Biomechanical Analysis of the Human Lumbar Spine
An Experimental and Computational Approach

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Thesis to obtain the Master of Science Degree in

Biomedical Engineering

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In theory there is no difference between theory and practice. In practice there is.

Jan L. A. van de Snepscheut
Acknowledgments

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Finally to all the other friends for all the support.
Abstract

The lumbar spine is a leaning site for spinal diseases to occur, particularly low back pain, a common condition involving multiple structures of spine, affecting a great portion of population. Besides being a health problem, it has a large economic burden on individuals and in government’s annual healthcare expenditures.

The biomechanical study of lumbar spine allows for a better comprehension of the mechanisms behind this condition. Developing patient-specific models offers a mean of preventing injuries to occur and predicting and monitoring the behaviour of spine structures during surgical interventions, when these cannot be avoided. Additionally, experimental methods, such as in vitro studies in animal models, supply valuable information when testing new medical devices or investigating pathological cases.

In computational models, mechanical properties play a major role in result’s reliability. In bone, most models opt for simplified representations of its components, since no clear boundary between cortical and trabecular bone exists. This work calculated the equivalent Young’s Modulus of the whole vertebra through compression testing until fracture of sheep samples.

Results were validated by constructing a finite element model, for which the samples’ geometry was obtained from a CT scan and the material properties and loading conditions of the compression tests were replicated.

Additionally, material properties’ sensitivity in a patient lumbar model segment was studied. Homogeneous and heterogeneous distribution of bone properties were initially compared followed by a contrast between linear and non-linear models of the intervertebral disc. The results were evaluated through stress distribution, range of motion and intradiscal pressure.

Keywords

Lumbar Spine; Experimental Work; Animal Model; Validation; Finite Element; Material Properties
Resumo

A coluna lombar é um local propenso à ocorrência de doenças, particularmente lombalgia, uma manifestação dolorosa que envolve múltiplos componentes da coluna e afecta grande parte da população. Adicionalmente possui grande impacto nas despesas anuais de saúde individuais e públicas.

O estudo biomecânico da coluna lombar permite uma maior compreensão dos mecanismos inerentes a esta condição. Modelos específicos do doente auxiliam não só na prevenção de lesões, mas também no planeamento e monotorização de intervenções cirúrgicas, quando estas são inevitáveis. Do mesmo modo, métodos experimentais, como modelos animais in vitro, fornecem informações valiosas no teste de novos dispositivos médicos ou na investigação de condições patológicas.

Em modelos computacionais, a escolha das propriedades mecânicas é fundamental para a fiabilidade dos resultados. No caso do osso, muitos modelos optam por uma representação simplificada dos componentes, pois não há uma divisão clara entre osso cortical e trabecular. Neste trabalho, o módulo de Young de vértebras de ovelha foi determinado por testes de compressão até fractura.

Os resultados experimentais foram validados pela construção de um modelo de elementos finitos, para o qual se obteve a geometria das amostras por aquisições de CT e replicou-se as propriedades materiais e condições do teste.

Paralelamente, estudou-se a sensibilidade das propriedades para um modelo humano de um segmento lombar. Inicialmente comparou-se a distribuição das propriedades mecânicas homogêneas e heterogêneas do osso, seguido pelo contraste entre um modelo linear e não linear do disco intervertebral. Os resultados foram avaliados pela distribuição de tensões, amplitude de movimento e pressão intradiscal.

Palavras Chave

Coluna Lombar; Trabalho Experimental; Modelo Animal; Validação; Elementos Finitos; Propriedades dos Materiais
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<tr>
<td>AF</td>
<td>Annulus Fibrosus</td>
</tr>
<tr>
<td>ALIF</td>
<td>Anterior Lumbar Interbody Fusion</td>
</tr>
<tr>
<td>ALL</td>
<td>Anterior Longitudinal Ligaments</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone Morphometric Protein</td>
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<tr>
<td>BV/TV</td>
<td>Bone Volume Fraction</td>
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<tr>
<td>CAD</td>
<td>Computer-aided Design</td>
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<tr>
<td>CL</td>
<td>Capsular Ligaments</td>
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<tr>
<td>COM</td>
<td>Center of Mass</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>EZ</td>
<td>Elastic Zone</td>
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<tr>
<td>FCL</td>
<td>Facet Capsular Ligament</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drugs Administration</td>
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<tr>
<td>FE</td>
<td>Finite Element</td>
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<tr>
<td>FEA</td>
<td>Finite Element Analysis</td>
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<tr>
<td>FEM</td>
<td>Finite Element Mesh</td>
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<tr>
<td>FSU</td>
<td>Functional Spinal Unit</td>
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<tr>
<td>GE</td>
<td>Geometrical Error Factor</td>
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<tr>
<td>HU</td>
<td>Hounsfield Unit</td>
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<tr>
<td>IAP</td>
<td>Intra-abdominal Pressure</td>
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<tr>
<td>IDP</td>
<td>Intradiscal Pressure</td>
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<tr>
<td>ISL</td>
<td>Interspinous Ligaments</td>
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<tr>
<td>ITL</td>
<td>Intertransverse Ligaments</td>
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<tr>
<td>IVD</td>
<td>Intervertebral Disc</td>
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<tr>
<td>KVP</td>
<td>Kilovoltage Peak</td>
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<tr>
<td>LBP</td>
<td>Low Back Pain</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>LF</td>
<td>Ligament Flavum</td>
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<tr>
<td>LN</td>
<td>Ligament Nuclae</td>
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<tr>
<td>MCA</td>
<td>Marching Cubes Algorithm</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NP</td>
<td>Nucleus Pulposus</td>
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<tr>
<td>NURBS</td>
<td>Non Uniform Rational Basis Spline</td>
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<tr>
<td>NZ</td>
<td>Neutral Zone</td>
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<tr>
<td>PLIF</td>
<td>Posterior Lumbar Interbody Fusion</td>
</tr>
<tr>
<td>PLL</td>
<td>Posterior Longitudinal Ligaments</td>
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<tr>
<td>PMMA</td>
<td>Poly (methyl methacrylate)</td>
</tr>
<tr>
<td>PVE</td>
<td>Partial Volume Effects</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
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<tr>
<td>ROM</td>
<td>Range of Motion</td>
</tr>
<tr>
<td>RP</td>
<td>Reference Point</td>
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<tr>
<td>SSL</td>
<td>Supraspinous Ligaments</td>
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<tr>
<td>STP</td>
<td>Standard Temperature and Pressure</td>
</tr>
<tr>
<td>TDA</td>
<td>Total Disc Arthroplasty</td>
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**List of Finite Element Types**

<table>
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<tr>
<th>Acronym</th>
<th>Type</th>
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<tr>
<td>C3D4</td>
<td>4-node linear tetrahedron</td>
</tr>
<tr>
<td>M3D3</td>
<td>3-node triangular membrane</td>
</tr>
<tr>
<td>M3D4</td>
<td>4-node quadrilateral membrane</td>
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<tr>
<td>SFM3D4</td>
<td>4-node quadrilateral surface element</td>
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List of Symbols

\begin{itemize}
  \item $A_0$ Initial cross-sectional Area
  \item $\sigma$ Stress
  \item $\varepsilon$ Strain
  \item $l_0$ Initial length
  \item $l_i$ Linear zone initial length
  \item $l$ Instantaneous linear length
  \item $\Delta l$ Total displacement
  \item $\Delta l_i$ Linear displacement
  \item $\Delta l_0$ Initial displacement adjustment
  \item $\mu$ Mean value
  \item $\sigma$ Standard deviation
  \item $x_i$ Position of node i in a mesh
  \item $\lambda$ Smoothing factor
  \item $HU_i$ Hounsfield CT number of material i
  \item $\mu_i$ Linear attenuation coefficient of material i
  \item $\rho_i$ Density of material i
  \item $CT$ Hounsfield measured CT number
  \item $CTH$ Hounsfield corrected CT number
  \item $E$ Young’s / Elasticity Modulus
  \item $\nu$ Poisson’s Ratio
\end{itemize}
$\Delta d_{\text{exp}}$ Experimental total displacement

$\Delta d_{\text{comp}}$ Computational total displacement

$e(\%)$ Relative error

$\bar{A}$ Average surface area

$R$ Elliptical radius of intervertebral disc

$T_L$ Lamella / Fibre bundle thickness

$N$ Number of lamellae

$A_B$ Cross-sectional area of one lamella

$\alpha$ Angle of inclination of collagen fibres

$s$ Spacing between each fibre bundle within the same lamella

$W_B$ Width of a fibre bundle

$t$ Number of fibre bundles per FE element

$U$ Strain energy

$C_{10}, C_{01}$ Material constants of hyperelastic Mooney-Rivlin model

$I_1, I_2$ First and second invariants of Cauchy-Green deformation tensor

$D_1$ Material constant to describe compressibility of the material

$J_{el}$ Elastic volume strain

$\mu_0$ Initial shear modulus

$k_0$ Initial bulk modulus
Contents

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1.2 Thesis Outline ............................................ 4
Low back pain (LBP) is a very common condition among the industrialized Western countries, from which it is estimated that 80% of the individuals will suffer from this condition one or more times during his lifetime [1] [2]. This condition may affect bone, muscle, joints or a combination of them. Playing the major role in bodyweight support and movement in daily routine, lumbar spine is commonly subjected to incorrect postures, unexpected loads or disc disease. These factors impel it to be a primary source of pain, being commonly a self-limiting and even disability state. LBP is thus not only a heath problem, but also causes an enormous economic impact on individuals and even in government’s annual health-care expenditures [3].

The basic pathophysiologic mechanisms of LBP lack of understanding by the scientific community. This is justified by the fact that LBP is not a specific medical condition but a symptom. However, an emerging evidence indicates that it is intimately connected with the so-called disc degeneration mechanism. For that reason, disc is a treatment target in order to reduce or even eliminate pain. The multiple approaches range from posture correction, to physical therapy, weight loss, medication prescription, and in severe cases, to surgical intervention [1] [4].

In lumbar spine, two types of surgery are commonly performed: lumbar decompression and spinal fusion. The first is divided into two procedures: microdiscectomy and lumbar laminectomy, both aiming to alleviate pain caused by a pinched nerve, by removing a portion of the bone and/or disc that are compressing the nerve root and easing the healing process. On the other hand, spinal fusion consists of joining two (or more) vertebrae recurring to a bone graft, which fully stops the motion at a painful motion segment. This allows the patient to increase his or her ability to function and enjoy everyday activities, but unfortunately does not “fix”low back [5].

Disc arthroplasty is an emerging technology alternative to spinal fusion technique. Seeking to restore the biomechanical functions of motion and stability preservation, intervertebral-height recover and conversion of shock-absorption properties, the idea behind it is to replace total or part of intervertebral disc by a non-biological device that enables all those functions [1].

Whichever the chosen treatment modality might be, the ultimate goal is to maintain the healthy spine function and a deeper study of the multiple variables relating LBP with disc degeneration remain controversial and not very well understood. This is revealed by some disparities in the clinical outcomes, some favourable, some not. In order to overcome this problem, efforts must be made in terms of methodologies adopted to study this relation and bias factors or differences in population should be identified in literature.

1.1 Motivation

From patient to patient, each one has different daily routines and his spine varies in size, shape, bone composition and density, which means that the spine biomechanical behaviour either in healthy and unhealthy conditions will be different. Naturally, one understands that a unique solution for all individuals is impossible to find and that opens the opportunity to study and come up with diverse solutions.
The solutions/devices that provide us the better choice can be evaluated through a biomechanical study. Three types of methodologies are currently available: *in vivo*, *in vitro* and *in silico* studies. The first is very oriented to subject-specific results, but it requires invasive approaches, which shortens its potential. *In vitro* models have been widely used and allow a more controlled environment than the latter [6], but are circumscribed to cadaverous specimen usage and likewise subjected to strict ethical regulations. The last is the most recent and most frequent applied method nowadays. *In silico* models are computer-based simulations that allow the user to have a level of control over the experimental variables that is usually not possible with both previous studies. [1]

A computational simulation usually starts with constructing a model and posteriorly analysing it with an appropriate program. The degree of anatomic detail of the model may vary depending on whether one intends to obtain precise results in a very specific response to a certain load condition or if one expects a broader model to represent several loading conditions or even represent a group of individuals. To illustrate this difference, one might mention the supposedly first *in silico* model representing an intervertebral disc. It was developed by Belytschko et al. [7] in 1974 and modelled assuming axial symmetry and linear orthotropic material properties. After that, several other models were published, varying the properties and including multiple segments and even the total spine. A worth mentioning contribution to this field were the several works of Shirazi-Adl et al. [8–15] in the eighties/nineties, where three dimensional non-linear models of lumbar segments took in consideration the complex composition of the intervertebral disc, studying the diverse mechanical responses under several loading conditions. [16] [17]

The present work analysed a computational model based on CT images from a thirty-year-old healthy woman. The modelling process was carefully designed so that its anatomy provided the most realistic results possible.

The lower spine levels, L4/L5 and L5/S1, carry the most weight and have the greatest movement, making the area more susceptible to develop injury and clinical problems [18] [19]. For that reason, this thesis focuses on L4-L5 segment.

To build a computational model, one has to assign the material properties to each component of it. Obviously, one is limited to replicate the exact properties in each infinitesimal distance, given the complex nature of living tissues, so a number of simplifications have to be done. This evokes the need of the model to be validated.

