (clinical, morphological and mechanical) are presented and in the following paragraphs the correlation of parameters of different groups. The results of the correlation analysis are reported in Appendix B.

3.2.1 Clinical parameters

It has been studied the relationship between BMD and TBS measured on the full vertebra and on the samples before the damage. In the first case there is a significant positive correlation (r = 0.82, p = 0.000), while in the latter analysis no correlation has been found (p = 0.161). This indicates that a well-structured trabecular bone (high TBS) corresponds a dense cortical bone and vice versa for an altered trabecular bone.

3.2.2 Morphological parameters

The results of the interrelationship between morphological variables are shown in the Table 3.6. There is a positive correlation between BV/TV and Tb.Th (r = 0.64, p = 0.000) and a negative correlation between BV/TV and Tb.Sp (r = -0.56, p = 0.000), in that an increase of the trabeculae’s size or a reduction of distance between the struts entail a higher bone volume fraction and vice versa. In view of the negative correlation between BV/TV and SMI (r = -0.67, p = 0.000), it appears that samples with a lower bone volume fraction are characterized by a smaller plate-to-rod ratio. BV/TV has a weak negative correlation with DA (r = -0.44, p = 0.004). This result indicates that the trabecular bones with higher bone volume fraction present an isotropic structure. Same type of tendency has been found between Tb.Th and DA (r = -0.53, p = 0.000), therefore an isotropic structure presents thicker trabeculae, but it does not present a different Tb.Sp from a anisotropic one (r = 0.14, p = 0.373). Contrary to what it can be supposed, Tb.Th is not related to Tb.Sp. Therefore, an increase of the distance between the trabeculae reduces the bone volume fraction, without leading to a further reduction of BV/TV caused by the thickness of the trabeculae that remains constant. Equivalent conclusions can be drawn for an increase of the trabeculae’s thickness. There is a negative
correlation between BS/BV and Tb.Th \( (r = -0.83, p = 0.000) \) and between BS/TV and Tb.Sp \( (r = -0.61, p = 0.000) \). These last two results are confirmed by the mathematical relationships derived from the Parfitt’s model for cancellous bone structure [20]. Another interesting outcome of the interrelationships between microarchitectural parameters is the dependence, although weak, of the SMI on Tb.Sp \( (r = 0.40, p = 0.010) \); in particular, a rod-like geometry requires a higher distance between trabeculae, vice versa for a plate-like structure, but no variation of the struts’ thickness \( (p = 0.403) \). A high correlation has been found between BV/TV and BS/BV \( (r = -0.81, p = 0.000) \), as consequence of their dependence on Tb.Th. All results listed in the Table 3.6 are confirmed by other similar studies.

**Table 3.6 – Pearson’s correlation coefficients between microarchitectural parameters**

<table>
<thead>
<tr>
<th></th>
<th>BV/TV</th>
<th>BS/BV</th>
<th>BS/TV</th>
<th>Tb.Th</th>
<th>Tb.Sp</th>
<th>SMI</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV</td>
<td>1</td>
<td>-0.81</td>
<td>0.56</td>
<td>-0.56</td>
<td>-0.67</td>
<td>-0.44</td>
<td></td>
</tr>
<tr>
<td>BS/BV</td>
<td>1</td>
<td>NS</td>
<td>-0.83</td>
<td>NS</td>
<td>0.48</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>BS/TV</td>
<td>1</td>
<td>-0.37</td>
<td>-0.61</td>
<td>NS</td>
<td>NS</td>
<td>-0.53</td>
<td></td>
</tr>
<tr>
<td>Tb.Th</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>1</td>
<td>0.40</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMI</td>
<td>1</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**3.2.3 Mechanical parameters**

*Table 3.7 shows the results of the interrelationship between the mechanical variables not depending on the total applied strain.***

**Table 3.7 – Pearson’s correlation coefficients between mechanical parameters**

<table>
<thead>
<tr>
<th></th>
<th>( E_0 )</th>
<th>( \varepsilon_y )</th>
<th>( \sigma_y )</th>
<th>( \varepsilon_{ult} )</th>
<th>( \sigma_{ult} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_0 )</td>
<td>1</td>
<td>-0.55</td>
<td>0.61</td>
<td>-0.49</td>
<td>0.76</td>
</tr>
<tr>
<td>( \varepsilon_y )</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( \sigma_y )</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{ult} )</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma_{ult} )</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

The initial elastic modulus is correlated with all the variables listed in the table. In particular the correlation is negative with the yield strain \( (r = -0.55, p = 0.001) \) and with the ultimate strain \( (r = -0.49, p = 0.012) \) and positive with the yield stress \( (r = 0.61, p = 0.000) \).
and with the ultimate stress \( r = 0.76, p = 0.000 \). It has been found a strong positive correlation between \( \sigma_y \) and \( \sigma_{ult} \) \( r = 0.88, p = 0.000 \). As in other similar studies, \( \varepsilon_y \) and \( \varepsilon_{ult} \) do not depend on \( \sigma_y \) and \( \sigma_{ult} \) \( p > 0.05 \) respectively. In some of those works it was observed a correlation between \( \varepsilon_y \) and \( \varepsilon_{ult} \) \( r = 0.30, p = 0.139 \).

The damage \( (D) \), defined in Eq. (2.5) as degradation in modulus, was in the range of 3.6% – 83.1%. It depends, in a monotonically increasing nonlinear fashion, on the magnitude of the plastic strain. As adopted in [15], it has been used the following type of function

\[
D = \frac{a \cdot \varepsilon_p}{b + \varepsilon_p}
\]

Eq. (3.1)

A nonlinear regression has been performed to fit the data. The \( R^2 \) resulting is 78.8% and the parameters estimate with the standard error and the 95% confidence interval are summerized in the table below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE Estimate</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>0.954</td>
<td>0.094</td>
<td>(0.787; 1.249)</td>
</tr>
<tr>
<td>(b)</td>
<td>1.388</td>
<td>0.398</td>
<td>(0.725; 2.734)</td>
</tr>
</tbody>
</table>

The Figure 3.1 shows the fitted curve that describe the relationship between the damage and the plastic strain.

![Figure 3.1 – Dependence of the damage on plastic strain](image)
The estimated parameters obtained in this study are different from the ones of [15] (a = 1.11, b = 0.751), because their samples were extracted from the lumbar spine of human cadavers. In fact the values computed in [14] (a = 1.17, b = 1.449), another work whose samples were taken from bovine proximal tibia, confirm that the source of the specimens influence the estimate of the parameter. The Figure 3.2 represents the different trends of the damage for porcine (our study), bovine (Keaveny et al., 1994) and human samples (Keaveny et al., 1997 and Kopperdahl et al., 2000). In all these studies the damage and the plastic strain are defined in the same way. It is interesting notice that inducing a plastic deformation of 5%, the human trabecular bone shows a damage of about 0.95, instead the porcine trabecular bone is affected by a damage of about 0.7. This means that the residual elastic modulus of the porcine samples loaded to 5% of strain is six times higher compared with the human one. This different resistance to deformation derived from different trabecular bone architecture. For example, as presented previously, the volume fraction of the samples used in this study is considerably greater than the human one.

![Figure 3.2 – Comparison of the damage between human and animal samples](image)

The plastic strain and the residual strain are strongly correlated (r = 0.95, p = 0.000). It has been prefered to adopt the plastic strain as independent variable in the nonlinear regression analysis, because it is available a greater number of plastic strains’ measurements.

As the damage, also the dissipation energy depend on the plastic strain (r = 0.93, p = 0.000) and there is a positive correlation between damage and dissipation energy (r = 0.79, p
3.2 Correlation analysis

This latter result is useful from a practical point of view, because the measurement of the energy can be performed after the first unloading of the specimen, carried out the mechanical tests quicker, and the damage can be computed as function of the dissipation energy.

3.2.4 Correlation between clinical, morphological and mechanical parameters

Table 3.9 shows the results of the correlation between clinical and morphological parameters.

<table>
<thead>
<tr>
<th></th>
<th>BV/TV</th>
<th>BS/BV</th>
<th>BS/TV</th>
<th>Tb.Th</th>
<th>Tb.Sp</th>
<th>SMI</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.61</td>
<td>-0.67</td>
<td>NS</td>
<td>0.63</td>
<td>NS</td>
<td>-0.42</td>
<td>NS</td>
</tr>
<tr>
<td>TBS</td>
<td>-0.46</td>
<td>0.38</td>
<td>NS</td>
<td>-0.35</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

There is a positive correlation between BMD and BV/TV \((r = 0.61, p = 0.000)\); as expected, this indicates that a DXA scan is not able to measure only the density of the trabeculae, but it takes into account also the voids of which the trabecular bone is composed. Unfortunately in this study it is not been spotted the relationship between BMD and Tb.Sp \((r = -0.04, p = 0.814)\), contrary to what obtained in other similar works, such as [8] and [25]. In support of what previously explained, BMD is positively correlated with Tb.Th \((r = 0.63, p = 0.000)\), that affects not only BV/TV but also BS/BV, as earlier observed. This explains the negative correlation between BMD and SMI \((r = -0.42, p = 0.007)\), confirming that a plate-like structure is more dense compared with a rod-like one.

TBS is a clinical parameter providing an indirect yet correlated evaluation of trabecular microarchitecture. Infact it is correlated with BV/TV \((r = -0.46, p = 0.003)\), with BS/BV \((r = 0.38, p = 0.016)\) and Tb.Th \((r = -0.35, p = 0.025)\). As for BMD, the correlation between TBS and Tb.Sp \((r = 0.13, p = 0.414)\) is not been found in this study, whereas from [8] and [27] it results that TBS is able to predict Tb.Sp. Lastly there is a weak correlation between TBS and SMI \((r = 0.30, p = 0.062)\).

In Table 3.10 the Pearson’s coefficients are reported, as results of the correlation between clinical and mechanical parameters.
Table 3.10 – Pearson’s correlation coefficients between clinical and mechanical parameters

<table>
<thead>
<tr>
<th></th>
<th>$E_0$</th>
<th>$\varepsilon_y$</th>
<th>$\sigma_y$</th>
<th>$\varepsilon_{ult}$</th>
<th>$\sigma_{ult}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.41</td>
<td>NS</td>
<td>0.49</td>
<td>NS</td>
<td>0.61</td>
</tr>
<tr>
<td>TBS</td>
<td>NS</td>
<td>-0.41</td>
<td>-0.38</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMD is positively correlated with $E_0$ ($r = 0.41, p = 0.011$), with $\sigma_y$ ($r = 0.49, p = 0.003$) and with $\sigma_{ult}$ ($r = 0.61, p = 0.001$). These results confirm the findings of other investigators, such as [25], that BMD provides a useful method for the prediction of the mechanical quality of trabecular bone.

TBS results to be weakly correlated with $\varepsilon_y$ ($r = -0.41, p = 0.015$) and with $\sigma_y$ ($r = -0.38, p = 0.027$). It is believed that these relationships obtained are a pure coincidence, because from the correlation analysis so far presented but also from the upcoming ones, $\varepsilon_y$ is linked only with $E_0$ and it is not predicted by other variables. Furthermore it makes no sense that TBS is correlated with $\sigma_y$ and not with $\sigma_{ult}$ ($r = -0.12, p = 0.567$), due to their strong dependence. Only few works discuss about this topic, mainly because they are focused on the relationship between BMD and the mechanical properties since TBS is used as support to BMD in the prediction with regard of the fracture risk. One example is [22] in which it has been spotted that TBS is correlated with $E_0$.

Lastly it has been studied the correlation between mechanical and morphological parameters. It has been found only a significant correlation between $\sigma_y$ and SMI ($r = -0.43, p = 0.011$), between $\sigma_{ult}$ and SMI ($r = -0.46, p = 0.020$), between $\sigma_{ult}$ and DA ($r = 0.43, p = 0.032$). The results obtained are not satisfactory, because the mechanical variables depend on the microstructure of the trabecular bone, as demonstrated by other studies.

Even if the aim of this work is not to analyze the relationships of the different variables, it is believed useful and interesting to present the principal results of the correlation analysis between mechanical and morphological variables, obtained from another similar study on porcine vertebral trabecular bone [24]:

“For mechanical properties obtained, all micro-architectural parameters except TV, TS and DA had some moderate correlation with $E_0$ ($R^2 > 0.5$). This observation is also similar for $\sigma_y$ and $\sigma_{ult}$. In contrast, no micro-architectural parameter correlated well with $\varepsilon_y$ and $\varepsilon_{ult}$.”

As expected, the microstructure of the trabecular bone affects its mechanical properties. In particular, an increase of stiffness and strength can be achieved with a greater volume
fraction. The characteristics of the trabeculae, as their size and shape, affect the mechanical response of the bone. In addition, the distance between the trabecular bone is a significant factor. The orientation of the substructures instead has slight correlation with $\sigma_y$ and $\sigma_{ult}$.

The Figure 3.3 summarizes the results of the correlation analysis carried out in this study. The positive correlations are expressed with a line and the negative correlations with a dash line.

*Figure 3.3 – Summary diagram of the correlation analysis’ results*
In this chapter, it is presented the results of the analysis carried out in order to understand which initial bone morphology has less tendency to damage, in other words it is wanted to figure out which morphology is indicative of a healthy bone. Furthermore, another question that is answered is which parameter is altered due to the compression applied on the samples. These two analyses are discussed in two different paragraphs.

4.1 Analysis on damage in relation to the initial morphology

The first part of this chapter is aimed to determine which morphological and clinical parameters indicate greater resistance to mechanical damage. This analysis is performed since the samples loaded to the same total strain underwent a different modulus reduction. Specimens with a greater value of damage (D) loaded to a certain total applied strain are weaker than specimens with lower D loaded in the same way. What distinguishes these specimens is their morphology. Figure 4.1 shows how the damage changes within the same mechanical group.
A multiple linear regression has been performed using Minitab®. The backward elimination approach has been adopted as suggested in the medical statistics field, in order to, starting with all candidate variables, delete the statistically insignificant variables. The Alpha-to-Remove significance level is set to 0.10. The dependent variables is damage (D) and independent variables, included in the initial model, are the total applied strain ($\varepsilon_t$), all the morphological variables, the trabecular bone score (TBS) and the bone mineral density (BMD). $\varepsilon_t$ has been included in every model. The normality distribution of the standardized residuals has been checked with an Anderson–Darling’s test and it has been also verified if there are any outlier in the analysis, checking that the standardized residuals belong to the interval [-3,+3]. It has also been ascertain that lack-of-fit is greater than 0.05. The results of the regression analysis are reported in Table 4.1.

<table>
<thead>
<tr>
<th></th>
<th>$DF$</th>
<th>$Adj SS$</th>
<th>$Adj MS$</th>
<th>$F$-Value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>2</td>
<td>1.38</td>
<td>0.69</td>
<td>63.47</td>
<td>0.000</td>
</tr>
<tr>
<td>BV/TV</td>
<td>1</td>
<td>0.10</td>
<td>0.10</td>
<td>9.07</td>
<td>0.005</td>
</tr>
<tr>
<td>$\varepsilon_t$</td>
<td>1</td>
<td>1.29</td>
<td>1.29</td>
<td>118.97</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>29</td>
<td>0.31</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>1.69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.1 Analysis on damage in relation to the initial morphology

The following model, whose adjusted $R^2$ is 80.12%, is obtained in the last step of stepwise procedure

$$D = 0.651 - 0.014 \frac{BV}{TV} + 0.136 \varepsilon_t$$ \hspace{1cm} Eq. (4.1)

As expected, $D$ depends on $\varepsilon_t$, and, among all the parameters in examination, $BV/TV$ is the only remaining factor. This can be explained considering the results of the correlation analyses which show that $BV/TV$ is correlated with all clinical and morphological parameters, whose effects on the damage is obscured by $BV/TV$. Figure 4.2 shows the main effect plot that illustrates how a response variable ($D$) relates to $BV/TV$ and $\varepsilon_t$. It is possible to see how $D$ is affected by $BV/TV$ and by $\varepsilon_t$. The slop of the line related to $\varepsilon_t$ is steeper, indicating that $D$ is more influenced by $\varepsilon_t$ than $BV/TV$. Figure 4.3 represents the fitted response related to two continuous variables. A contour plot provides a two-dimensional view where all points that have the same response are connected to produce contour lines of constant responses.