*Ex vivo* may provide the bone properties necessary for the model. Ideally this data would come from human vertebral specimens, but ethical regulations among with other factors latter described, are commonly replaced by animal specimens, which supply *in vivo, in vitro* and *ex vivo* data. [1]

For spinal research, the use of quadruped animals such as sheep, porcine, calf or deer is largely reported by literature but there can be other animal models held for the same purpose. Undoubtedly, differences in anatomy and morphology of the animals and human spines exist, but morphometric studies show a qualitative equivalence between calf and human spines, immediately followed by porcine. Sheep spines are often employed in *in vitro* tests, such as the famous work of Wilke et al. [20], to evaluate ROM.
The present work made use of sheep cervical spines to determine the equivalent Young’s modulus of the whole vertebra by performing compressive tests on prepared test samples. Along with the experimental work, a computational model of the sheep spine was made based on previous acquired CT scans from the same cervical sample and the conditions were recreated in order to validate the model.

1.2 Thesis Outline

Despite pursuing the same goal of studying the biomechanical behaviour of spine, the work approaches the issue by two possible paths: by a computational model of the human lumbar spine and by an experimental validation of the sheep cervical spine. This thesis is presented in 9 chapters, including this introduction to explain the relevance of its execution.

The second chapter presents the human spine, in a way to become acquainted of its functions and the several elements that compose it. The normal biomechanical behaviour is described as well as when the events turn pathological.

The third chapter explains the possible options currently available to restore the normal state of the spine. It describes in detail the lumbar fusion option, when and how the surgery is performed and its instrumentation. An alternative to fusion surgery is also presented, lumbar arthroplasty, which has been gaining supporters in the medical community.

In the fourth chapter, the experimental work is made clear. From the animal under testing choice to the compressive trials and calculations done to obtain the experimental elasticity modulus of the total vertebra, the explanation will facilitate the computational validation done afterwards.

In the fifth chapter, the geometrical model is developed. The several steps, from the CT image acquisition to the material properties distribution, are systematized. Note that different approaches were implemented for the sheep and human vertebrae. Additionally, the intervertebral disc is also designed for the human model.

The sixth chapter is the finite element validation of the experimental work. After obtaining the geometrical models of the sheep vertebrae, the loading and boundary conditions are replicated as accurately as possible, inserting the data provided experimentally. The analysis quantifies therefore the validity and reliability of the obtained data for future purposes.

The seventh chapter presents the other approach, based on the computational model of the human spine. It illustrates how a biomechanical model is implemented and the different studies that can be done when studying material and geometrical influences in the stress distribution, range of motion and disc pressure in the human lumbar spine. The results were compared with others coming from literature.

The eighth and ninth chapters gather the discussion and work conclusions.
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2.1 Spine Anatomy

The spine, or vertebral column, is a complex mechanical structure composed by a series of axially jointed bones, named vertebrae, that articulate themselves with each other and with the soft tissues in order to support the upper body’s weight while allowing for biomechanical functions such as bending and turning. It also provides a point of attachment for the ribs, back and abdominal muscles, as well as protection of the spinal cord and other soft tissues from damage. Extending from the skull to the pelvis, it has a natural S-shaped curvature in the sagittal plane with two convex anteriorly and concave posteriorly curves at the neck and low back (cervical and lumbar, respectively) and two concave anteriorly and convex posteriorly curves at the chest and pelvis (thoracic and sacrocccygeal, respectively). This feature is particularly important in the human spine since it permits to stand in orthostatic posture providing stability and proper stiffness at the intervertebral joint level, while increasing flexibility and shock-absorption capabilities.

The vertebral column is composed by 33 or 34 vertebrae and is divided into five regions: seven cervical vertebrae (C1-C7); twelve thoracic vertebrae (T1-T12); five lumbar vertebrae (L1-L5); five fused sacral vertebrae, which constitute the sacrum; and four or five fused coccygeal vertebrae, which form the coccyx. The two previous structures are joined during adulthood, whilst the remain vertebrae maintain their original format.

The vertebrae are intercalated by intervertebral discs and anchor ligaments and muscle insertions so that the vertebral column remains aligned and stabilized.
In the present work, focus will be given to the lumbar region, detailing the biomechanical behaviour of the Functional Spinal Unit (FSU) L4-L5. A FSU corresponds to the smallest representation in a given region of the spine constituted by two adjacent vertebrae with the intervening disc and ligaments intact. \[26\]
2.2 The Vertebra

The vertebrae that constitute the vertebral column may vary in size, shape and detail depending on which spine region they belong to. Nevertheless, they share common features. Each vertebra consists of two main regions: the anterior part segment, the body, and the posterior element, the neural arch. Both elements surround the vertebral foramen, a triangle aperture, that, when combined by the superposition of vertebrae, forms the spinal canal through which passes the spinal cord. [21] [22]

![Figure 2.2: The lumbar vertebra in side view on the left and in superior view on the right ](25).

The vertebral body has a kidney shaped profile, with approximately 40-50 mm in lateral diameter and 30-35 mm sagittal diameter, proper for weight-bearing. The outer surface of the body is comprised of cortical bone, estimated around 0.35 mm thickness, although the upper and lower surfaces are thicker, with approximately 0.5 mm. Thus, these stiffer regions are called endplates. The interior of the body is filled with trabecular (or cancellous) bone which is a more porous structure, giving more flexibility and resilience to the vertebra. [27] [21] [22] [24]

The posterior elements have thicker coverings of cortical bone and consist of the following structures:

- two pillar-like pedicles projected from the posterior surface of the vertebral body;
- two laminae, flattened plates extended from the pedicles to fuse centrally posteriorly;
- four articular processes. Two of them project downwards and two upwards from the pedicle-lamina junctions. The facets of the superior process of one vertebra articulate with the facets of the inferior process of the next vertebra in the zygapophysial joint. This complements the role of the intervertebral discs by resisting compressive forces and allowing for movement;
- two transverse processes, bony protrusions that project laterally from the pedicle-lamina junction;
- and the spinous process, a slender structure arising from the two laminae junction. Along with the transverse processes, these structures offer attachment sites for the muscles, which move the vertebral column, and ligaments, which stabilize it. [21] [28]
Between the pedicles of adjacent vertebrae there are intervertebral foramina on both sides of the column through which a single spinal nerve exits the spinal canal. [21] [28]

The lumbar vertebrae must bear the weight of the trunk, absorb the stress exerted when carrying or lifting objects and control its movements on the pelvis in the upright posture. Therefore, their vertebral bodies are not only the widest and strongest of the movable vertebrae, but also the most flexible due to the lack of ribs [21] [22]. The processes are short and thick with the spinous processes adapted to anchor the back muscles and there might be additional processes to attach more muscle endings. Examples of these are the mamillary process in the superior articular processes and the accessory process in the transverse processes [21]. Likewise have the laminae greater height over its length. Despite being fitted to their mechanical role, the lumbar region is the most likely to have problems.

### 2.3 The Intervertebral Disc

Depending on age, time of day, occupation and disease state, the intervertebral discs (IVD) make up approximately 15% -20% of the spine’s length. Intercalating the adjoining vertebrae, discs absorb biomechanical forces, allow for movement and flexibility. While flexibility decreases with age, movement can be critically affected through all stages of life. [1]

From the axial view, the IVDs are classified as amphiarthrosis (joint that allows for limited movement), presenting a biconvex lens form, with 40-45 mm in lateral diameter and 35-40 mm sagittal diameter in the lumbar region. [21]

The disc’s thickness varies upon the region where it is located, being approximately 9 mm in lumbar region. Here and in cervical region, the posterior part is thicker than the anterior part, conversely to the thoracic region, where the reverse occurs. This explains the natural S-shaped form of the spine previously mentioned. [21] One should note that the lumbar region presents the thickest discs due to its weight-bearing and wider motion functions.

These structures present themselves complex tissues comprising an outer circumferential ring of fibrocartilage, the annulus fibrosus (AF) which encloses a central proteoglycan-rich core, the nucleus pulposus (NP). The nucleus is sandwiched superiorly and inferiorly by the cartilage endplates of the contiguous vertebrae. [1]

![Figure 2.3](image-url) Schematic view of a FSU with the intervertebral disc. On the left it can be seen the nucleus pulposus (NP) surrounded by the lamellae of the annulus fibrosus(AF) and sandwiched by the cartilage endplates. On the right it is shown the fibers orientation in a criss-cross pattern on the outermost layer of the AF [25].
The nucleus pulposus is an oval gelatinous part that occupies the center part of the IVD. Its position and cross-sectional area relies on the spine level considered, ranging from 30% to 50%. It is made up by clustered cartilage cells, randomly arranged collagen fibers and radially arranged elastin fibers embedded in a large extracellular substance. A healthy nucleus is made up of 70-90% water content, proteoglycans (65% of dry weight) and the loose network of collagen type II (10-20% dry weight). The proteoglycans are long chains of subunits of glycosaminoglycans and proteins linked to hyaluronic acid chains. Its ionic nature promotes hydrophilic behaviour and hence an osmotic pressure in the hydrated tissue. Coupled with the high fluid content, the NP is usually considered as an incompressible fluid like material responsible for maintaining the disc pressurized, distributing loads to the AF and keeping disc’s height. The water composition decreases with ageing process and the NP tends to be more compressed reducing the distance between vertebrae, which is macroscopically verified with the overall decrease in individual’s height.

The annulus fibrosus is a ring structure that encloses the NP. It is build up of 20 to 62 lamellae made up of parallel collagen fibers in each lamella and embedded in ground matrix. The ground matrix contains mostly water (70-80% of its weight), proteoglycans and other proteins. The reinforcement of the matrix is given by the collagen fibers, mostly type I on the outer region and type I and II closer to the NP. The collagen type I is observed in tissues that experience tensile or compressive loads. Its content and fiber orientation angle vary from the innermost to the outermost layer, as suggested by several authors. The angle is changed in such a way to form a criss-cross pattern between successive layers (±30° relatively to a horizontal plan) and elastin fibers lie in between to restore the disc’s original configuration and to keep the lamellae together since they only account for stiffness in radial position. In terms of mechanical properties, the outer anterior portion of the AF is stiffer than the inner posterolateral regions. The inner and middle annulus account for compression whereas the outer part resists to bending and torsion. The collagen bands act as internal ligaments, increasing the nucleus pressure and counterbalance the shear loads in the annulus.

As a footnote reminder, it should be said that there is no clear boundary between the NP and AF, they rather merge continuously from one to the other and this becomes more evident with ageing since the nucleus becomes more fibrotic.

The last constituent of the IVD are the cartilage endplates. They cover the top and bottom of the IVD, usually with less than 1 mm thick, constituted by horizontal collagen layers that are aligned with the bands of the AF. These are permeable structures that permit diffusion of nutrients from the bone marrow through the IVD.
2.4 Ligaments and muscles

Ligaments are uniaxial structures attached at each spine level which establish limits on the physiological motion, protecting the neural structures and absorbing excessive energy when trauma occurs. A mechanical load applied in the direction of their collagen fibres will have the most effective response. Nevertheless, regarding the fibres orientation, a single ligament is capable of resisting to tensile forces in a range of directions. Moreover, adjoined vertebrae are connected by several types of ligaments and this diversity allows for the distinct responses observed in the physiological motion.

Overall, one might find nine ligaments in the spine, even though they are not mutually present in all spine regions: the anterior (ALL) and posterior (PLL) longitudinal ligaments, the interspinous (ISL) and supraspinous (SSL) ligaments, the ligament Flavum (LF), the intertransverse ligaments (ITL), the capsular ligaments (CL), the ligament nuchae (LN) and the facet capsular ligament (FCL). On the lumbar region, only the first seven are contemplated. A brief summary of their composition and location is given in table 2.1 followed by the illustrative figure 2.4:

<table>
<thead>
<tr>
<th>Ligament name</th>
<th>Composition</th>
<th>Location</th>
<th>Principal Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Longitudinal Ligament (ALL)</td>
<td>Collagen fibres</td>
<td>It covers the anterior surfaces of the vertebral bodies and discs from the entire spine. It is attached strongly to the vertebral bone, being wider, and weakly to the discs, where it shows as a narrower structure.</td>
<td>It resists bending of the spine, specifically extension.</td>
</tr>
<tr>
<td>Posterior Longitudinal Ligament (PLL)</td>
<td>Collagen fibres</td>
<td>It covers the posterior aspects of the vertebral bodies and discs from the entire spine. It is attached strongly to the discs, being wider, and weakly to the bone, where it shows a narrower structure.</td>
<td>It resists bending of the spine, specifically flexion.</td>
</tr>
<tr>
<td>Ligament Flavum (LF)</td>
<td>Elastin</td>
<td>It connects the lower and upper ends of the internal surfaces of the adjacent laminae, closing the gap between the consecutive laminae.</td>
<td>It limits the interlaminal distances in flexion and extension movement.</td>
</tr>
<tr>
<td>Intertransverse Ligaments (ITL)</td>
<td>Thin collagen fibres</td>
<td>Connect the transverse processes.</td>
<td>Limit lateral flexion.</td>
</tr>
<tr>
<td>Interspinous Ligaments (ISL)</td>
<td>Thin collagen fibres</td>
<td>Connect the spinous processes.</td>
<td>Effective under flexion movement.</td>
</tr>
<tr>
<td>Supraspinous Ligaments (SSL)</td>
<td>Tendinous fibres</td>
<td>Connect the peaks of adjacent spinous processes.</td>
<td></td>
</tr>
<tr>
<td>Capsular Ligaments (CL)</td>
<td>Collagen fibres</td>
<td>Connect the circumferences of the joining articular facet joints, being perpendicular to the surface of the joints.</td>
<td>Most effective when distracted along the fibres direction.</td>
</tr>
</tbody>
</table>
Figure 2.4: Schematic view of the main ligaments in lumbar spine. ITL and CL are not discriminated in this figure. Image adapted from [33].