![Main effects plot for Damage](image1)

**Figure 4.2 – Main effects plot for Damage**

![Contour Plot Damage vs BV/TV; Total Applied Strain](image2)

**Figure 4.3 – Contour Plot Damage vs BV/TV; Total Applied Strain**
In order to understand if a relationship of an individual variable with the modulus degradation exists, different multiple linear regression analyses have been carried out. In all of them the dependent variable is the damage and as independent variable the total applied strain and a different variable for each analysis. These regression analyses have been performed because, as mentioned before, the effects of the micro-architecture parameters (both morphological and clinical) are obscured by BV/TV in the previous regression analysis. The results are summarized in the following table.

Table 4.2 – Linear regression analysis: Damage vs Variable; Total Applied Strain

<table>
<thead>
<tr>
<th>Variables</th>
<th>p-value</th>
<th>( \varepsilon_t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.033</td>
<td>0.000</td>
</tr>
<tr>
<td>TBS</td>
<td>0.448</td>
<td>0.000</td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.005</td>
<td>0.000</td>
</tr>
<tr>
<td>BS/BV</td>
<td>0.014</td>
<td>0.000</td>
</tr>
<tr>
<td>BS/TV</td>
<td>0.641</td>
<td>0.000</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>0.085</td>
<td>0.000</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>0.247</td>
<td>0.000</td>
</tr>
<tr>
<td>SMI</td>
<td>0.014</td>
<td>0.000</td>
</tr>
<tr>
<td>DA</td>
<td>0.931</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Looking at Table 4.2, not only BV/TV affects damage, but also other morphological properties: BS/BV and SMI are significant factors. It can be noticed that also BMD is statistically significant. The following regression equation \( (R_{adj}^2 = 77.75\%) \) is obtained.

\[ D = 0.476 - 0.886 \text{BMD} + 0.123 \varepsilon_t \]  

\( Eq. (4.2) \)

Therefore, it can be concluded that BMD can be used as predictor of bone resistance to load and fracture. On the other hand, TBS is not correlated with \( D \), indicating that TBS cannot be adopted as index of predisposition to damage.

The Figure 4.4 and Figure 4.5 are the main effects plot and the contour plot obtained from the linear regression analysis with BMD and \( \varepsilon_t \) as predictor variables. Making a comparison with the similar plots related to BV/TV and reported above, it can be concluded that the effect of BV/TV and of BMD on \( D \) is quite similar. In fact, considering the range of values obtained in this study, the slope of their line is comparable. In particular, the estimated effect of changing BV/TV from 32.5% to 49.6% (minimum and maximum value of BV/TV respectively) is a reduction of damage of 0.155 (between 0.074 and 0.384 with 95%
confidence) and the estimated effect of changing BMD from 0.292 g/cm$^2$ to 0.533 g/cm$^2$ is a reduction of damage of 0.194 (between 0.018 and 0.408 with 95% confidence).

![Main effects plot for Damage](image1.png)

**Figure 4.4 – Main effects plot for Damage**

![Contour Plot Damage vs BMD; Total Applied Strain](image2.png)

**Figure 4.5 – Contour Plot Damage vs BMD; Total Applied Strain**

As mentioned previously, SMI can be used as predictor variable of damage. This indicates that also the shape of the trabeculae is a significant morphological indicator able to estimate the strength and the tendency to damage. Passing from rod-like architecture to a plate-like one, that is reducing SMI, it can be observed a reduction of damage. The following regression equation ($R^2_{adj} = 79.04\%$) is obtained

$$D = -0.030 + 0.087 \text{ SMI} + 0.143 \epsilon_t$$

*Eq. (4.3)*

The estimated effect of changing SMI from 2.143 to 0.340 is a reduction of damage of 0.158 (between 0.006 and 0.322 with 95% confidence).
4.2 Influence of the compression on the measured variables

DXA scans and μCTs have been performed twice, before and after the mechanical test, in order to measure the variation of the clinical and morphological parameters due to the induced damage.

The following tables report the mean values (Table 4.3) and the standard deviations (Table 4.4) of the parameters measured before and after the compression test divided in the four groups loaded with a different value of strains (1%, 2%, 3.5% and 5%).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1%</th>
<th>Group 2%</th>
<th>Group 3.5%</th>
<th>Group 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD [g/cm²]</td>
<td>0.45 0.42 0.41 0.37 0.42 0.38 0.41 0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBS [-]</td>
<td>1.22 0.81 1.19 0.47 1.21 0.50 1.15 0.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E [MPa]</td>
<td>1687.38 1674.04 1607.04 845.28 1627.12 672.08 1544.41 383.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV [%]</td>
<td>44.64 37.30 39.57 31.98 40.98 33.16 43.63 35.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS/BV [1/mm]</td>
<td>9.55 9.20 11.00 10.67 10.60 10.34 10.41 9.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS/Tv [1/mm]</td>
<td>4.19 3.32 4.33 3.39 4.33 3.37 4.51 3.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.31 0.29 0.26 0.24 0.26 0.26 0.25 0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.Sp [mm]</td>
<td>0.46 0.48 0.47 0.50 0.45 0.48 0.38 0.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMI [-]</td>
<td>1.06 0.78 1.49 1.03 1.38 0.96 0.82 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA [-]</td>
<td>0.51 0.55 0.54 0.61 0.57 0.63 0.58 0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1%</th>
<th>Group 2%</th>
<th>Group 3.5%</th>
<th>Group 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD [g/cm²]</td>
<td>0.08 0.07 0.05 0.05 0.05 0.05 0.06 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBS [-]</td>
<td>0.18 0.22 0.08 0.19 0.14 0.13 0.10 0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E [MPa]</td>
<td>545.97 595.04 532.98 262.05 423.00 222.28 381.22 120.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV [%]</td>
<td>6.28 7.27 4.58 3.53 2.55 4.36 3.99 4.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS/BV [1/mm]</td>
<td>1.40 1.81 1.22 0.86 0.59 1.61 0.99 1.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS/Tv [1/mm]</td>
<td>0.34 0.23 0.49 0.24 0.26 0.18 0.23 0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.07 0.05 0.03 0.01 0.02 0.04 0.03 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.Sp [mm]</td>
<td>0.05 0.06 0.08 0.06 0.05 0.03 0.10 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4 – Standard deviation of variables measured before and after the damage

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1% Before</th>
<th>Group 1% After</th>
<th>Group 2% Before</th>
<th>Group 2% After</th>
<th>Group 3.5% Before</th>
<th>Group 3.5% After</th>
<th>Group 5% Before</th>
<th>Group 5% After</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMI [-]</td>
<td>0.57</td>
<td>0.78</td>
<td>0.37</td>
<td>0.38</td>
<td>0.42</td>
<td>0.51</td>
<td>0.51</td>
<td>0.66</td>
</tr>
<tr>
<td>DA [-]</td>
<td>0.09</td>
<td>0.07</td>
<td>0.08</td>
<td>0.03</td>
<td>0.08</td>
<td>0.06</td>
<td>0.10</td>
<td>0.07</td>
</tr>
</tbody>
</table>

The following figures represent for each variable on the left the mean values computed for each group before (light colors) and after the mechanical test (dark color) with the standard deviations and on the right the percent variations of that variable as reported below.

\[
\text{Percent variation } (G_i) = \frac{\text{mean}(G_i)_{\text{after}} - \text{mean}(G_i)_{\text{before}}}{\text{mean}(G_i)_{\text{before}}} \quad \text{Eq. (4.4)}
\]

Since the samples were grouped into four groups loaded until a different value of strains, the variables are represented divided into the four groups. In order to draw a graph of the collected data, it is not possible to include the effects of the animal and of the vertebra that have been considered in the statistical analysis described afterwards.