Regarding muscles, these can be classified as postvertebral or prevertebral muscles depending on their location. Additionally the postvertebral muscles are subdivided into three categories: deep, intermediate and superficial. The following table summarizes their description:

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Subcategory</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postvertebral</td>
<td>Deep</td>
<td>Short muscles connecting adjacent spinous and transverse processes with laminae. More diffused muscles linking the transverse processes of each vertebra to the spinous process of the upper vertebra. Collectively named <em>erector spinae</em>.</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Superficial</td>
<td></td>
</tr>
<tr>
<td>Prevertebral</td>
<td>-</td>
<td>Abdominal muscles in which three of them involve the abdominal region and one in the other located anteriorly in the midline.</td>
</tr>
</tbody>
</table>
2.5 Biomechanics of the Lumbar Spine

As previously said, the vertebral column, as a biomechanical structure, plays three major roles: stabilization, flexibility and movement and protection of adjacent structures from damaging forces and motions. The lumbar spine takes the leading role concerning these functions: it bears the greatest loads, forces and moments while offering the greatest mobility.

The loads acting on the lumbar spine are divided into two classes: physiologic loads, resulting from daily activity, and traumatic loads, which generally occur without warning and with great amplitude. The loads can also be classified according to its origin. The gravity load is a compressive one increasing downwards and depends on the mass of the individual and acceleration that he/she is subjected. The muscles, being active tissues, contract and relax governed by the nervous system. The muscle forces help to stabilize the column but also subject it to high compressive and tensile forces, which can double the load concerning the body weight. The ligaments counterbalance the effect of muscles, being passive elements, and responding to tensile forces to release energy. [27]

The intra-abdominal pressure (IAP) decreases the spinal compression because of abdominal muscle activity. In fact, holding breath and wearing abdominal belt are two techniques used both by athletes and pregnant women to increase IAP and in consequence stabilizing the spine. Still, disc pressure varies with position in which the load acts and with different activities. Moreover, the force distribution and its distance to the lumbar spine will affect disc pressure [27]. Visual explanations are given below in figure 2.8:

![Figure 2.8: (a) Centre of mass (COM) shifting with gestation weeks. (1) The normal S-shaped curvature in women is altered (2) as the fetal load increases, moving the COM forwards and modifying the arching of the vertebrae (3) such that the COM is realigned with the hips, ankles and knees. [35] (b) The influence of holding breath mechanism in intradiscal pressure. [36]](image)

With respect to the anatomical planes, the spinal movements are (1) the flexion/extension in the sagittal plane, (2) the left and right lateral bending in the frontal plane and (3) the left and right rotation around the longitudinal axis of the spine.
The range of spinal motions has been extensively measured both \textit{in vivo} and \textit{in vitro}. A reproducible standard testing frame has been proposed and three parameters are usually employed to quantify the spinal motion: (1) the \textit{neutral zone} (NZ), in which the specimen moves essentially free from applying a load, (2) the \textit{elastic zone} (EZ), in which the deformation is measured from the neutral zone to the point of maximal loading, and (3) the \textit{range of motion} (ROM), the sum between NZ and EZ in one direction of motion. \cite{26}

Beholding a general lumbar segment, the flexion/extension motion is increased from L1-L2 to L4-L5 in the range of $12^\circ$ - $16^\circ$, followed by $6^\circ$ in lateral bending and about $2^\circ$ in axial rotation. These ranges are affected by several parameters, such as geometry, hydration, age, degeneration or stiffness of the IVD, the state of the ligaments, articular facet joints and posterior bone elements. The IVD should be highlighted, given that its diameter and height influence greatly the ROM. Smaller diameters and larger heights enhance ROM. The lumbar region, having the greatest disc height proportion in the spine, has therefore the greatest mobility \cite{23} \cite{1}. Figure 2.9 summarizes the previous information:

![Diagram of spinal motions](image)

\textbf{Figure 2.9:} On the left it is shown the anatomic reference frame for \textit{in vitro} testing of a FSU with all possible loads and pure motion components (rotations and translations). On the right, the ROM for the three rotations is displayed for each lumbar segment. The information was adapted from works of Wilke and White and Panjabi \cite{26} \cite{23}.

\section*{2.6 Lumbar Disorders}

No other human tissue or organ experiences such a deep degeneration process or starts so soon (mostly during puberty) as the intervertebral disc. Despite being major interest in clinical practice and research, the definition of disc degeneration remains unclear. Certainly, this has to do with the extensive methodologies adopted to evaluate the degree of degeneration. According to a recent review, there are at least 22 grading systems to quantify it. \cite{37} \cite{1}. Either way, a broader definition for degeneration is accepted: it means a structural and/or cell-mediated change in the normal composition, structure and function of the spine. \cite{27}

As it can be seen, the definition does not compel the observer to discern whether those changes are pathological or consequence of normal ageing. However, it has been divided into two classes: (1) long-term age-related degeneration and (2) shorter-term environmental degeneration. The former
may lead to annular tears, disc prolapse and herniation or osteoporotic failures in cancellous bone. The latter are usually result of a mechanical event and occur unexpectedly. This includes vertebral failures, endplate fractures, annulus buckling or internal disc disruption during compression overloads; spondylosis (hypertrophic changes in apophyseal endplates and zygapophyseal joints) due to shear overload in excessive lordosis or heavy loads on the back; impact in articular joints and anterior annulus tears resulting from torsion; sprains (stretch and/or tear) in ligaments caused by forward bending; impact of neural arch and posterior disc bulging in backward bending; and sprains and articular joint impaction in lateral bending. The most alarming injuries happen though when there is a combination of the previous mechanical effects. [27] [38] [39]

Cell death/proliferation and structural disc alterations frequently lead to water and nutrient cessation which, in turn, induce an increase in stress and anomalous stress concentrations. Osteoporosis, damage in muscles and ligaments and osteoarthritis may be consequences of disc degeneration but may also be the cause for the limited function and load tolerance of the spine segments, and more critically, lumbar segments.

Figure 2.14: Several types of disc degeneration. Images obtained from [39]. (a) Fissures in annulus fibrosus. (b) Bulging disc. On the left a normal disc (for comparison) where the disc material does not extend beyond the periphery, contrary to the disc on the right. (c) Protrusion in herniated disc. Axial and sagittal views. The protrusion can even disrupt the disc and loose connection with the disc of origin. Compression of the nerve roots usually causes back pain. (d) Spondylosis deformans, on the left, is manifested by apophyseal osteophytes, with preservation of the disc space. Intervertebral osteochondrosis, on the right shows disc space narrowing, fissuring and cartilage erosion.
In general, whenever conservative treatments such as medication, physical therapy, injections or chiropractic adjustment fail to treat lower back pain, surgery should be considered. To do that, the physical origin of the pain must be identifiable within the L1-S1 segment. Two types of lumbar spine surgery comprise the most common surgical procedures for the lower back: lumbar decompression and lumbar fusion.

In lumbar decompression, the surgeon attempts to relieve pain caused by nerve root pinching, usually caused by lumbar herniated disc or lumbar spinal stenosis. The procedure consists of a bone portion removal over the nerve root and/or disc material from under the nerve root to decrease pressure in the nerve and allow the nerve to heal.

In lumbar fusion, one wants to stop rather than relieve the pain at a painful motion segment in the lower back. This type of surgery is usually employed for pain and disability caused by lumbar degenerative disc disease or a spondylolisthesis. A bone graft is used to stop the motion at the vertebral segment and, as so, to diminish pain generated from the joint. In addition to the traditional one-level fusion or decompression surgery, other alternatives are currently available, including lumbar arthroplasty, which has recently gained the interest of the medical community. This procedure comprises the removal of the painful disc and replace it by a prosthetic implant made of metal which preserves range of motion in the referred functional spinal unit. Lumbar fusion and lumbar arthroplasty are hereby briefly discussed and their pros and cons presented.

3.1 Lumbar Fusion

Robinson and Smith described intervertebral body fusion technique for the first time in 1955 to treat disc degeneration with nerve root compression, maintaining spine posture, decreasing pain but also reducing spinal movement. Although the technique is described for anterior portion of spine, it is also extended to posterior and lateral approaches. Basically, all of them consist of removing the IVD and replacing it by a bone graft, which causes two vertebral bodies to grow together into one long bone. With time, the graft provides foundation and environmental conditions to grow new bone and stabilize it properly. Since bone does not form at the time of surgery, instrumentation is also required to provide additional stabilization after the surgery and until the regeneration process is completed.

3.1.1 Bone Grafts

There are several options for bone graft: autograft - the donor is the same as the receiver -, allograft - donor is human but different from the receiver-, xenograft - the donor is an animal-, using a bone graft substitute or bone morphogenetic protein (BMP). Combinations of the previous grafts are also available.

The bone graft to use depends on several factor among which the type of fusion technique, the number of levels of the spine involved, local of fusion, patient risk factors for non-fusion, surgeon
experience and preference. Autografts are considered the best option since they fulfil osteoconductive and osteoinductive requirements, osteogenic cells, strength, growth factors and no immune response. However, autologous bone extraction is limited to a small extraction site (mainly iliac crest) and some complications might arise there. Hence, allografts from cadaver banks are used to overcome these complications. Nonetheless, they do not contain living bone cells nor growth factors and there might be a risk of disease transmission. Among the bone graft substitutes, solutions using bone morphogenetic proteins (BMP) have been widely employed to induce bone formation in spinal fusion models [1] [41].

3.1.2 Types of Interbody Fusion

As said, adding to segment level of fusion and bone graft options, the approach side from which the surgical procedure takes place will also vary: from the front (anterior), the back (posterior), both front and back, and/or from the side.

The posterior approach (PLIF) allows for the placement of all instrumentation in a single incision, but a smaller disc space evacuation than the anterior approach (ALIF). While the first may cause damage to neural structures, the latter might lead to vascular injury. On the other hand, the latter has a faster rate of fusion, since the graft is placed in a compression site and bone tends to form faster in compression states [42].

Another alternative has recently emerged, the lateral approach. It is a minimal invasion technique which provides minimal tissue damage, blood loss and post-operation discomfort, avoids main ligaments, yet providing full access to the disc site for removal. The only disadvantage of this technique in comparison to PLIF or ALIF is the access limitation, for example, in segment L5-S1 due to the pelvic brim, deformities or anatomy irregularities [43].

3.1.3 Fixation

Lastly, after guaranteeing enough blood supply and contact between the bone graft and bone, internal fixation is required to immobilize this area. Fixation is attained resorting to instrumentation, such as pedicle screws, rods and/or interbody cages. The degree of immobilization shall not change during the healing process, otherwise the implants might loosen, break or pull out of bone.

Cages are porous biologically inert structures with high resistance to fatigue and impact placed in between the bones of the intervention site and can be combined with the bone grafts. They stabilize this area, maintain intervertebral space height and have good inflammatory response. Cages exist in several designs - screw structure (horizontal cylinders), box and cylinder (vertical ring) - and in different materials - titanium, carbon or high weight thermoplastic material (PEEK) [44].
The goal of intervertebral body fusion is to reduce back pain rather than to repair disc function. Therefore additional stability can be attained using pedicle screws that anchor to vertebrae’s points and are connected with rods. The materials of choice for these adjunct fixations are stainless steel and titanium. The pedicle screws are placed in successive levels and a common rod connects them, thus disabling the area of any type of motion and promoting spinal fusion. However, the high stiffness offered by these constructs may accelerate degeneration in adjacent levels (stress shielding) and mechanical failure or migration of the device may occur. To overcome stress shielding events, semi-rigid fixation was developed in which the rods are made of a polymeric or composite material [1].

More recently, stability of the spine can be attained through dynamic stabilization. This technique makes use of pedicle screws and non-rigid rods or elastic bands to provide flexible connections which allow for movement and alter load bearing pattern of the spinal segment in a positive manner, controlling the abnormal motion of the segment but without fusion. This type of devices are specially useful for younger patients since it maintains flexibility at their backs. Devices such as Dynesis system, or Graf system achieve these goals but clinical outcomes are still being evaluated, with literature supporting that dynamic stabilization does not overcome standard fusion techniques [43].

Figure 3.2: Posterior dynamics stabilization devices. On the left the Graf system, on the center the Dynesis system and on the right a radiography with Dynesis system [43].
3.2 Lumbar Arthroplasty

Although lumbar fusion surgery is still the most prevalent option for spine surgeons nowadays, the idea of motion preservation in response to disc disease and degeneration led to the development of disc prostheses. Among them, total disc arthroplasty (TDA) intends to preserve the biomechanical motion patterns of the healthy disc, restore intervertebral height, stabilize the motion segment and convert shock-absorption properties to avoid the abnormal stress concentrations in the adjacent levels by replacing the diseased disc by an artificial one [1] [46] [47].

Despite its early implementation with Fernstörm's steel balls in the 1950's, the first devices for lumbar disc replacement were only approved by U.S. Food and Drugs Administration (FDA) in 2004. Current literature status remains inconclusive about the success of TDA over spinal fusion, since only short-term results are available and long-term clinical results, durability and complications remain unknown. Nevertheless, data provided by FDA studies support the claim that artificial discs provide results that are not inferior to fusion surgery [1].

The implants placement involves a similar approach to ALIF. The surgeon reaches the lumbar segment through an incision at the front of the abdomen, and moves the abdominal contents aside, such as major blood vessels, in order to reach the lumbar segment (usually performed by a general/vascular surgeon). The degenerated disc is removed, space between two vertebrae is created and the prosthesis is fitted in between [49].

Modern disc arthroplasty devices have a prothesis-bone interface consisting of a rigid end-plate made of metal that maximize the contact between both structures to ensure successful osteointegration and fixation. Stainless steel, cobalt chrome and titanium alloys are the materials of choice to TDA devices due to either their wear resistance or biocompability characteristics. In terms of bearing surface, the articular surface must allow for mobility, load distribution, low friction and high wear resistance, which is accomplished by metal-on-polymer or metal-on-metal articulations. Polyethylene and polyurethane are the two primary polymers used for TDA. The polymer core design exists in two forms: single-gliding surface with a securely fixed polymer or one with a metal end-plate or a double-gliding surface with a mobile polymer core sandwiched between two metal end-plates. [1] [46] [49]

Examples of these devices are shown below in figure 3.3.
As a final remark, one should note that lumbar disc replacement is not indicated for every patient. The primary indication is \textit{isolated mechanical discogenic back pain without radiculopathy or instability at L3-L4, L4-L5, or L5-S1 intervertebral levels} \cite{1}. Degenerative disc disease must be identifiable as the primary symptom in CT and MRI studies. TDA is contraindicated in patients with obesity, alterations in the lumbar segment, or already submitted to a fusion surgery.

Despite clinical outcome of this recent technique, even conservative estimates that 30\% to 50\% of present spine fusion surgeries will be replaced by spinal disc arthroplasty and two thirds of the current market lumbar spinal fusion market will be supplanted by disc replacement or other arthroplasty technologies \cite{50}. 
4
Experimental Part

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Low back pain itself is the most expensive disease process in the 30-50 year age range, with direct costs estimated at $20 billion in the USA. In the last decade, surgeons, research scientists and development executives from spinal implant companies have been making an effort to standardize preventive measures, treatment modalities and methodologies to access the study of this organ. However, the understanding and modelling of this field is still lacking.

Three types of methodologies are available. In vitro measurements provide the simplest scenarios of loading conditions allowing to investigate the kinematics (ROM, neutral zone, elastic zone), mechanical and structural properties of the vertebral body and discs, and comparison of healthy and pathological conditions. In vivo measurements provide true subject-specific information and physiological state, notwithstanding they have gradually been replaced by in silico testing methods. These methods are based in finite element (FE) analysis, in which the detailed anatomy is modelled and carefully chosen boundary conditions are assigned. Being a numerical method, it will solve problems of engineering and mathematical physics and provide results that can be used to prepare surgical interventions or evaluate the patient’s state of disease. It is also common to perform experimental validation of the results, meaning, attempt to reproduce as faithfully as possible the experimental conditions in the computational model and examine if the results are similar or not.

In the experimental study of the biomechanical behaviour of human spine and testing of new implant devices, anatomy and size are unquestionably important. Therefore, human cadaveric spine is the most reliable model, yet the tissues are difficult to obtain for in vitro studies as well as performing in vivo testing, being both subjected to strict ethical regulation.

The use of animal models presents a good alternative for biomechanical testing. Conversely to humans, where the specimens may result from a population with great variability due to gender, age, pathologies, ethnicity or even loading history, breed animals present more homogeneous properties. Plus, the assessment of a younger population is easier in animal specimens rather than in human models. And finally, the operators performing the experimental work are less exposed to bacterial and viral infections (e.g. AIDS and hepatitis) than when manipulating human material.

The use of quadruped animals such as sheep, porcine, calf or deer is largely reported by literature since the availability of specimens is greater for breed animals. Nevertheless, other animals such as dog, primates and mice have also been used in order to access biological processes and more human-like scenarios, where anatomy or metabolic processes play leading roles.

This thesis follows a work previously done in the LAETA laboratory in IST, for which two sheep cervical spines were provided by Évora’s Veterinary Hospital. The supplied specimens came from mature healthy sheep that were sacrificed for an anatomy class. They were dissected and frozen during the class and later brought to IST’s laboratory. It has already been advised that acquisition of animal material by the IST’s laboratory in the future should follow regulation number 1069/2009 with a permission request to Direcção Geral de Veterinária, specifying the location where the tests will take place and what the laboratory intends to do with the disposals. Regarding the disposals, it has been agreed that they will return to Évora’s Hospital for proper handling.

The goal of the work was to determine the Young’s modulus of the whole vertebra, since it would be
difficult to identify where the frontier between cortical and trabecular bone in the computational model would be. The experimental Young's modulus was obtained after performing compressive tests in the vertebrae until fracture. Then, this value was assigned as a material property in the computational model and the results of total displacement applying the same compressive forces were compared.

4.1 Comparison of the Sheep and Human Spines

Before explaining the experimental procedure, attention must be given to the comparison between sheep and human spines, focusing mainly on the vertebrae, rather than IVDs.

The clearest difference lies on the load distribution, that one should expect to be higher in humans due to its upright position, opposing to the horizontal position of a quadruped animal. This difference in axial load distribution should be found when examining the vertebrae and, according to Wolff’s Law, the vertebrae bone density should be higher in humans than in sheep. However, this is not verified. In fact, sheep have a more denser trabecular network and a higher bone mass than human. The reason for this is that the spine in quadrupeds is optimized for bending and has to support loadings due to the body weight while maintaining the spine posture. Therefore, the axial loads play an essential role in this support and provide similar conditions to those observed in humans. [1] [52] [53] [55]

Anatomically, the number of vertebrae is larger in sheep than in humans (7 cervical vs 7, 12-14 thoracic vs 12, 6-7 lumbar vertebrae vs 5). The vertebral body in humans is two times wider than tall, having a cylindrical shape, while in sheep it is taller than wide, having a conical shape. Additionally, the vertebral body height in sheep decreases from the cervical to the lumbar regions, converse to human where lumbar vertebral bodies have greater height than in cervical region. Lastly, the cortical shell is thicker for sheep than for humans. The pedicles follow the same rationale being narrower and higher in sheep. As for the facet joints, they present an angle of 28° between the front plane and the articular surfaces, while in humans this angle is around 58°. The transverse processes are usually longer than in humans so that paraspinal muscles have a stronger support to bear the weight of abdominal viscera and preserve spinal movements. To conclude, spinal canal in humans is wider and deeper than in sheep. [1] [20] [52] [58]

Though the IVD study was outside the range of this thesis, the wedge shape is common to ovine and human discs, but the height varies, being 5-6.3 mm in sheep and 8 - 16 mm in human. Water and collagen content of the annulus and collagen fibre orientation angles are similar in lumbar discs from sheep and humans [1] [20] [59].

Wilke et al. [20] compared the ROM of ovine and human spine at the cervical, thoracic and lumbar levels, showing similar in vitro ROMs for flexion-extension, lateral bending and axial rotation, yet larger in humans than in sheep. Figure 4.1 shows the differences in ROMs for both species at the cervical region and clarifies the morphometric differences described above.
4.2 Materials

After receiving the sheep spines from Évora’s Veterinary Hospital, they were frozen at -20°C in the laboratory. The preparation of the vertebrae for mechanical testing was made by a previous colleague, Catarina Soares Pires, during her master thesis [57].

The material was acquired from VWR [61], a global laboratory supplier, from which the following items were bought:

- Disposable Nitrile Gloves - used to handle the animal material;
- Plastic bags - to reserve biological waste to be send back to Évora’s Veterinary Hospital and the biological material of interest. Each specimen was kept in a separate bag and properly labelled;
- 5L container - used to leave the spine and latter the testing samples in water whenever they were not being used nor frozen;
- Pursept A desinfectant - Needed to clean all the instruments used and the workspace after work;
- Hy-G-Clenz Soap - An anti-bacterial soap, to wash hands after working with biological material.
- Dissection Kit - Including a scalpel, a scissor, a spatula, tweezers, a needle and a lancet. This instruments were needed for muscle removal from the spine and separation of individual vertebrae.
- Extra scalpels - The scalpels’ blades are very sharp, though its wear rapidly makes them disposable and, to keep precision in vertebrae isolation, changing scalpels is mandatory.

Note that the two latter items were not used during this work as they are only necessary for the spine preparation.
One of the reasons why this work follows a previous one is that the testing machine available at the laboratory, an universal INSTRON 5544, could only be accessed to a load cell with maximum limit of 2000 N. Since the ultimate force could not be achieved with this load, another two machines were used, kindly provided by Professor Fátima Vaz and Professor Pedro Amaral. The first was a electro-mechanical INSTRON 5566 operating with a load cell of 10 kN and the second was a servohydraulic INSTRON 8800 operating with a load cell of 100 kN. The justification for using two testing machines is related to technical and maintenance issues that succeeded simultaneously to the experimental stage. Fracture of the samples was only achieved with the 100 kN cell.

4.3 Methods

4.3.1 Spine Preparation

As said before, the spine was prepared by a previous colleague and the procedure’s details are given in her thesis works, here referenced and suggested as further reading [57]. A brief description is given anyway.

The spine was defrosted in a water bath at 4°C for 24 hours long. Afterwards, all muscle tissue and ligaments were harvested. The tissues of interest - bone and IVD - should be kept at room temperature for the least amount of time possible and always hydrated. The number of thawing and frosting cycles have been reported to have low effect in tissues’ properties [26] and specimens should not be kept over 20 hours in thawing conditions. Therefore, whenever one must perform the mechanical tests, the frosting - defrosting cycles should be done as many times as needed and the samples should be kept in the water bath when they are not being tested.

After separating the vertebrae and discs, the latter were stored and the former were taken to the IST workshop to be cut. Since no cement pot was used to achieve uniform boundary conditions, special care was taken with the geometry of the cut, trying to reach as much as possible two parallel surfaces. A total of three vertebrae were cleaned and separated, and one of them was split in half, from which resulted two samples. A total of four samples led to the results presented in the following chapters.

Figure 4.2: The prepared samples, labelled as V11, V12, V2 and V3 from left to right and top to bottom.
4.3.2 Vertebral Testing

Since no vertebral experimental compressive tests have ever been done in the IST laboratory, the testing criteria had to be adapted from an existing article in the literature: Buckley et al. [62]. The described method has already been cited in reviews and communications about standardization of in vitro testing. [52] [63]

Nonetheless, some modifications were required. Buckley et al. [62] start by potting the vertebrae in poly (methyl methacrylate) (PMMA) bone cement with a layer thickness varying between 1-3 mm to assure uniformly plano-parallel surfaces and perform ten pre-test loading cycles between the [100, 250] N interval at 0.1 Hz in order to avoid compliance of the PMMA layer and, hence, dissimilarities in inter-specimen stiffness. For the present case, no cement was used, justifying the need for performing a perfect parallel cut in vertebrae’s surfaces. Using the first testing machine, this pre-test was also performed within the same load interval, but velocity condition was modified to 1 N/s because no frequency (Hz) command option was available in the used software. In the second testing machine, the adjustment is automatically performed by a feedback loop control mechanism conducted intrinsically by the machine, so no pre-test was required.

Figure 4.3: (top left) INSTRON 5566 with experimental setup. (top right) The pre-loading cycles to perform the adjustment. (bottom left) INSTRON 8800 with experimental setup. (bottom right) No pre-loading is required since the machine is regulated by a feedback loop mechanism that automatically performs the adjustment. Note that no PMMA layer was used, but thin plastic sheets were placed under and over the sample surfaces to protect them from the metal.
Posterior to pre-testing, the article details the actual compression at a 1 mm/min velocity until reaching ultimate force of 5 kN. For the current work, in the first testing machine the velocity condition as replicated, but for the second machine this was not possible and therefore, an estimation of velocity conditions was made. Since the former spent 4.5 minutes until reaching the stopping criterion of 10 kN, the velocity assigned for the latter was \( \frac{10\,\text{kN}}{4.5\,\text{min}} = 2.22\,\text{kN/min} \).

For the sake of specimen safety, two stopping criteria were assigned. The first was, as just said, the maximum load allowed. For the first machine, having a 10 kN load cell, it stopped at 9800 N. For the second, despite having a 100 kN loading cell, an estimation of the compressive ultimate force based on literature Young’s modulus was made and a value of 30 kN was assigned. Even so, when the operator saw that the test had already reached the fracture region, manual stopping was allowed. The second criterion was left as the 40% load reduction criterion in the first machine, which is the default option, considered the consequence of specimen breakage. This option does not apply to the second machine, since it is load-driven and therefore only displacement during each trial was visually controlled.

In the first testing machine, several tests were made to gain some load sensibility: 100 N, 750 N, 1980 N, 3000 N and 9800 N, but non of them reached the elastic limit and no signs of fracture were visible nor microfracture were detected on the graphics. In the second machine, since after attaining the fracture region, the specimen must be discarded, only one trial per sample was done. The results were saved in a .csv file acquiring time (s), displacement (mm) and force (kN), for latter processing and analysis. In the following sections only results from the fracture trials are presented due to thesis extension limit.

### 4.3.3 Area Measurement

In order to calculate Young’s Modulus, there are only left two missing constants: cross-sectional area \((A_0)\) and initial length \((l_0)\). Initial length was measured before initiating the tests with a paquimeter between both surfaces at different places and average was taken for each sample. Cross-sectional area was calculated using an open source program, ImageJ [64], which can import image data and, through pixel parametrization, allows for area measurement. For that, the picture must be taken in a frontal perspective view and a distance has to be included to serve as the picture’s scale.

Since the vertebrae’s geometry are too irregular, the area of both surfaces was calculated and averaged. Figure 4.4 describes the procedure for ROI selection, done with the help of some ImageJ tutorials [64].
4.4 Results

The .csv file was imported to a Matlab routine that drew the force-displacement (kN/mm) and stress-strain (MPa/(mm/mm)) curves. Stress, given in MPa is obtained according to:

$$\sigma = \frac{F}{A} \text{[MPa]}$$ \hspace{1cm} (4.1)

Force is given in Newton and area in squared millimetres. The strain is obtained by the formula:

$$\varepsilon = \frac{\Delta l - \Delta l_0}{l_0}$$ \hspace{1cm} (4.2)

where $\Delta l = l - l_0$ and $\Delta l_0 = l_i - l_0$, with $l$ being the instantaneous displacement at a given point in the curve, $l_0$ the initial length and $l_i$ being a chosen value by the operator where it is considered to begin the elastic region. It has been reported that at small strains, trabecular bone exhibits nonlinear behavior responsible for a toe region at the beginning of the curve and this response may vary with the anatomic site [65]. Hence, a point considered being inside the elastic region and another considered...
to be just before the apparent compressive yield strain were chosen to represent the interval of the linear region. A linear fit was done by the Matlab routine and the $R^2$ values were calculated. This step was repeated for each sample as many times as needed until a good fit was achieved. The results for vertebra V1.1 are shown in figure 4.5.