Figure 4.6 – (Left) Comparison of the variables measured before (light colors) and after (dark colors) the damage with the standard deviation. (Right) Percent variation of the analyzed variables.
ANALYSIS ON MORPHOLOGICAL AND CLINICAL PARAMETERS AND EFFECT OF INDUCED DAMAGE
4.2 Influence of the compression on the measured variables

- Bone Surface to Bone Volume
- Bone Surface to Total Volume
- Trabecular Thickness

Graphs showing the percent variation of BV/TV and BSDV/TV for different compression levels (G1%, G2%, G3.5%, G5%).
ANALYSIS ON MORPHOLOGICAL AND CLINICAL PARAMETERS AND EFFECT OF INDUCED DAMAGE
Influence of the compression on the measured variables

4.2

From the chart of the elastic modulus, it can be ensured that the damage is been induced experimentally. In fact the modulus degradation, that is a direct measure of mechanical damage which is related to microdamage accumulation in the bone tissue, is evident for the group 2%, 3.5% and 5%. As expected the samples belonging to the group 1% are not damaged, because they have been loaded to 1% of strain which is the yield strain.

It can be noticed that SMI is reduced after the compression test. A first reason that explain this reduction is a shape variation of the trabeculae that assume a plate-like geometry. Secondly the rod-like trabeculae can be broken as a result of the applied load and can be cleaned off by the saline solution used during the second series of μCT. Basically the latter reason justifies the variation of SMI as a percentage reduction in the number of rod-like trabeculae.

The hypothesis of the broken trabeculae can justify the reduction of the BMD, in fact the load applied during the compression test is not supposed to vary density, but only the bone microstructure.

The bone loss entails the reduction of bone volume fraction and of the bone surface to total volume. The drop of number of trabeculae implicate a greater space between them, in fact this can be observed by the chart relative to the trabecular spacing.

Looking at the percent reduction chart of the trabecular thickness, it can be noticed a trend. It is believed that this trend is illusory, because the percentage of reduction, which is in the range of -7.5% and 4.5%, can be associated to the variability due to the uncertainty of the measurement. Same considerations can be considered valid for the bone surface to bone volume. For the degree of anisotropy, it is difficult to interpret its chart, but there seems to be an increase.

It is clear from the charts that TBS is able to detect the damage of the bone however without quantify it, since the group 2%, 3.5% and 5% have similar values of percent reduction. It can be noticed that also the samples of group 1%, loaded to yield point, have lower TBS values compared to the ones measured before the compression test. This worsening of the trabecular microstructure is attributed to the bone loss and not to the degradation of the mechanical properties, in fact the percent modulus reduction of the group 1% is almost zero.

After analyzing the data through a qualitative description of the graphs above reported, statistics analyses are carried out in order to verify whether the variables change after the compression test and check if exist a group to which is associated a greater variation.

An analysis of covariance (ANCOVA) has been performed with SAS in order to test if the variation of the measured variables differs between the groups. The animal, the vertebra and the group are the factors and the variable measured before the mechanical testing (Pre-damage Var.) is the covariance. The dependent variable is the difference of the variable in examination between after and before the compression test. The least squares means (LS-means) of the estimated main effects has been computed (using LSMEANS statement in SAS) as an
estimation of the mean variation of each group. When missing values occur, as in this study, the LS-mean are preferred to arithmetic means, because they reflect the model that is being fit to the data. LS-means are also used when a covariate appears in the model such as in ANCOVA. One-sample Student’s t-test has been carried out (with the MIXED procedure) to test the null hypothesis that LS-means equals zero. The resulting p-values are listed below. Adopting the Bonferroni correction, the significance level is set to 0.0125.

### Table 4.5 – Table of p-values from one-sample Student’s t-test

<table>
<thead>
<tr>
<th>Variables</th>
<th>G1%</th>
<th>G2%</th>
<th>G3.5%</th>
<th>G5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.266</td>
<td>0.013</td>
<td>0.010</td>
<td>0.003</td>
</tr>
<tr>
<td>TBS</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>E</td>
<td>0.306</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.011</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>BS/BV</td>
<td>0.042</td>
<td>0.866</td>
<td>0.266</td>
<td>0.048</td>
</tr>
<tr>
<td>BS/TV</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>0.278</td>
<td>0.028</td>
<td>0.701</td>
<td>0.136</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>0.037</td>
<td>0.000</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>SMI</td>
<td>0.430</td>
<td>0.241</td>
<td>0.045</td>
<td>0.000</td>
</tr>
<tr>
<td>DA</td>
<td>0.105</td>
<td>0.055</td>
<td>0.000</td>
<td>0.010</td>
</tr>
</tbody>
</table>

All the parameters, apart from BS/BV and Tb/th, underwent a change as a consequence of the induced damage, as it can be observed from Figure 4.6. TBS, BV/TV and BS/TV vary in every groups and the remaining parameters (BMD, E, Tb.Sp, SMI, DA) are affected only for some groups, in particular for G1% E, as expected, is not changed (p = 0.306). Since Tb.Th remains unvaried, the trabeculae are not deformed due to the compression. Therefore the reason that explain the reduction of SMI for the samples belonging to the group G5% is the break of the rod-like trabeculae. In other words the trabecular number (Tb.N) is reduced. The parallel plate model for trabecular bone structure supports this hypothesis, in fact, applying it, the trabecular number is given by

\[ \text{Tb. N} = \frac{1}{Tb. Th + Tb. Sp} \]

\textbf{Eq. (4.5)}

Since Tb.Th remains constant and Tb.Sp is increased, consequently \textbf{Eq. (4.5)} confirms the reduction of Tb.N.

The results of the ANCOVA test are summerized in the following table, in which the statistically significant effect are highlighted.
Table 4.6 – Table of p-values from ANCOVA tests

<table>
<thead>
<tr>
<th>Variables</th>
<th>Animal</th>
<th>Vertebra</th>
<th>Pre-damage Var.</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>0.075</td>
<td>0.014</td>
<td>0.001</td>
<td>0.047</td>
</tr>
<tr>
<td>TBS</td>
<td>0.680</td>
<td>0.214</td>
<td>0.000</td>
<td>0.054</td>
</tr>
<tr>
<td>E</td>
<td>0.222</td>
<td>0.521</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.036</td>
<td>0.643</td>
<td>0.001</td>
<td>0.463</td>
</tr>
<tr>
<td>BS/BV</td>
<td>0.010</td>
<td>0.389</td>
<td>0.024</td>
<td>0.374</td>
</tr>
<tr>
<td>BS/TV</td>
<td>0.004</td>
<td>0.157</td>
<td>0.000</td>
<td>0.878</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>0.021</td>
<td>0.010</td>
<td>0.125</td>
<td>0.069</td>
</tr>
<tr>
<td>Tb.Sp</td>
<td>0.052</td>
<td>0.022</td>
<td>0.000</td>
<td>0.418</td>
</tr>
<tr>
<td>SMI</td>
<td>0.036</td>
<td>0.060</td>
<td>0.003</td>
<td>0.200</td>
</tr>
<tr>
<td>DA</td>
<td>0.000</td>
<td>0.463</td>
<td>0.000</td>
<td>0.194</td>
</tr>
</tbody>
</table>

From the above table, it can be concluded that the variation associated to BV/TV, BS/TV, Tb.Sp, SMI and DA do no depend on the group. A Tukey’s HSD test has been adopted as multiple comparison procedure to test which group is associated with a greatest variation. The test has been performed only for BMD, TBS, E and Tb.Th, because the p-values for Group is less than 0.05 or its value is borderline. In order to present the results, reported in the following table, it has been assigned for each level a letter; those levels that do not share a letter are significantly different.

Table 4.7 – Results of Tukey’s HSD test

<table>
<thead>
<tr>
<th>Variables</th>
<th>G1%</th>
<th>G2%</th>
<th>G3.5%</th>
<th>G5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>TBS</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>E</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

The results of Tukey’s HSD tests confirm for TBS and Tb.Th and underline for BMD that Group is not a statistically significant factor. Instead for E, G1% is statistically different from G2%, G3.5% and G5%, which instead do not differ.

Comparing the results of one-sample Student’s t-test (Table 4.5) and the ones of the ANCOVA and Tukey’s HSD test (Table 4.6 and Table 4.7), it can be noticed a inconsistency for BMD, Tb.Sp, SMI and DA. Anyway it can be stated that BMD, Tb.Sp, SMI and DA underwent
a variation only for some groups, but there is no evidence to conclude that those variation are different, probably due to low power of test (ANCOVA). Same conclusion can be drawn for TBS, whose chart shows a difference, between G1% and the remaining group, that is not statistically significant, probably due to the high dispersion of the collected data.