![Figure 4.5: Stress-strain curve and linear fit for Vertebra 1 slice 1, starting at strain zero, from the optimal chosen linear region.](image)

The resulting Young's Modulus of around 187 MPa is within the typical reported values in literature for trabecular bone of 100 MPa and for cortical bone of 12000 MPa [66]. Additionally the yield strength lies between 20 MPa and 25 MPa, the point where plastic deformation starts to be noticeable. This is important because it is known that dry bone has a higher Young’s Modulus, but the strength and strain to failure decrease significantly with hydration and hence, mechanical tests are generally performed with wet bones [67].

For this sample the $R^2$ value was 0.9890, which shows the good fitting of the elastic region for the experimental values as a proportional relation, as theorized in Hooke’s Law. The graphics for the other tests showed a similar behaviour and can be seen in Appendix A. A summary of the data and results can be found in table 4.1.
Table 4.1: Summary of experimental data and results.

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Area (mm$^2$)</th>
<th>Height (mm)</th>
<th>Stiffness (kN/mm)</th>
<th>Young’s Modulus (MPa)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 Slice 1</td>
<td>1043.981</td>
<td>9.95</td>
<td>18.778</td>
<td>186.84</td>
<td>0.9890</td>
</tr>
<tr>
<td>V1 Slice 2</td>
<td>965.900</td>
<td>10.25</td>
<td>20.709</td>
<td>219.76</td>
<td>0.9917</td>
</tr>
<tr>
<td>V2</td>
<td>941.339</td>
<td>15.78</td>
<td>28.549</td>
<td>475.54</td>
<td>0.9958</td>
</tr>
<tr>
<td>V3</td>
<td>924.199</td>
<td>11.25</td>
<td>14.678</td>
<td>178.67</td>
<td>0.9720</td>
</tr>
<tr>
<td>Mean Value ($\mu$)</td>
<td>20.696</td>
<td>265.20</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Deviation ($\sigma$)</td>
<td>5.039</td>
<td>122.409</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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In order to obtain a Finite Element Model from the acquired CT images, several stages should be undertaken. After the CT image acquisition, Mimics is used for image processing and segmentation, which combined with 3d-Matic, provide a pre-processing of the superficial meshes that allows for triangle reduction and elimination of artifacts. A Point Cloud File is obtained and imported into Solidworks, and with the help of Scanto3D Module, the superficial mesh is restored and smoothed. This is followed by a conversion into surfaces and finally into a solid model. A Parasolid file is then imported into Abaqus, where the finite element analysis can be attained.

For the human model, density-based vertebrae models were developed. For this purpose, a specific mesh was chosen and again imported back to Mimics. This allows for the assignment of the density distribution and material properties through the mesh elements. The mesh is now returned to Abaqus and the analysis proceeds. Figure 5.1 summarizes the previous description.

![Figure 5.1: Methodology used for the developed model.](image-url)
5.1 Medical Images

Medical Imaging has revolutionized medicine. It allows physicians to see inside the body without making an incision and from that to measure, manage, diagnose, treat, and even think about medical illnesses and conditions. Among the several image modalities, three are on the list of the top five innovations that have changed medicine: computed tomography (CT), magnetic resonance imaging (MRI) and minimally invasive interventional techniques. [68]

The Computed Tomography is based on the absorption of X-Ray by different tissues in the body. For that, a X-ray beam with a given energy is emitted by a source and passes through the patient, being detected by film or ionization chamber on the opposite side of the body. Contrast revealed in the image between different tissues arises from the X-Ray attenuation of each tissue. Such value is particularly accentuated in bone, since it absorbs almost completely in this energy range, and not so much in soft tissues.

Quantitatively, the amount of linear attenuation in each "voxel" of a 3D image is described by the Hounsfield number (HU), owing the name to the considered inventor of the image technique, Dr. Godfrey Hounsfield, rewarded in 1979, together with Cormack, with the Nobel Prize. The HU scale is a linear transformation of the original linear attenuation coefficient measurement in one in which the radiodensity of distilled water at standard pressure and temperature (STP) is defined as zero, while the radiodensity of air at STP is defined as -1000 HU. Further details will be discussed later. [69]

The X-ray source is composed by two electrodes within a tube: a negatively charged cathode made of a heavy metal (electron source) with a high melting point and an anode containing the metal target. When a potential difference is applied (between 15 to 150 kV depending on the application) between the electrodes, streams of electrons are accelerated from the cathode to the anode and produce X-rays as they strike the anode. The potential difference comes in the form of a rectified alternating voltage and is characterized by its maximum value, the kilo voltage peak (kVP). Another important parameter is the tube current (mA), which quantifies the number of electrons per second that travel inside the tube. [70]

The patient lies inside a tunnel with the scanner source and detector positioned $180^\circ$ across each other. A X-ray collimated beam will pass through a thin slice, producing an unidimensional projection. The source and detectors rotate to reconstruct a two dimensional image of each slice and after completing a full cycle, the bed slides to obtain the measurements of the next slice. A computer receives the data and reconstructs the three dimensional anatomy.
Figure 5.2: Basic functioning of CT [71].

Since acquiring one slice at one time would be very inefficient and would imply patient exposure to a large radiation dose, most modern CT machines offer helical CT modality and are able to continuously take pictures while the patient’s bed is still moving.

The CT scan of the sheep cervical spine segment was obtained by Prof. João Gamelas in Clínica dos Quadrantes in Miraflores and the CT scan for the complete upper body of a 33 year old woman was performed in Ecorad Clinic. Both set of images show no pathology of the target regions.

| Table 5.1: Several parameters in the acquisition of sheep and human’ CT scans. |
|---------------------------------|--------|--------|
| File Format                    | Dicom  | Dicom  |
| Manufacturer’s Model Name       | Siemens SOMATOM Definition AS+ | Toshiba Aquillion |
| Study Date                     | 2014/01/30 | 2013/03/22 |
| KVP (kV)                       | 120    | 135    |
| X-ray Tube Current (mA)        | n/a    | 350    |
| Voxel Size (px)                | 512x512x403 | 512x512x291 |
| Pixel Size (mm)                | 0.246094 | 0.25 |

5.2 Image Segmentation

The following modelling step is used to extract or “segment” the relevant information of the acquired images. Image segmentation is defined a partition of an image into non-overlapping regions, each defined according to an homogeneous criterion of an image feature, such as intensity or texture. In the medical field this is especially useful in anatomy structure delineation, tumour identification and volume measurement [72].
For bone segmentation, three techniques can be employed: global thresholding, semi-interactive methods based on edge detection and manual segmentation. However, only thresholding and manual techniques were explicitly implemented, being the edge detection methods implicitly used in the manual selection tools. Mimics Materialise is a powerful tool that allows for the bridge between image data and 3D-mesh data [73]. After loading the DICOM images into the software, the user defines the range of intensities that contains the information of interest within a volume histogram of pixel intensities. Thus, the user is applying a threshold, in which the region of interest (ROI) is selected and the values above or below the threshold are cancelled. This technique though depends on the user experience and does not take into account spatial information. Therefore it only gives reasonable results when applied to images with high contrast and it is not sensitive to noise nor artefacts [74].

Figure 5.3: ROI definition using threshold and manual segmentation techniques for (a) Vertebra 2 (C4) in sheep and (c) L4 in human models. In (b) the operator can define the mask’s threshold.
Mimics offers advanced manual editing tools, such as crop mask, morphology operations, multi-slice editing and 3D mask editing, but also automatic methods. Unfortunately, a full automatic segmentation was not possible, so a semi-automatic segmentation was implicitly used with the live wire shape in the previous tools. It is based on a minimum-cost path search from a single seed point on a graph provided by the user, which allows greater control through selection of the a desired path from multiple candidate paths. Further information might be found in bibliography [75].

At last, segmentation of the intervertebral discs in the human model were difficult to implement due to the low values of linear attenuation in soft tissues. Hence, manual segmentation was the chosen method to identify these structures. The resulting models can be seen below in figure 5.4.

![Figure 5.4](image_url)
5.3 Pre-Processing of Superficial Meshes

The next step is the conversion of the segmentation into a 3-D superficial mesh. Mimics performs this using the "Convert mask to 3D solid" feature, in which a triangular mesh is assigned using an adapted Marching Cubes Algorithm (MCA) that takes into account Partial Volume Effects (PVE). The voxels of the 3-D image are considered as three dimensional nodes with an associated intensity value. The voxels that have intensity values within the user-specified range contribute to the isosurface. The algorithm marches through each and every parallelepiped and establishes a surface boundary composed by triangular elements [74].

After the superficial mesh is finished, it is imported into 3-matic, a software that combines CAD tools with pre-processing capabilities. Concern must be given to two consequences of the MCA: (i) a staircase shape surface which is different from the natural surface curvature, and (ii) an excess of nodes and facets that expresses extra and/or erroneous information. One might overcome these features using smoothing and decimation, respectively. While smoothing was here performed just for the matter of visualization, decimation was here implemented more accurately with triangle reduction. This function uses a geometrical error factor (GE) to define the solution points that can be eliminated without decreasing the accuracy between the scanner data and the model. It is recommended to use 1/8 of the pixel size. So in both sheep and human models it was defined $GE = 0.25/8 = 0.03125$ mm [76]. The results are shown in figure 5.5.

The surface was remeshed and upon it a volume mesh was created. The file was exported as a Point Cloud File (.txt). In each step the mesh quality was evaluated.

5.4 Conversion into a Solid

When the point cloud is imported to Solidworks, Scanto3D, a very useful module, has two functions, called Mesh Wizard and Surface Wizard, that can be used to finalize the geometric model. The former allows once again to simplify and smooth the mesh, which is regenerated from the imported point cloud. Since decimation has already been made, the wizard deals now with the staircase effect. Therefore, the surface requires a low-pass filtering. Smoothing alters the overall mesh appearance by changing the node coordinates relative to each other but with no change in the number of nodes nor triangular elements. Laplacian smoothing is a common filter to rectify the staircase effect, which for a single node $p_i$ at position $x_i$, will move $p_i$ to a new position $x_i + 1$ according to

$$x_{i+1}^{n} = x_{i} + \lambda \sum (x_{j} - x_{i}), \forall j : 0 \leq j \leq n$$

(5.1)

where $x_j$ are the positions of the $n$ neighbouring nodes $p_j$ connected to $p_i$, and $\lambda$ a user-specified parameter that controls the degree of smoothing applied to the surface mesh. A bigger $\lambda$, leads to a smoother mesh [74]. Since no elements nor nodes are eliminated from this process, the definition for the curves’ reconstruction remains high. In Solidworks, the iterations are performed manually as many times as desired and the degree of smoothness is given by a sliding bar.
Figure 5.5: Performing triangle reduction in the superficial meshes using remeshing features in 3-matic software in C4 sheep vertebra (c) and human L4 vertebra (a). After triangle reduction avoiding geometrical error, the operator can evaluate the quality of the triangles using thresholds (in this case height/base relation) in the histogram to properly export the pre-processed mesh (b) (d). Note that some extra-erroneous coming from segmentation was removed.
Afterwards, *Surface Wizard* is launched in order to define the surface patches that interpolate the mesh surface and will form the solid model. In Scanto3D toolbox, groups of NURBS patches are generated and the user must define the amount of detail of the patches. These group of NURBS patches form regions on the surfaces that are editable by feature lines. The user is able to choose the lines location and in this fashion control the distribution of the patches. Ideally, feature lines should coincide with mesh curvature transitions to obtain an accurate representation. Due to the complexity of the anatomy, an automatic method was employed, all the errors were deleted and the solid was created. The solid output was finally ready to be imported into Abaqus to generate the FE mesh.

![Figure 5.6: Mesh Prep and Surface Wizards for human L4 solid model generation. The upper three images reveal the smoothing operation whereas the bottom three show the errors in the first, being corrected by editing the feature lines to form the final solid model.](image)

![Figure 5.7: Mesh Prep and Surface Wizards for sheep C4 solid model generation. The upper three images reveal the Mesh Prep operations and the bottom the Surface Wizard operations.](image)
5.5 Bone Mineral Density Distribution and Material Properties Assignment

The majority of the studies reported by the literature concerning human spinal models relate only to the geometry of the model including the FE mesh and boundary conditions. In these studies, the effect of morphology changes in the mechanical response of the model is ascertained. One example is the thickness of the cortical shell that is assumed to be constant around the surface of the vertebral body. The authors usually simplify the material properties of the model by assigning only two types of materials: the cortical shell, having a given Young’s Modulus and Poisson’s Ratio, and the trabecular bone, having other values for these material properties [27]. Regularly, mesh convergence studies are also performed and the mechanical response is compared with experimental data in order to be validated.

Fewer number of studies are found relating to the sensitivity of specimen-specific material properties. In spine investigation, the combination of FEA and CT data sets can give valuable information about, for example, strength, stiffness or fracture risk, assuming that each element of the mesh contains an amount of CT grid points and these represent the real physical properties of the material [51] [77] [78].

In the bone structure field, two different methods are generally reported for meshing: (1) volume based approach and (2) greyscale approach. The first uses $\mu CT$ images to segment only trabecular bone without any trabecular space or interstitial fluid and each element will have material properties based on bone volume fraction (BV/TV). The latter can be obtained by any type of CT modality and each element will be assigned with the average greyscale off all voxels that belong to it and this scale is related with density, from which the material properties are calculated [51]. In this work, the greyscale approach was chosen, since it takes into account the spatial variation in bone properties (regions of higher mineralization are visually brighter) and the specimen data was acquired in vivo, meaning, in a fully hydrated state with marrow intact.