As a conclusion, it can be stated that the plastically deformed specimens, belonging to the group G2%, G3.5% and G5%, have not been damaged differently; in fact, the variations of E for the groups G2%, G3.5% and G5% are not statistically significant. This may be the reason why there is no evidence that a dependence between loading condition and morphological variations exists.
CHAPTER 5
CONCLUSIONS AND FUTURE DEVELOPMENTS

This concluding chapter of the dissertation aims to give a global point of view about this research work, highlighting what has been done and future developments.

In this study several and different kinds of measurements have been performed on samples from the lumbar porcine vertebrae, to obtain a vast amount of information about the effective bone resistance. BMD and TBS (clinical parameters) were obtained by DXA measurements. Then micro-computed tomography (μCT) have been adopted to measure the most important morphological parameters that describe the three-dimensional structure of the trabecular bone. Besides, compression test on the samples allowed to determine the mechanical strength and stiffness of the bones.

The relationship between the measured parameters has been study with a correlation analysis and the main results are here summerized. BV/TV is well correlated with all the morphological and clinical parameters. BMD is a good predictor of the mechanical properties, as SMI, and principal 3D bone microarchitecture parameters.

The main topic of this study is the accumulation of damage. This topic is important because vertebral fractures in nontraumatic events are the consequences of damage accumulation and permanent deformation in the vertebral body. The accumulation of damage forms microcracks resulting in degradation of stiffness and permanent residual strain. From this study it has been demonstrated that BV/TV and BMD are good and similar index of resistance to damage, proving that BMD is a clinical measure to quantify fracture risk. In addition SMI results to be a good predictor of the modulus degradation. This last conclusion confirms what was assumed in the introduction of this dissertation, namely that the propensity
to brittle fracture is explained not only by BMD but also by SMI. This calls for orientating a research to identify a non-invasive technique for the measurement of the SMI.

To consider the effect of the accumulation of damage on the different parameters, DXA and μCT measurements were performed on a set of samples after the mechanical damage tests. Comparing the values of parameters before and after the compression test, the result is that the micro-architecture is changed. Considering the reduction of SMI and BV/TV, it has been assumed that the rod-like trabeculae were broken as a consequence of the induced damage and washed away by the saline solution used for the μCT. This assumption explains the increase of Tb.Sp and the reduction of BMD, BS/TV. DA results to be higher after the compression test probably as a consequence of the degradation of the micro-architecture. TBS is the parameter that underwent the maximum reduction. Tb.Th was not altered, indicating that the compression test is not able to deform the trabeculae.

In this study the samples were embedded in aluminium endcaps, because in preliminary compression test the samples without endcaps underwent a localized deformation at their top. The alignment between sample and endcap, even if it was done with a homemade tool, was not perfect for same samples. This is believed be a source of error which has not allowed, for example, to verify the correlation between mechanical and morphological parameters. In order to avoid the application of the endcaps, it would have been better to consider the full vertebra that likely would not undergo a localized deformations due to the presence of the the cortical bone.

The design of experiment adopted to mechanically test the samples was similar to an unbalanced Latin Square design with animals and vertebra as blocking factor and groups as factor. But the groups were tested with different number of samples. Maybe it would have been better from a statistical point of view to test the groups with greater and equal number of samples.

In order to study the accumulation of damage a fatigue test can be performed, instead of a compression test. A series of DXA and μCT can be performed after a predefined number a cycles, in order to monitor with more attention the variations of the morphological and clinical parameters. Moreover the fatigue test can simulate better than a compression test the load at which bones are subjected in real-life.
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Table 4.6 – Table of p-values from ANCOVA tests
Table 4.7 – Results of Tukey’s HSD test


A.1 Computation of the external boundary

clear all
clc

% Load the datasheet
datasheet = readtable('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Datasheet.xlsx');

% Folder of the images
folder_image = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\CT scans\jpeg_individual_cropped_samples';

% Folder of where you want to save the results
folder_results = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\CT scans\Area - BV';

% Folder of the modified images without background
folder_no_background = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\CT scans\images_no_background';

% Number of samples
n_samples = 40;

% Set the resolution
resolution = 25.68*1e-3;

% Insert the samples whose background has to be deleted
samples_background = [13,18,19,20,21];

for i = 1:n_samples
    name_sample = char(datasheet.Name(i));
    name_sample = name_sample(9:end);
    folder = fullfile(folder_image,name_sample);
    file_samples = dir(fullfile(folder,'*.tif'));

% Create a folder for the images whose background has to be deleted
if sum(i==samples_background) == 1
    new_folder = strcat(name_sample,'_no_background');
    mkdir(fullfile(folder_no_background,new_folder))
end

for j = 1:length(file_samples)
    name_with_folder = fullfile(folder,file_samples(j).name);
    BW = imread(name_with_folder);
    % Conversion of BW in a matrix of 1s and 0s
    BW = im2double(BW);
    % Eliminate particle with size lower than 30 pixel
    BW30 = bwareaopen(BW,30);
    % Create a matrix data n by 2. The first column contain the x-value
    % of the points and the second column the y-value
    [row,col] = find(BW30);
    row = size(BW30,1)-row+1;
    data(:,1) = col;
    data(:,2) = row;
    % Calculation of the boundary and the area with a shrink factor of 0.1
    [k,area(j,1)] = boundary(data(:,1),data(:,2),0.1);
    area(j,1) = area(j,1)*(resolution^2);
    % Create the mask for canceling what is outside the boundary
    mask30 = roipoly(BW30,data(k,1),data(k,2));
    mask30 = 1-mask30;
    mask30 = mask30([size(BW30,1):-1:1],:);
    BW_clean = BW-mask30;
    matrix_minus_1 = BW_clean==1;
    BW_clean = BW_clean+matrix_minus_1;
    % Calculation of BV
    BV(j,1) = sum(sum(BW_clean))*(resolution^3);
    if sum(i==samples_background) == 1
    % BW_modified is obtained subtracting mask to the BW
    BW_modified = mask30+BW;
    matrix_plus_2 = BW_modified==2;
    BW_modified = BW_modified+matrix_plus_2;
    % Save the image for the calculation of the trabecular spacing
    name_samples_modified = strcat(file_samples(j).name(1:end-4),'_no_background.tif');
    path_name_modified = fullfile(fullfile(folder_no_background,new_folder),name_samples_modified);
    imwrite(BW_modified,path_name_modified);
    end
    clear data
    fprintf('
%s',strcat(name_sample,'=',num2str(j)))
end
results_table = table(BV,area,'VariableNames',{BV,'area'});
name_file_variable = strcat(name_sample,'.txt');

writetable(results_table(fullfile(folder_results,name_sample),',',','))

clear area
clear BV

end

A.2 Function for the segmentation of the preliminary and loading cycles

function [points,strain_limit] = points(time_filtered,strain_filtered)
% INPUT: time_filtered is the a vector related of the time filtered
% strain_filtered is the a vector related of the strain filtered
% OUTPUT: points is a vector 13x1
% Point 1: 1st point of the 1st preliminary cycle
% Point 2: point corresponding of the max strain value in the
1st preliminary cycle
% Point 3: last point of the 1st preliminary cycle that is the
1st point of the 2nd preliminary cycle
% Point 4: point corresponding of the max strain value in the
2nd preliminary cycle
% Point 5: last point of the 2nd preliminary cycle that is the
1st point of the 3rd preliminary cycle
% Point 6: point corresponding of the max strain value in the
3rd preliminary cycle
% Point 7: last point of the 3rd preliminary cycle that is the
1st point of the 1st loading cycle
% Point 8: point corresponding of the max strain value in the
1st loading cycle
% Point 9: last point of the 1st loading cycle that is the 1st
point of the 2nd loading cycle
% Point 10: point corresponding of the max strain value in the
2nd loading cycle
% Point 11: last point of the 2nd loading cycle that is the 1st
point of the 3rd loading cycle
% Point 12: point corresponding of the max strain value in the
3rd loading cycle
% Point 13: last point of the 3rd loading cycle
% strain limit is a vector 13x1, similar to the vector points but
% with the strain value

% The point 1 is the first point of the test
points(1,1) = 1;
strain_limit(1,1) = strain_filtered(1);