To get quantitative data from a CT machine, it is possible to extract the CT number. CT numbers are directly related to the linear attenuation coefficient for the X-ray beam, when the energy of the beam is fixed. The voxels obtained are usually represented in 12-bit binary numbers, which results in $2^{12} = 4096$ possible values. The machine is calibrated for water and air linear attenuation coefficients such that their HU numbers correspond to 0 HU and -1024 HU, respectively. Thus the scale extends from -1024 HU to +3071 HU and the HU number for any material with a linear attenuation coefficient $\mu_x$ is given by:

$$HU = \frac{\mu_x - \mu_{\text{water}}}{\mu_{\text{water}} - \mu_{\text{air}}} \times 1000$$

(5.2)

where $\mu_{\text{water}}$ and $\mu_{\text{air}}$ are the linear attenuation coefficients for water and air, respectively. A linear relation between the HU number and the apparent density ($\rho_{\text{app}}$) of the tissue can be found. A linear trend line can be found between the no material condition which corresponds to air ($\rho_{\text{air}} = 0 \text{ gcm}^{-3}$) and the bone condition which corresponds to full mineralized cortical bone ($\rho_{\text{bone}} = 1.92 \text{ gcm}^{-3}$). The apparent density at any point in the bone is then found by linear interpolation of HU values [69].
After importing the geometrical model to Abaqus, a mesh convergence study for the human FSU was performed for the simplified model, meaning, not taking into account density and material properties heterogeneity in vertebrae. The procedure is described in chapter 7. When the desired mesh density was achieved, a .inp file only containing the mesh information was imported back to Mimics with the help of FEA Mesh. In this module, the operator can toggle the "Material Assignment" option. A total of 25 types of materials were selected and the density values were calculated. Along with density distribution, mathematical relationships between Young’s Modulus and density were also assigned as illustrated by figure 5.8. The elasticity-density relationships are a bit more challenging since they might depend on the experimental techniques needed to measure the mechanical properties in a highly porous anisotropic material such as bone [79]. Further details will be given below.

Figure 5.8: Material Assignment procedure. On the left the vertebrae's meshes are shown as imported from Abaqus. On the center the final meshes with all material properties assigned. On the right it is highlighted the number of materials option and the mathematical relationships that relate density with HU numbers and elasticity modulus with density. Poisson ratio was assigned 0.3 for all materials. Below both histograms for elements and materials versus HU numbers are displayed. The values can be seen and/or edited clicking on the "Material Editor" tab.

Despite the procedure being the same, two different methods for density assignment were employed, which will be discussed now.

5.5.1 No Calibration Method

For this case, it was assumed that no non-linearities in the detector system or other physical factors would affect the linearity of the HU-μ relationship, though one should keep in mind that this is not true [69, 80, 81]. Therefore, a distinction will be made in notation, where CT correspond to the “measured HU values” and CTH correspond to the “correct HU values”. For a non-calibrated system equation 5.3 assumes CT = CTH so it is applied directly in HU-ρ relationship:

\[ \rho = a + bCT \]  

(5.3)

where \( a \) and \( b \) are constants resulting from the regression law. In order to find the HU value for air in the CT data set a region of interest (ROI) was selected in Mimics containing only air. This can be
done by creating a threshold segmentation mask considering only the lowest values of the whole set. Drawing a voxel histogram, the lowest CT value found was -981, so $CT_{air} = -981$ corresponding to $\rho_{air} = 0 \text{gcm}^{-3}$.

**Figure 5.9:** ROI selection for air and respective histogram with the lowest CT value in the whole CT data set.

The same rationale was applied to find cortical bone CT value. The higher CT value was chosen within the selected ROI leaving $CT_{bone} = 1442$ to $\rho_{bone} = 1.92 \text{gcm}^{-3}$.

Solving a linear system with two equations and two unknowns ($a$ and $b$), the final relation obtained for HU-$\rho$ was:

$$\rho = 0.777350392 + 7.924061081 \times 10^{-4}CTH[\text{gcm}^{-3}]$$

(5.4)

### 5.5.2 Phantom-less Calibration Method

Available commercial CT scanners produce energetic beams in the order of kilovoltage. In this range, photoelectric effect and coherent scattering play substantial roles. These effects are observed because of the properties of the targeted and surrounding tissues and even the nature of the beam itself. Therefore, the relationship between HU scale and effective density for body tissues must be calibrated to maximize accuracy of the results. This is usually achieved with the help of an external reference phantom, which contains a material similar to those present in body tissues, with known density and linear attenuation coefficient throughout the photon energy spectrum. Phantoms are placed under the patient during scanning and equivalent densities are determined by comparison between the values observed for them and the body tissues [69].

Although the method accounts for scan-to-scan and scanner-to-scanner variability, the peripheral placement of the calibration system results in a measurable degradation in precision due to patient-moderated artifacts, partial volume averaging and repositioning errors [82] [80] [81].

Patient-moderated artifacts account for scatter and beam hardening. The former occurs because non-symmetrical objects appear in the X-ray path, resulting in different path lengths. The latter relates
to the polychromatic nature of the beam in which for the same material, the edges appear brighter than the center. This occurs because the lower energy spectrum is more readily attenuated than the higher energies. The end result is a beam that, though diminished in overall intensity, has a higher average energy than the incident beam. Partial Volume Effects (PVE) occur due to tissue inhomogeneity, since one pixel might contain several substances/tissues, but since its value will be the average, blurring will inevitably exist and propagate to its neighbouring pixels. Positioning errors also happen because manually chosen ROI's are weakly reproducible [80-82].

To improve reproducibility and accuracy of the results, over the last two decades, several authors have proposed a phantom-less method that uses the patient body tissues as the calibration reference. The proximity of the tissues to the vertebral bodies, for instance, avoid the reported errors and its easy implementation would have widespread clinical utility and cease quantitative information in any type of CT scanner that is currently available but not utilized [69,80-84].

As seen, water is the substance for which the HU scale is usually calibrated. Unfortunately, it may not be available during the scanning and its effective energy is not always known. By effective energy, one means "the single photon energy at which the linear attenuation coefficient of a particular material equals the linear attenuation coefficient in the polyenergetic beam". It depends on the material being scanned and on the position within the object. Containing water, fat and skeletal muscle have fixed chemical compositions and known linear attenuation coefficients as a function of X-ray effective energy, thus providing an alternative to use as reference tissues to plot a standard calibration curve [85].

Using equation 5.2, the difference between the HU values for muscle and fat is given by

\[
HU_{\text{muscle}} - HU_{\text{fat}} = \frac{\mu_{\text{muscle}} - \mu_{\text{fat}}}{\mu_{\text{water}}} \times 1000
\]

and considering the real value of \( HU_{\text{water}} = CTH_{\text{water}} = 0 \) one can find the following ratio

\[
\frac{\frac{-HU_{\text{fat}}}{HU_{\text{muscle}} - HU_{\text{fat}}}}{\frac{\mu_{\text{water}} - \mu_{\text{fat}}}{\mu_{\text{muscle}} - \mu_{\text{fat}}}} = \frac{\mu_{\text{water}} - \mu_{\text{fat}}}{\mu_{\text{muscle}} - \mu_{\text{fat}}}
\]

to be a remarkable consistent value of 0.77 for measured CT values between 60 keV and 80 keV of effective energy, which correspond to tube peak voltages in the range of 120-140 kVp [83]. The measured HU values \( CT \) are then related to the corrected HU values \( CTH \) by

\[
CTH = kCT + B
\]

where \( k \) is the slope and \( B \) is the offset [83].

To obtain the CT values for muscle and fat, two ROIs were submitted to threshold and manually selected containing only each constituent. The data was inserted in a Matlab routine to draw the histogram and to obtain the most frequent CT value in each ROI, as depicted in figure 5.10. One should note that the most frequent and not the average value was used in order to reduce ROI repositioning errors [80]. The values found for fat and muscle were \( CTFat = -97 \) and \( CT_{\text{muscle}} = 53 \), respectively.
Figure 5.10: ROI selection for fat (red) and paraspinal muscle (pink) and respective histogram with the most frequent CT value for each.

Solving the system for a tube voltage of 135 kVp, which corresponds approximately the average of the effective energy range of 70 keV, the corrected values were given by

\[ CTH = 1.0019CT - 17.361 \]  

(5.8)

and this adjustment was inserted in equation 5.4 to give the new HU-\( \rho \) relation:

\[ \rho = 0.791081265 + 7.909033917 \times 10^{-4}CT[gcm^{-3}] \]  

(5.9)

The calculations are detailed in Appendix B.

5.5.3 Young’s Modulus Mathematical Relationship

While the relationship between CT attenuation coefficients and density can be established by direct calibration, mathematical relationship between density and mechanical properties of bone is more challenging. Several empirical density-elasticity relationships have already been established but Helgason et al. [79] discuss the validity of the results, since standardized testing procedures were only proposed last year [63]. Additionally, the extrapolation of these relations to cortical bone might be also misleading. Based on this review, this work employed all the relations reported for human spine and the results were compared. A summary of the mathematical relationships is presented in table 5.2.
Table 5.2: Density-elasticity mathematical relationships used [79].

<table>
<thead>
<tr>
<th>Study</th>
<th>Site</th>
<th>Type of bone</th>
<th>(E) [MPa]</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morgan et al. (2003)</td>
<td>Human vertebrae</td>
<td>Trabecular</td>
<td>(E = 4730\rho_{app}^{1.56})</td>
<td>0.73</td>
</tr>
<tr>
<td>Keller (1994)</td>
<td>Human spine</td>
<td>Trabecular</td>
<td>(E = 1890\rho_{ash}^{1.92})</td>
<td>0.702</td>
</tr>
<tr>
<td>Kopperdahl and Keaveny (1998)</td>
<td>Human vertebra</td>
<td>Trabecular</td>
<td>(E = 2100\rho_{app} - 80)</td>
<td>0.61</td>
</tr>
<tr>
<td>Carter and Hayes (1977)</td>
<td>Pooled</td>
<td>Cortical and trabecular</td>
<td>(E = 3790\dot{\varepsilon}^{0.06}\rho_{app}^3)</td>
<td>NR</td>
</tr>
</tbody>
</table>

Ash density = \(\rho_{ash} = 0.6\rho_{app}\); Strain rate = \(\dot{\varepsilon} = 1\)
Computational Validation of the Experimental Work

Contents

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There is a growing consensus in the scientific community that biological tissues can be modelled by finite-element (FE) models and comply with the experimental data in multiple load regimes. Along this line, the results obtained in the experimental study were introduced in the commercial FEA software Abaqus to verify whether the measured parameters were reached or not.

Still holding on Solidworks, after generating the three sheep vertebrae, these were cut as much alike as possible to the cuts performed for the compression tests. This implied to slice each vertebra with two parallel surfaces with 9.95 mm, 10.25 mm, 15.68 mm and 11.25 mm height.

![Figure 6.1: Comparison between the V2 sample and the model. Front and back perspectives are given.](image)

The geometries were exported as Parasolid files (.x_t) and imported into Abaqus as solid parts, being the surfaces already subdivided. Remaining errors were corrected using geometry edit tool and the small surfaces were removed using the virtual topology toolset, since they were not useful and would posteriorly hinder the mesh generation.

To mimic the compression plates' function, two analytical rigid parts were created. They were sketched as rigid surfaces, so that their deformation was negligible. A reference point (RP) was assigned to each plate and being an analytical part, this means that every property of this point will propagate to the whole part. Likewise, no mesh is necessary to a analytical rigid part.

In the Property module the vertebrae were assigned as homogeneous solid sections and a mechanical elastic and isotropic material was specified. The Young's Modulus for each slice determined experimentally was assigned and a Poisson's ratio of 0.3 was employed for all. Once again there was no distinction between cortical and trabecular bone since the limits cannot be easily determined. Therefore, a simpler model had to be made.

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Young's Modulus [MPa]</th>
<th>Poisson's Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 Slice 1</td>
<td>186.84</td>
<td></td>
</tr>
<tr>
<td>V1 Slice 2</td>
<td>219.76</td>
<td>0.3</td>
</tr>
<tr>
<td>V2</td>
<td>475.54</td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>178.67</td>
<td></td>
</tr>
</tbody>
</table>

Postliminary the plates and the vertebra slice were assembled and interaction properties between them were created. The RP in the bottom plate was defined as encastre (boundary condition) and the RP in the upper plate was restrained to move only in Z-direction. A tangential frictionless and a normal hard contact properties were assigned between the master analytical surfaces and the slave.
surfaces wrapping the slice. A concentrated force of 18 kN was applied on the RP of the upper plate, in the Z-direction, perpendicular to the slice, just like in the compression tests. This magnitude was chosen because it is commonly displayed in the linear region for all the samples.

A set was created for Reaction Force (RFz) in the bottom RP and another set was created to measure total Displacement (Uz) in the upper RP. History output for both variables was specified and XY Data was created to visualize the evolution of these variables.

### 6.1 Mesh Convergence Study

The main idea behind a finite element (FE) method is to subdivide a continuous domain into multiple smaller finite regions, the elements, that share common points, the nodes. These nodes must obey force equilibrium equations and the assemblage of their displacement and force solutions should give the overall solution for the continuous domain that contains them. A set of elements connected all together is called a mesh.

For a general linear mechanical system, the relationship between forces and displacements is given by:

\[
[K]{u} = {f}
\]

(6.1)

where \( {f} \) = vector of external applied forces and moments at nodes, 
\([K]\) = stiffness matrix and 
\({u}\) = vector of unknown displacements and rotations at nodes.

The stiffness matrix depends on the geometry of the structure and on the elastic material properties of the system. Loading conditions, such as forces and moments, are inserted into vector \( {f} \) and boundary conditions, such as displacements and rotations, are solved in vector \( {u} \). This is achieved by calculating the inverse of the stiffness matrix \( (K)^{-1} \) and finding equilibrium solutions in successive iterations at a given increment. One should note, though, the solution process for linear and nonlinear systems is distinctly different, as briefly discussed in the next chapter.

Before getting into the validation, a few decisions had to be made. The first concerns with the type of elements that Abaqus will use to build the mesh. Tetrahedral (tet) elements were chosen over hexahedral (hex) elements due to the complexity of the model’s geometry. Nonetheless, they have poorer convergence rate than hex, typically require finer meshes to give good results and have problems with incompressible materials, plasticity and bending. On the other hand, tet linear elements were chosen over quadratic ones since they work better with interactions between bodies using pressure contact, because the latter have zero contact force at their corner nodes, leading to poor predictions of the contact pressures. However, second order elements give more accurate results and stress concentration issues.

Consequently, despite not being the most suitable elements, the analysis was carried out with four node linear tetrahedron elements (C3D4), as depicted by figure 6.2.
The second decision one should make is the degree of mesh refinement. In the analysis, one should use a sufficiently detailed mesh to ensure adequate results, still not overloading the computer resources required to perform the analysis \[87\]. Coarser meshes cannot detect areas where high gradients exist and might mislead the output results. Plus, finer meshes tend to diminish the mesh distortion, which is obviously desired.

In order to select the final mesh, a convergence study was done. Since the geometry of the problem is so irregular, a global mesh refinement was chosen rather than a local refinement. The mesh control used was seeding. Seeds are markers that were placed as much uniformly as possible in the part’s edges, by defining the global seed size. Initially, a big value was chosen for the seed size (3.8), which forms a coarse mesh, only to evaluate where the stress concentrations would be. A cut was made in the middle of one of those regions and the node's coordinates with higher stress were picked.

To save those coordinates while the convergence study proceeded, a path with a point list was saved for those coordinates and XY Data was created to get the Von Mises stress at that point throughout the convergence study. The stress evolution is plotted in a Stress vs Mesh Density graphic and the analysis is repeated until the two successive different meshes give the same results, in other words.
words, the convergence is achieved. Ideally, the convergence study should resemble figure 6.4.

![Figure 6.4: Stress vs Mesh Density typical convergence curve.](image)

The mesh was remade twelve times until seeds reached a global size of 1 and the process repeated: create a path with the coordinates found in the first mesh, save the number of elements and the von Mises stress for that point, assign a new global seed size, perform the analysis. For the matter of visualization, the convergence curve of Vertebra 2 was inserted in an independent graphic, given the higher number of elements.

![Figure 6.5: Convergence curve for Vertebra 2 and respective logarithmic trend line.](image)

From the previous figure, stress decreases quickly at the beginning and after 50000 elements the stress curve stabilizes at value slightly lower than 15.5 MPa. The explanation might be that the picked point was in a high gradient region and since the coarse mesh could not detect this gradient, the large element was assigned with a high von Mises stress. But after lowering the seed size, the importance
of mesh refinement is stressed out. To avoid overload the CPU resources, the operator should pick a lower number of elements within the region considered to be stable. For vertebra 2 the marked point containing 57470 elements was chosen with a global seed size of 1.8.

The same analysis is shown for the remain samples immediately. Notice that being different models, with different material properties and geometries, different points from the one picked above had to be chosen to perform the cycling procedure described previously.

**Figure 6.6:** Convergence curve for Vertebra 1, slice 1 and 2, and Vertebra 3. The dashed lines represent the respective logarithmic trend lines.

As it can be seen, vertebra 1, slice 1, will have no big changes in stress for meshes with greater number of elements than 40000, which also corresponds to a seed size of 1.8. For slice 2, the convergence is not easily understandable, although it follows the logarithmic trend line. The size value of 1.5 was chosen, since values of stress for higher element numbers tend to marginally oscillate around the value of 14.17 MPa. Vertebra 3 shows a nice convergence behaviour and stress of 12.99 MPa is practically repeated between seed size of 1.5 and 1.2. Therefore, 1.5 size, containing 55065 elements was elected. A summary of the meshes’ final sizes is given in table 6.2.

**Table 6.2:** Final number of elements of each sample.

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>Seeds global size</th>
<th>Number of Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 Slice 1</td>
<td>1.8</td>
<td>38783</td>
</tr>
<tr>
<td>V1 Slice 2</td>
<td>1.5</td>
<td>50137</td>
</tr>
<tr>
<td>V2</td>
<td>1.8</td>
<td>57470</td>
</tr>
<tr>
<td>V3</td>
<td>1.5</td>
<td>55065</td>
</tr>
</tbody>
</table>
6.2 Displacement Analysis

Displacement in vertebrae during the compressive tests was replicated in computational model and is analysed below in Figure 6.7.

Figure 6.7: Displacements [mm] on (a) \(V1_1\), (b) \(V1_2\), (c) \(V2\) and (d) \(V3\), after applying a 18 kN vertical load on the upper plate.

Poisson’s ratio effect was noticed in samples’ extremities. These regions suffer bigger transverse displacements, and since Figure 6.7 shows magnitude of displacement, these were the zones which suffered higher total displacements. Hence, comparison between the results of the experimental work and the validation model was made plotting reaction force (RFz) in the lower plate vs displacement (Uz) of the upper plate, which is analogous to the measurement done by the testing machine. Saving the history output data returned by Abaqus, this was saved in a .txt file and a similar graphic was sketched by a Matlab routine to find the trend line and added to the experimental curve, already presented.

Note that only the experimental linear region up to 18 kN was plotted. Although the equation for the trend line of experimental values is represented, it does not represent the experimental stiffness since the whole linear range is not considered.
Figure 6.8: Force-Displacement graph for Abaqus and experimental work with maximal displacement at 18 kN for $V_{1,1}$.

Figure 6.9: Force-Displacement graph for Abaqus and experimental work with maximal displacement at 18 kN for $V_{1,2}$. 
Figure 6.10: Force-Displacement graph for Abaqus and experimental work with maximal displacement at 18 kN for V2.

Figure 6.11: Force-Displacement graph for Abaqus and experimental work with maximal displacement at 18 kN for V3.

With exception for vertebra 1 slice 2, all validation displacement values are lower than the experimental ones. The different behaviour of the first vertebra might be explained by a previous test performed on the sample to verify if the test machine was operating correctly. Therefore, there was not enough time for the initial tissue adaptation (toe region) to restore the initial length.
## 6.2.1 Results Comparison

Table 6.3: Experimental and computational displacements for the samples at 18 kN. The error is calculated as 
\[ e(\%) = \frac{|\Delta d_{\text{exp}} - \Delta d_{\text{comp}}|}{\Delta d_{\text{exp}}} \times 100. \]

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>(\Delta d_{\text{exp}}) [mm]</th>
<th>(\Delta d_{\text{comp}}) [mm]</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 Slice 1</td>
<td>0.869</td>
<td>0.827</td>
<td>4.83%</td>
</tr>
<tr>
<td>V1 Slice 2</td>
<td>0.907</td>
<td>1.031</td>
<td>13.67%</td>
</tr>
<tr>
<td>V2</td>
<td>0.860</td>
<td>0.778</td>
<td>9.53%</td>
</tr>
<tr>
<td>V3</td>
<td>1.414</td>
<td>1.233</td>
<td>12.80%</td>
</tr>
</tbody>
</table>

First of all, material properties selection for Abaqus analysis are chosen as mechanical elastic and isotropic materials when in reality bone is way more complex. Besides having a cortical shell, which is a denser and stiffer material than trabecular bone, one could model it as anisotropic and porous material. The density could also be calculated knowing the total mass of bone and the sample total volume. This could be easily obtained using Mimics to estimate the volume, and a density-elasticity relationship could be used to take into account the heterogeneity of bone.

One also notices that the trend line does not follow the toe region, where nonlinearity is prevalent. This is why the elasticity modulus was determined without considering this region. One option would be to calculate the nominal stress and strain for several points and calculate the Young’s modulus so that they could follow the experimental curve. In Abaqus, this is achieved employing an hyperelastic material that allows for uniaxial test data usage.

Concerning experimental errors during the procedure, the cross-sectional area used to calculate Young’s modulus is a mere average of the upper and bottom surfaces, which does not represent the reality. Plus, the cut performed in Solidworks tried to be as most faithful to reality as possible. Nevertheless, this cannot be achieved and for comparison, the average of bottom and upper surfaces was taken to compare the geometry errors, displayed in table 6.4.

Table 6.4: Average experimental and computational areas for the samples. Computational areas were estimated identically to the experimental values, calculating the upper and lower surface areas from each sample. The error is calculated as 
\[ e(\%) = \frac{|\bar{A}_{\text{exp}} - \bar{A}_{\text{comp}}|}{\bar{A}_{\text{exp}}} \times 100. \]

<table>
<thead>
<tr>
<th>Vertebra</th>
<th>(\bar{A}_{\text{exp}}) [mm²]</th>
<th>(\bar{A}_{\text{comp}}) [mm²]</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 Slice 1</td>
<td>1043.981</td>
<td>1037.965</td>
<td>0.58%</td>
</tr>
<tr>
<td>V1 Slice 2</td>
<td>965.900</td>
<td>1004.845</td>
<td>4.03%</td>
</tr>
<tr>
<td>V2</td>
<td>941.339</td>
<td>931.72</td>
<td>1.02%</td>
</tr>
<tr>
<td>V3</td>
<td>924.199</td>
<td>933.91</td>
<td>1.05%</td>
</tr>
</tbody>
</table>

Finally, Poisson’s ratio \((\nu)\), not having such a great impact in \(E\) calculation, but showing how a material expands in two transverse directions when it is being compressed in a third perpendicular...
direction, is other simplified variable in Abaqus model. It is given by

\[ \nu = -\frac{\varepsilon_x}{\varepsilon_z} = -\frac{\varepsilon_y}{\varepsilon_z} \]  

(6.2)

and for a linear isotropic elastic material varies between \(-1 < \nu < 0.5\), with 0.5 being a perfectly incompressible material. In literature, cortical and trabecular bone are assumed to have different \(\nu\), being 0.3 and 0.2, respectively, and this was not taken into account during the validation. Varying Poisson’s ratio within this interval, one can observe an increase in displacements on the computational model as exemplified by figure 6.12.

Figure 6.12: Poisson’s ratio variation for vertebra V3.

However, it still does not reach the experimental value and for V1_2 it even moves further from the desired value. One can verify that as Poisson’s ratio decreases, so does stiffness, nonetheless, within the considered interval this effect is not very accentuated.

6.2.2 Von Mises Stress Distribution and Experimental Observations

Qualitatively, it is interesting to verify whether the stress distribution in the computational model coincides or not with the failure regions observed in reality. There are several fracture criteria, depending on the type of material we are facing. For a ductile behaviour von Mises criterion is usually employed whereas for a brittle behaviour Mohr’s criterion using principal stresses is more adequate. Due to its complex microstructure, trabecular bone displays frequently different fracture patterns, so it was opted to represent both von Mises and Principal stress. Figure 6.13 unveils these similarities/differences. Better agreement between fracture sites and Maximum principal stress was found for V1_1 (a) and V1_2 (b), whereas for V3 (d) von Mises appeared to have more similarities. V2 (c) showed poorer agreement with computational validation, perhaps due to stress concentration issues.
Figure 6.13: Comparison between regions where compressive fracture occurred and von Mises stress [MPa], Maximum and Minimum Principal stresses [MPa] distributions in Abaqus model when applying a 18 kN compressive load in (a)V1_1, (b)V1_2, (c)V2 and (d)V3.
Finite Element Analysis

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7.1 Simplified Model

7.1.1 Material and Initial Conditions

For the simplified model, sections had to be assigned to both disc and vertebrae. To improve
accuracy of measurements, the disc was divided into nucleus pulposus (NP) and annulus fibrosus
(AF) in Solidworks, where superficial area and centroid can be determined. This way one was able to
center an ellipse shape in the disc model and perform a partition of 30% of the total cross sectional
area to model NP and the exterior part to model AF [23]. The separation of cortical from trabecular
bone was made using an homogeneous membrane section with thickness of 0.35 mm in the Property
module in Abaqus. This allows to assign only one mesh to the part but different elements are used in
the cortical layer (M3D3) and in the trabecular region (C3D4). The material properties and element
types are specified in table 7.1.

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Young’s Modulus [MPa]</th>
<th>Poisson’s Ratio</th>
<th>Element type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Bone</td>
<td>12000</td>
<td>0.3</td>
<td>M3D3</td>
</tr>
<tr>
<td>Trabecular Bone</td>
<td>100</td>
<td>0.2</td>
<td>C3D4</td>
</tr>
<tr>
<td>Annulus Fibrosus</td>
<td>4.2</td>
<td>0.45</td>
<td>C3D4</td>
</tr>
<tr>
<td>Nucleus Pulposus</td>
<td>Incompressible fluid – filled cavity</td>
<td></td>
<td>SFM3D4</td>
</tr>
<tr>
<td>Cartilage Endplates</td>
<td>24</td>
<td>0.46</td>
<td>M3D4</td>
</tr>
</tbody>
</table>

Table 7.1: Material properties and element types for the simplified model.

Note that in the present case, the materials were considered linear elastic and isotropic. For the
heterogeneous bone density distribution case no partition on vertebrae was needed. Nevertheless,
other partitions had to be made to apply interactions and link the parts of the assembly together.

Tie constraints were used between vertebrae and respective contacting surface of the disc and also
between the NP and AF, which allow for these surfaces with different meshes to move together. An
incompressible fluid cavity interaction was created in NP surface using $\rho = 1.125 \text{ gcm}^{-3}$. Cartilage
endplates on top and bottom of NP were defined as a membrane section of thickness 1. Finally, an
interaction between the vertebral facets of L4 and L5 was established to provide tangential motion.

Two reference points were assigned in the upper surface of L4 and lower surface of L5 with cou-
pling constraints, which allow for the continuum distribution of motion from this points to the respective
surfaces. These points were used to apply loads and displacements during the analysis. The bottom
reference point associated to the lower surface of L5 was defined as encastré.