% The computation of the points is based on finding where there is a jump
% in the time vector
% Set the minimum difference in order to define the jump
Appendix A
MATLAB CODE

toll = 0.0005;
t = 2;
for i = 2:length(strain_filtered)
    strain_old = strain_filtered(i-1);
    strain_new = strain_filtered(i);
    if abs(strain_new - strain_old) > toll
        strain_limit(t,1) = strain_filtered(i-1);
        strain_limit(t+1,1) = strain_filtered(i);
        t = t+2;
    end
end

j = 2;
for i = 1:length(strain_filtered)
    if abs(strain_filtered(i)-strain_limit(j)) < 0.000001
        points(j) = i;
        j = j+1;
    end
    if j > length(strain_limit)
        break
    end
end

A.3 Computation of the stiffness

% Folder of the damage test
folder_damage_test = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Porcine Damage test\Tests';

% Folder of the damage test filtered
folder_damage_test_filtered = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Filtered damage test';

% Folder of the stress-strain curve with the regression curve
folder_regression = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Stress_strain curve with E';

% Folder of the stress-strain curve with the regression curve - Damaged stiffness
folder_regression_damaged = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Stress_strain curve with E damaged';

% Position of the mechanical parameters excel file
mech_par_excel = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx';

% Load the datasheet
datasheet = readtable('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Datasheet.xlsx');

% Load the max strain value at which I stopped the valuation of the stiffness for the FAILED test
max_strain_datasheet = readtable('C:\Users\Dade\Documents\Università\Anno \Semestre II\Tesi\Ossa\Mechanical properties\max_strain_stiffness_FAILED.xlsx');

% Load the txt damage test names
file_samples = dir('C:\Users\Dade\Documents\Università\Anno \Semestre II\Tesi\Ossa\Porcine Damage tests\*.txt');

% Load the txt damage test filtered names
file_samples_filtered = dir('C:\Users\Dade\Documents\Università\Anno \Semestre II\Tesi\Ossa\Mechanical properties\Filtered damage test\*.txt');

% Number of samples
n_samples = 40;

r = 1;
for i = 1:size(file_samples_filtered,1)
    % Load the damage test filtered
    damage_test_name_filtered = file_samples_filtered(i).name;
    damage_test_filtered = dlmread(fullfile(folder_damage_test_filtered,damage_test_name_filtered),',',1,0);
    all_damage_test_name(i,1) = cellstr(damage_test_name_filtered(1:end-13));

    % Change the name of the value filtered
    time_filtered = damage_test_filtered(:,1);
    strain_filtered = damage_test_filtered(:,2)/100;
    load_filtered = damage_test_filtered(:,3);
    stress_filtered = damage_test_filtered(:,4);

    % Compute the points in the time-strain graph
    [points,strain_limit] = points(time_filtered,strain_filtered);

    % Compute the stiffness with the 2nd and 3rd preliminary cycles
    first_point = points(3);
    last_point = points(7);
    if last_point ~= 0
        mdl_1=fitlm(stress_filtered(first_point:last_point),stress_filtered(first_point:last_point));
        E_1(i,1) = mdl_1.Coefficients{2,1};
        intercept_1(i,1) = mdl_1.Coefficients{1,1};
    else
        E_1(i,1) = 0;
        intercept_1(i,1) = 0;
    end

    % Compute the stiffness with the 1st loading cycle
    [max_stress index] = max(stress_filtered);
    j = 0;
    E_2_all = 0;
    intercept_all = 0;
    last_strain = 0;
    if length(damage_test_name_filtered(1:end-13)) == 15
        while last_strain < strain_filtered(index)*0.95
first_strain = strain_filtered(j+points(7));

k = 1;
while strain_filtered(k+j+points(7)) - strain_filtered(j+points(7)) <= 0.002
    k = k+1;
end

last_strain = strain_filtered(k+j+points(7));
mdl_2=fitlm(strain_filtered(j+points(7):k+j+points(7)),stress_filtered(j+points(7):k+j+points(7)));
    E_2_all(j+1) = mdl_2.Coefficients{2,1};
    intercept_all(j+1) = mdl_2.Coefficients{1,1};
    j = j+1;
end
comparison(i,1:2) = [last_strain strain_filtered(index)];
[E_2(i,1) index_E] = max(E_2_all);
intercept_2(i,1) = intercept_all(index_E);

h = 0;
else
    while last_strain < max_strain_datasheet.Max_strain(r)
        first_strain = strain_filtered(j+points(7));
        k = 1;
        while strain_filtered(k+j+points(7)) - strain_filtered(j+points(7)) <= 0.002
            k = k+1;
        end

        last_strain = strain_filtered(k+j+points(7));
        mdl_2=fitlm(strain_filtered(j+points(7):k+j+points(7)),stress_filtered(j+points(7):k+j+points(7)));
            E_2_all(j+1) = mdl_2.Coefficients{2,1};
            intercept_all(j+1) = mdl_2.Coefficients{1,1};
            j = j+1;
        end

        comparison(i,1:2) = [last_strain max_strain_datasheet.Max_strain(r)];
        [E_2(i,1) index_E] = max(E_2_all);
        intercept_2(i,1) = intercept_all(index_E);
        r = r+1;
        h = 1;
    end

    % Compute the stiffness after the damage
    if length(points) > 11
        first_point = points(11);
        last_point = points(12);

        % Checking if in the first part of the third cycle, the strain decreases and in this
        % case I start the evaluation of the stiffness from the lower value
        % of strain
Computation of the stiffness

\[ \text{[initial\_strain\_3rd\_cycle index\_min\_strain]} = \min(\text{strain\_filtered(first\_point:last\_point)}) \]

% Change the first point of this analysis
first\_point = points(11)+index\_min\_strain-1;

j = 0;
E\_damaged\_all = 0;
intercept\_damaged\_all = 0;
last\_strain = 0;

while last\_strain < strain\_filtered(last\_point)
    first\_strain = strain\_filtered(j+first\_point);
    k = 1;
    while strain\_filtered(k+j+first\_point) - strain\_filtered(j+first\_point) <= 0.002
        if k+1+j+first\_point == length(strain\_filtered)
            break
        end
        k = k+1;
    end
    last\_strain = strain\_filtered(k+j+first\_point);
    mdl\_damaged = fitlm(strain\_filtered(j+first\_point:k+j+first\_point),stress\_filtered(j+first\_point:k+j+first\_point));
    E\_damaged\_all(j+1) = mdl\_damaged.Coefficients{2,1};
    intercept\_damaged\_all(j+1) = mdl\_damaged.Coefficients{1,1};
    j = j+1;
end

[E\_damaged(i,1) index\_E] = max(E\_damaged\_all);
intercept\_damaged(i,1) = intercept\_damaged\_all(index\_E);

scatter(strain\_filtered(first\_point:last\_point)*100, stress\_filtered(first\_point:last\_point),10, 'bo','filled')
hold on
plot([strain\_filtered(first\_point)*100:0.01:strain\_filtered(last\_point)*100],...
    intercept\_damaged(i,1)+E\_damaged(i,1)/100*[strain\_filtered(first\_point)*100:0.01:strain\_filtered(last\_point)*100], 'r')
xlabel('Strain [%]')
ylabel('Stress [MPa]')
print(fullfile(folder\_regression\_damaged, strcat(damage\_test\_name\_filtered(1:end-13), '_damaged')), '-dpdf', '-r300')
close
else
    E\_damaged(i,1) = 0;
    intercept\_damaged(i,1) = 0;
end

if h == 0
    if comparison(i,1) < 1.3
        scatter(strain\_filtered*100, stress\_filtered,10, 'bo','filled')
        hold on
        plot([0:0.01:1], intercept\_2(i,1)+E\_2(i,1)/100*[0:0.01:1], 'r')
        plot([0:0.01:1], intercept\_1(i,1)+E\_1(i,1)/100*[0:0.01:1], 'g')
xlim([0 1])
xlabel('Strain [%]')
ylabel('Stress [MPa]')

print(fullfile(folder_regression,damage_test_name_filtered(1:end-13)),'-dpdf','-r300')
close
else
scatter(strain_filtered*100,stress_filtered,10,'bo','filled')
hold on
plot([0:0.01:2],intercept_2(i,1)+E_2(i,1)/100*[0:0.01:2],'r')
plot([0:0.01:2],intercept_1(i,1)+E_1(i,1)/100*[0:0.01:2],'g')
xlabel('Strain [%]')
ylabel('Stress [MPa]')
xlim([0 2])

print(fullfile(folder_regression,damage_test_name_filtered(1:end-13)),'-dpdf','-r300')
close
end
else
if comparison(i,1) < 1.3
scatter(strain_filtered*100,stress_filtered,10,'bo','filled')
hold on
plot([0:0.01:1],intercept_2(i,1)+E_2(i,1)/100*[0:0.01:1],'r')
plot([0:0.01:1],intercept_1(i,1)+E_1(i,1)/100*[0:0.01:1],'g')
line(max_strain_datasheet.Max_strain(r-1)*100, [0 max(stress_filtered)*1.15])
xlim([0 1])
xlabel('Strain [%]')
ylabel('Stress [MPa]')

print(fullfile(folder_regression,damage_test_name_filtered(1:end-13)),'-dpdf','-r300')
close
else
scatter(strain_filtered*100,stress_filtered,10,'bo','filled')
hold on
plot([0:0.01:2],intercept_2(i,1)+E_2(i,1)/100*[0:0.01:2],'r')
plot([0:0.01:2],intercept_1(i,1)+E_1(i,1)/100*[0:0.01:2],'g')
line(max_strain_datasheet.Max_strain(r-1)*100, [0 max(stress_filtered)*1.15])
xlim([0 2])
xlabel('Strain [%]')
ylabel('Stress [MPa]')

print(fullfile(folder_regression,damage_test_name_filtered(1:end-13)),'-dpdf','-r300')
close
end
end

clear points
clear strain_limit

% Save the results in the excel file mechanical_parameters
sheet = 1;
xlRange = 'A2';
A.4 Computation of the residual strain

```matlab
% Folder of the damage test shifted
folder_damage_test_shifted = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test';

% Folder of the stress-strain curve with the plastic strain
folder_plastic_strain = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Stress_strain curve with plastic strain';

% Load the txt damage test shifted names
file_samples_shifted = dir('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test\*.txt');

% Load the mechanical parameters excel file
datasheet_mech_par = readtable('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx');

% Position of the mechanical parameters excel file
mech_par_excel = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx';

for i = 1:size(file_samples_shifted,1)
    % Load the damage test shifted
damage_test_name_shifted = file_samples_shifted(i).name;
damage_test_shifted = dlmread(fullfile(folder_damage_test_shifted,damage_test_name_shifted),',',',',1,0);

    time_shifted = damage_test_shifted(:,1);
    strain_shifted = damage_test_shifted(:,2);
    load_shifted = damage_test_shifted(:,3);
    stress shifted = damage_test_shifted(:,4);

    % Load the E damaged and the intercept damaged
    E = datasheet_mech_par.E_damaged(i)/100;
    intercept = datasheet_mech_par.intercept_damaged(i);

    % Load the shifting deformation
    shifting_def = datasheet_mech_par.shift_def_to_left(i);

    % Calculation of the plastic strain using the regression line of the third cycle
    y = E_damaged * (x + shift_def_to_left) + intercept_damaged
    plastic_strain = -(intercept_damaged / E_damaged) - shift_def_to_left
    % Note: to shift a line towards left, I have to sum the x values
    if E ~= 0
        plastic_strain_1(i,1) = -(intercept/E) - shifting_def;
    else
        plastic_strain_1(i,1) = 0;
    end
```

A.4 Computation of the residual strain

```matlab
par = [E_1 intercept_1 E_2 intercept_2 E_damaged intercept_damaged];
```

```matlab
sheet = 1;
xlRange = 'B2';
xlswrite(mech_par_excel,par,sheet,xlRange)
```

```matlab
A.4 Computation of the residual strain

% Folder of the damage test shifted
folder_damage_test_shifted = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test';

% Folder of the stress-strain curve with the plastic strain
folder_plastic_strain = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Stress_strain curve with plastic strain';

% Load the txt damage test shifted names
file_samples_shifted = dir('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test\*.txt');

% Load the mechanical parameters excel file
datasheet_mech_par = readtable('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx');

% Position of the mechanical parameters excel file
mech_par_excel = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx';

for i = 1:size(file_samples_shifted,1)
    % Load the damage test shifted
damage_test_name_shifted = file_samples_shifted(i).name;
damage_test_shifted = dlmread(fullfile(folder_damage_test_shifted,damage_test_name_shifted),',',',',1,0);

    time_shifted = damage_test_shifted(:,1);
    strain_shifted = damage_test_shifted(:,2);
    load_shifted = damage_test_shifted(:,3);
    stress_shifted = damage_test_shifted(:,4);

    % Load the E damaged and the intercept damaged
    E = datasheet_mech_par.E_damaged(i)/100;
    intercept = datasheet_mech_par.intercept_damaged(i);

    % Load the shifting deformation
    shifting_def = datasheet_mech_par.shift_def_to_left(i);

    % Calculation of the plastic strain using the regression line of the third cycle
    y = E_damaged * (x + shift_def_to_left) + intercept_damaged
    plastic_strain = -(intercept_damaged / E_damaged) - shift_def_to_left
    % Note: to shift a line towards left, I have to sum the x values
    if E ~= 0
        plastic_strain_1(i,1) = -(intercept/E) - shifting_def;
    else
        plastic_strain_1(i,1) = 0;
    end
```
% Calculation of the plastic strain using the last point of the test
if E ~= 0
    plastic_strain_2(i,1) = strain_shifted(end);
stress_residual_2 = stress_shifted(end);
else
    plastic_strain_2(i,1) = 0;
end

% Calculation of the strain level as the maximum shifted strain
[strain_level(i,1) index] = max(strain_shifted);
stress_level(i,1) = stress_shifted(index);
if E ~= 0
    figure
    scatter(strain_shifted,stress_shifted,10,'bo','filled')
    hold on
    plot([0:0.01:5],E*[0:0.01:5]+intercept,'b')
    plot([0:0.01:5],E*[0:0.01:5]+intercept,'r')
    scatter(plastic_strain_1(i,1),stress_residual_1,50,'ko','filled')
    scatter(plastic_strain_2(i,1),stress_residual_2,50,'go','filled')
    scatter(strain_level(i,1),stress_level(i,1),50,'ro','filled')
    xlim([-0.5 max(strain_shifted).*1.1])
    ylim([-2 max(stress_shifted)])
    xlabel('Strain [%]')
    ylabel('Stress [MPa]')
    grid on
    print(fullfile(folder_plastic_strain,strcat(damage_test_name_shifted(1:end-21),'_plastic_strain')),'-dpdf','-r300')
    close
else
    figure
    scatter(strain_shifted,stress_shifted,10,'bo','filled')
    hold on
    scatter(strain_level(i,1),stress_level(i,1),50,'ro','filled')
    xlabel('Strain [%]')
    ylabel('Stress [MPa]')
    grid on
    print(fullfile(folder_plastic_strain,strcat(damage_test_name_shifted(1:end-21),'_plastic_strain')),'-dpdf','-r300')
    close
end

% Save the results in the excel file mechanical_parameters
sheet = 1;
xlRange = 'I2';
xlswrite(mech_par_excel,[plastic_strain_1 plastic_strain_2
strain_level],sheet,xlRange)

A.5 Computation of the yield and ultimate points

% Folder of the damage test shifted
folder_damage_test_shifted = 'C:\Users\Dade\Documents\Università\Anno
\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test';

% Folder of the stress-strain curve with yield and ultimate value
Computation of the yield and ultimate points

folder_yield_ult = 'C:\Users\Dade\Documents\Università\Anno IV\Semestre II\Tesi\Ossa\Mechanical properties\Stress_strain curve with yield and ult';

% Load the txt damage test shifted names
file_samples_shifted = dir('C:\Users\Dade\Documents\Università\Anno IV\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test\*.txt');

% Load the mechanical parameters excel file
datasheet_mech_par = readtable('C:\Users\Dade\Documents\Università\Anno IV\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx');

% Position of the mechanical parameters excel file
mech_par_excel = 'C:\Users\Dade\Documents\Università\Anno IV\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx';

for i = 1:size(file_samples_shifted,1)
    % Load the damage test shifted
    damage_test_name_shifted = file_samples_shifted(i).name;
    damage_test_shifted = dlmread(fullfile(folder_damage_test_shifted,damage_test_name_shifted),',',',',1,0);
    time_shifted = damage_test_shifted(:,1);
    strain_shifted = damage_test_shifted(:,2)/100;
    load_shifted = damage_test_shifted(:,3);
    stress_shifted = damage_test_shifted(:,4);

    % Import the E_2 and the intercept_2
    E = datasheet_mech_par.E_2(i);
    intercept = datasheet_mech_par.intercept_2(i);

    % Find the ultimate stress and strain
    [max_stress index] = max(stress_shifted);
    max_strain = strain_shifted(index)*100;

    % Compute le position of the starting point of the loading cycles
    [points_value,strain_limit] = points(time_shifted,strain_shifted);

    % Compute the stress-strain curve of the first loading cycle
    if length(points_value) > 7
        strain_curve = strain_shifted(points_value(7)+1:points_value(8));
        stress_curve = stress_shifted(points_value(7)+1:points_value(8));
    else
        strain_curve = strain_shifted(points_value(7)+1:end);
        stress_curve = stress_shifted(points_value(7)+1:end);
    end

    % Create the strain vector for the construction of the line
    strain_line = [0.002:0.0001:strain_curve(end)];

    % Load the shifting deformation
    shift_def = datasheet_mech_par.shift_def_to_left(i)/100;

    % Calculate the stress for the line shifted of 0.002 mm/mm toward right
    stress_line = E*(strain_line-0.002+shift_def)+intercept;

    F_curve = griddedInterpolant(strain_curve,stress_curve,'spline') ;
    dif = stress_line - F_curve(strain_line);
    t = 1;
for j = 2:length(dif)
    dif_old = dif(j-1);
    dif_new = dif(j);
    if dif_old*dif_new <= 0
        yield_stress_multi(t) = stress_line(j);
        t = t+1;
    end
end
if t > 1
    yield_stress(i,1) = max(yield_stress_multi);
    yield_strain(i,1) = strain_line(find(stress_line==yield_stress(i,1)))*100;
    ult_strain(i,1) = max_strain;
    ult_stress(i,1) = max_stress;
else
    yield_stress(i,1) = 0;
    yield_strain(i,1) = 0;
    ult_strain(i,1) = 0;
    ult_stress(i,1) = 0;
end
figure
scatter(strain_curve*100,stress_curve,5,'bo')
hold on
plot(strain_line*100,stress_line,'r')
plot([0:0.001:max(strain_line)*100],E/100*([0:0.001:max(strain_line)*100]+shift_def*100)+intercept,'g')
if t > 1
    scatter(yield_strain(i,1),yield_stress(i,1),80,'ko','filled')
    scatter(ult_strain(i,1),ult_stress(i,1),80,'mo','filled')
end
xlim([0 max_strain*2])
ylim([0 max_stress*1.5])
xlabel('Strain [%]')
ylabel('Stress [MPa]')
grid on
print(fullfile(folder_yield_ult,strcat(damage_test_name_shifted(1:end-21),'_yield_ult')),'-dpdf','-r300')
close

% Save the results in the excel file mechanical_parameters
sheet = 1;
xlRange = 'L2';
xlswrite(mech_par_excel,[yield_strain yield_stress ult_strain ult_stress],sheet,xlRange)

A.6 Computation of the dissipation energy

% Folder of the damage test shifted
folder_damage_test_shifted = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test';

% Folder of the stress-strain curve with yield and ultimate value
folder_energy = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Stress_strain curve with energy';

% Load the txt damage test shifted names
Computation of the dissipation energy

```matlab
file_samples_shifted = dir('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\Shifted damage test\*.txt');

% Load the mechanical parameters excel file datasheet_mech_par = readtable('C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx');

% Position of the mechanical parameters excel file mech_par_excel = 'C:\Users\Dade\Documents\Università\Anno V\Semestre II\Tesi\Ossa\Mechanical properties\mechanical_parameters.xlsx';

for i = 1:size(file_samples_shifted,1)
    % Load the damage test shifted
    damage_test_name_shifted = file_samples_shifted(i).name;
    damage_test_shifted = dlmread(fullfile(folder_damage_test_shifted,damage_test_name_shifted),',',1,0);

    time_shifted = damage_test_shifted(:,1);
    strain_shifted = damage_test_shifted(:,2)/100;
    load_shifted = damage_test_shifted(:,3);
    stress_shifted = damage_test_shifted(:,4);

    % Compute the position of the starting point of the loading cycles
    [points_value,strain_limit] = points(time_shifted,strain_shifted);
    if length(points_value) > 7
        last_point = points_value(8);
    else
        last_point = length(strain_shifted);
    end

    % Find the minimum value of the strain and start the analysis from that point in order to exclude from the analysis the initial part of the loading cycle in which the strain decrease
    [min_strain index] = min(strain_shifted(points_value(7):last_point));
    first_point = points_value(7)+index-1;

    % Compute the linear regression considering the first ten points
    mdl=fitlm(strain_shifted(first_point:first_point+10),stress_shifted(first_point+10));
    m = mdl.Coefficients{2,1};
    q = mdl.Coefficients{1,1};
    x_0 = -q/m;

    % Shifting all the points with the quantity x_0
    strain_shifted_2 = strain_shifted - x_0;

    % Create a vector for the calculation of the energy, that it'll be calculated from 0 to max_strain
    incr = 0.00001;
    xx = [0:incr:max(strain_shifted_2)];

    % Add the point [0,0]
    strain_spline = [0; strain_shifted_2(first_point:last_point)];
    stress_spline = [0; stress_shifted(first_point:last_point)];

    % Calculation of the energy absorbed
    pp = spline(strain_spline,stress_spline);
    yy = ppval(pp,xx);
```
energy_value(i,1) = integral(@(x)ppval(pp,x),0,max(strain_shifted_2));

figure
scatter(strain_shifted_2(first_point:last_point)*100,stress_shifted(first_point:last_point),'ro')
hold on
plot(xx*100,yy,'b')
xlabel('Strain [%]')
ylabel('Stress [MPa]')
grid on
print(fullfile(folder_energy,strcat(damage_test_name_shifted(1:end-21),'_energy')),'-dpdf','-r300')
close

end

% Save the results in the excel file mechanical_parameters
sheet = 1;
xlRange = 'P2';
xlswrite(mech_par_excel,energy_value,sheet,xlRange)
<table>
<thead>
<tr>
<th>p-Value Pearson's r</th>
<th>BMD</th>
<th>TBS</th>
<th>E₀</th>
<th>ε₀</th>
<th>σ₀</th>
<th>ε₀ ult</th>
<th>σ₀ ult</th>
<th>BV/TV</th>
<th>BS/TV</th>
<th>Tb.Th.</th>
<th>Tb.Sp.</th>
<th>SMIB</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td>0.000</td>
<td>0.162</td>
<td>0.011</td>
<td>0.691</td>
<td>0.003</td>
<td>0.494</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.387</td>
<td>0.000</td>
<td>0.814</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>1.000</td>
<td>-0.251</td>
<td>0.410</td>
<td>-0.071</td>
<td>0.487</td>
<td>-0.143</td>
<td>0.612</td>
<td>0.606</td>
<td>-0.668</td>
<td>-0.140</td>
<td>0.632</td>
<td>-0.038</td>
<td>-0.419</td>
</tr>
<tr>
<td>TBS</td>
<td>0.162</td>
<td>0.000</td>
<td>0.754</td>
<td>0.015</td>
<td>0.027</td>
<td>0.740</td>
<td>0.567</td>
<td>0.003</td>
<td>0.016</td>
<td>0.562</td>
<td>0.025</td>
<td>0.414</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>-0.251</td>
<td>1.000</td>
<td>0.053</td>
<td>-0.414</td>
<td>-0.380</td>
<td>-0.070</td>
<td>-0.120</td>
<td>-0.457</td>
<td>0.380</td>
<td>-0.095</td>
<td>-0.355</td>
<td>0.133</td>
<td>0.298</td>
</tr>
<tr>
<td>E₀</td>
<td>0.011</td>
<td>0.754</td>
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