7.1.2 Mesh Convergence Study

Just like in the sheep case, mesh was subjected to a convergence study. At first, the two vertebrae
were studied in order to get the final mesh that would receive the material properties for the hetero-
genous models supplied by Mimics, as described in Chapter 5.5. Two sets containing a point in the
anterior face of the vertebral body and a point in the top of the posterior elements, having the greater
stress values, were chosen and the top surface of L4’s vertebral body was subjected to a compressive
force of 460 N. A mesh of size 2 was given to the whole disc. The curves can be seen in figure 7.1.

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A seed global size of 3 was chosen for both vertebrae since they show stabilization from this value on. The shift observed in L4 anterior and L5 posterior to lower stress values in small seed sizes is related to the interaction definitions, since vertebrae were assigned as being the master surfaces and disc as the slave. One requirement of Abaqus is to always define the master surface having larger seeds than the slave, so that one can avoid penetration of the former into the latter.

The disc requires a different approach. Since the two components are so different and Abaqus internally assigns the fluid elements automatically, which cannot be evaluated by von Mises stress, only AF curve is displayed. A higher mesh seed is assigned to NP because this was chosen as master surface in the tied constraint between NP and AF.

The mesh study starts at an equal seed size of the vertebrae, i.e., at 3. Despite having an instantaneous rise at 1.5, the chosen seed size was 2 due to the stress similar behaviour to lower values. And, to conclude, the NP was assigned to 2.5. A summary of the mesh properties is given in table 7.2.

**Figure 7.1:** Mesh convergence study for L4 and L5.

**Figure 7.2:** Mesh convergence study for the AF.
After completing the mesh selection, a job file (".inp") was created, containing in the assembly only
the vertebrae, and this was imported to Mimics to get the material properties. Then, it was imported
back again into Abaqus as individual parts and the assembly was remade.

<table>
<thead>
<tr>
<th>Table 7.2: Final number of elements of the assembly.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vertebra</strong></td>
</tr>
<tr>
<td>Vertebra L4</td>
</tr>
<tr>
<td>Vertebra L5</td>
</tr>
<tr>
<td>Nucleus Pulposus</td>
</tr>
<tr>
<td>and Endplates</td>
</tr>
<tr>
<td>Annulus Fibrosus</td>
</tr>
</tbody>
</table>

7.2 Nonlinear Disc Model

7.2.1 Material and Initial Conditions

Linear elasticity and isotropic formulation are often used to simplify soft tissue modelling, decre-
asing its computational cost and avoid numerical difficulties in contact formulations. However, soft
tissues are fibre reinforced with a predominant natural fibre direction, which dictates a more accurate
disc’s modelling [17] [88] [89].

As mentioned in Chapter 2, the AF contains collagen bundles arranged in 15 to 25 concentric
layers (lamellae) which run obliquely from one vertebral body to the other. The angle of inclination
decreases from the innermost to the outermost layer and the relative strength increases, being the ou-
termost layer essentially composed of collagen type I. Surrounding the lamellae, the annulus ground
substance behaves as a nonlinear near-incompressible material that shows high level of strain.

Based on previous IVD’s models from Little [90], the FEM model was adapted to be 8 lamellae
instead of the average 20 existent in vivo and all geometrical parameters were modified to translate
the mechanical properties to this configuration, assuming the layers to be evenly spaced through the
AF’s radius.

After determining the smaller elliptical radius \( R \) in Solidworks, the layer thickness \( T_L \) was calcu-
lated by dividing the radius by the total number of lamellae \( N \) and sketching splines that intermediate
the inner and outermost layers of the AF. From these splines, one could extrude surfaces, which were
saved as a .x file and imported as a Part into Abaqus. In the Assembly module, the AF part was cut
with this geometry and the eight lamallae of the AF were obtained.

In the surface sections now created, the collagen fibers were modelled as rebar elements. This
avoids to introduce additional stiffness to the annulus ground substance besides that of the collagen
fibers. The rebar elements require the definition of some parameters, such as their cross-sectional
area \( A_B \), their angle of inclination \( \alpha \) and their spacing \( s \) between the fibers for each lamella.
These values were derived from experimental work from Marchand and Ahmed [30]. The cross-
sectional area of each bundle is considered to be elliptical, with a width \( W_B \) being 59% of \( T_L \) and a
thickness ($T_B$) of 82.35% of $T_L$. $A_B$ is given by

$$A_B = \pi \times \frac{W_B T_B}{2}$$  \hspace{1cm} (7.1)

The spacing between each bundle within the same rebar element was assumed to be 0.23 mm and the number of bundles per finite element, would be limited by the IVD’s height and

$$t = \frac{A_B}{s}$$  \hspace{1cm} (7.2)

Figure 7.3: Implemented model for 8 collagen layers and respective calculations, based on the works of Marchand and Ahmed [30] and Little [90].

The angle inclination of the fibres and their relative strength were calculated by linearly interpolating the values reported in literature for the inner and outer layers of AF. The outer layer corresponds to a 100% relative stiffness of the collagen fibre, and its Young Modulus is reported to be 655 MPa. The layer adjacent to NP is reported to be 65% relative strength. In terms of inclination, the outer, stiffer layer presents a 25° to the vertical direction and the inner layer around 35°. Note also that each layer is distributed in a criss-cross pattern, thus $\alpha$ will be intercalated between positive and negative values. The final properties of the rebar elements are shown in table 7.3.

Table 7.3: Collagen rebar layers properties as function of radial distance.

<table>
<thead>
<tr>
<th>Circumferential Layer</th>
<th>$\alpha$ (degrees)</th>
<th>E (MPa)</th>
<th>Poisson’s Ratio</th>
<th>Relative Strength (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+35</td>
<td>425.75</td>
<td>0.3</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>-35</td>
<td>425.75</td>
<td>0.3</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>+32</td>
<td>504.35</td>
<td>0.3</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>-32</td>
<td>504.35</td>
<td>0.3</td>
<td>77</td>
</tr>
<tr>
<td>5</td>
<td>+28</td>
<td>576.40</td>
<td>0.3</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>-28</td>
<td>576.40</td>
<td>0.3</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>+25</td>
<td>655</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>-25</td>
<td>655</td>
<td>0.3</td>
<td>100</td>
</tr>
</tbody>
</table>

The initial material orientation of the fibres is shown in figure 7.4.
Collagen fibres in soft tissues are stiffer in tension than in compression [17]. This fact was taken into account and in the material properties, a tension-only state was assigned. The compressive stress state is carried out by the ground substance. The ground substance behaves as a nonlinear elastic stress-strain (hyperelasticity) material, which is described by the large strain theory. A strain energy function is used to define the material. Several formulations have been employed in previous works, however, in the present work, a more general model was used. The Mooney-Rivlin model presents the strain energy function as a linear combination of two invariants of the left Cauchy-Green deformation tensor and is usually valid for strains up to 100% [89]. It has the form:

\[
U = C_{10}(\bar{I}_1 - 3) + C_{01}(\bar{I}_2 - 3) + \frac{1}{D_1}(J_{el} - 1)
\]  \hspace{1cm} (7.3)

where \(C_{10}\) and \(C_{01}\) are material constants obtained experimentally and \(D_1\) describes the compressibility of the material and depends on

\[
\mu_0 = 2(C_{10} + C_{01})
\]  \hspace{1cm} (7.4)

\[
\nu = \frac{3k_0}{\mu_0} - 2
\]  \hspace{1cm} (7.5)

and

\[
k_0 = \frac{2}{D_1}
\]  \hspace{1cm} (7.6)

where \(\mu_0\) is the initial shear modulus, \(\nu\) is the Poisson’s ratio and \(k_0\) is the initial bulk modulus. The material constants were based on previous sensitivity tests made by Little (2004) [90], having values of \(C_{10} = 0.5\) and \(C_{01} = 1\). To contrast the nonlinear AF with the linear one, \(\nu = 0.45\), hence a compressible material was assigned to the AF and \(D_1 = 0.06897\).
7.3 Results

Two types of analysis resulted from the two problems formulations described previously: one concerning the effect of heterogeneity in bone density distribution and other concerning the nonlinear effects of intervertebral disc. Validation of the obtained results in the following sections comes from the review article of Dreischarf et al. (2014) [66], from which figures 7.10, 7.11 and 7.12 are an adaptation to ease the comparison.

7.3.1 Linear Disc Model

In this section, the comparison between the linear isotropic homogeneous model and the heterogeneous models described by the \( \rho - E \) equations will be evaluated in terms of stress distribution, NP pressure and ROM. The effects of calibration are as well juxtaposed.

7.3.1.A Stress Distribution

Applying a compressive force of 460N in the upper surface of L4, the stress distribution is observed for the three model types in figure 7.5. The stress distribution varies from the homogeneous model to the heterogeneous models, whereas between the calibrated heterogeneous model and the non-calibrated one the differences are less evident.

In the homogeneous model, stresses tend to concentrate in the outer cortical shell, mostly in the vertebral body rather than in posterior elements. Being a compressive force, they are anteriorly distributed, with higher magnitude in L5 vertebra and also in the anterior part of the AF. Note that, as the material limits are well defined, so does the rapid change in stress distribution from the cortical to the trabecular bone.

In the heterogeneous models, for the several density-elasticity relationships, the stress patterns are very similar, showing a bigger spread of the stress from the outer surface to the inner material. However, the higher stresses appear to form a shell boundary near the surface, suggesting regions of higher density similar to the one simplified in the homogeneous model. The stresses in vertebrae are one order of magnitude lower than in the first model, but the stresses in the AF are in the same order. Between the calibrated and non-calibrated model, the variation in stress occurred only in the third decimal order for all \( \rho - E \) relations except for Kopperdahl. The differences for Kopperdahl relation are shown in Figure 7.5.

7.3.1.B Intradiscal Pressure

IDP is evaluated in three compressive load magnitudes of 300N, 460N and 600N to preview its linearly growing behaviour. The values for the homogeneous and heterogeneous linear models show a growing linear trend when applying greater loads. Comparing to the image adapted from Dreischarf et al. [66], figure 7.10, either homogeneous and heterogeneous models show greater IDP than the in vivo values, but are within the limits of the computational models reviewed in the article, which avoids the invalidation of the model.
Figure 7.5: Von Mises stress distribution when applying a compressive force of 460N in (a) cortical shell of the homogeneous model, (b) trabecular bone of the homogeneous model, (c) AF of the homogeneous model, (d) and (g) calibrated Kopperdahl model, (e) and (h) non-calibrated Kopperdahl model, (f) calibrated and (i) non-calibrated Kopperdahl AF model.
For the present study, the homogeneous model shows lower IDP values than the heterogeneous models, and between the calibrated and non calibrated equations, the IDP values appear to be the same. In terms of density-elasticity relations, the Keller model shows closer values to the linear model and also the in vivo values of the article. The several IDP are organized in table 7.4.

<table>
<thead>
<tr>
<th>Compressive Force</th>
<th>Linear model</th>
<th>Carter and Hayes (MPa)</th>
<th>Keller (MPa)</th>
<th>Kopperdahl (MPa)</th>
<th>Morgan (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300N</td>
<td>0.336</td>
<td>0.375</td>
<td>0.375</td>
<td>0.369</td>
<td>0.368</td>
</tr>
<tr>
<td>400N</td>
<td>0.515</td>
<td>0.575</td>
<td>0.575</td>
<td>0.564</td>
<td>0.564</td>
</tr>
<tr>
<td>600N</td>
<td>0.670</td>
<td>0.750</td>
<td>0.750</td>
<td>0.735</td>
<td>0.736</td>
</tr>
</tbody>
</table>

Table 7.4: Intradiscal pressure for the homogeneous model and for the calibrated and non-calibrated models with different density-elasticity relationships. All values are in MPa.

7.3.1.C Range of Motion

The ROM is tested in each axis direction displaying a movement within the physiological range. Additionally a pre-load is applied to allow for the disc’s compression, which plays an important role in its behaviour. Except for flexion, all movements in the three models show similar values to the ones exhibited in vivo, as depicted in figure 7.11.

Nevertheless, notice that the linear model usually over-estimates the ROM values and may even exceed the physiological range. This is especially true for the axial rotation, whereas the heterogeneous models do not display such exaggerated behaviour. Juxtaposing the calibrated and non-calibrated models, the values are mainly different in flexion/extension movement and in lateral bending, not varying as much in axial rotation.

<table>
<thead>
<tr>
<th>Flexion 7.5Nm + 1175N</th>
<th>Linear model</th>
<th>Carter and Hayes</th>
<th>Keller</th>
<th>Kopperdahl</th>
<th>Morgan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Cal</td>
<td>Cal</td>
<td>No Cal</td>
<td>Cal</td>
<td>No Cal</td>
</tr>
<tr>
<td>Flexion</td>
<td>8.96</td>
<td>7.03</td>
<td>6.30</td>
<td>7.11</td>
<td>7.03</td>
</tr>
<tr>
<td>Extension 7.5Nm + 500N</td>
<td>3.24</td>
<td>1.81</td>
<td>1.80</td>
<td>2.80</td>
<td>1.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extension</th>
<th>Linear model</th>
<th>Carter and Hayes</th>
<th>Keller</th>
<th>Kopperdahl</th>
<th>Morgan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Cal</td>
<td>7.03</td>
<td>6.58</td>
<td>7.07</td>
<td>7.07</td>
<td></td>
</tr>
<tr>
<td>Cal</td>
<td>7.07</td>
<td>2.40</td>
<td>1.86</td>
<td>1.85</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.5: Range of motion during flexion/extension movement. All units in degrees.

Figure 7.6: Flexion movement on the left when subjecting the FSU to a 7.5 Nm and a compressive force of 1175 N and extension movement on the right when applying a 7.5 Nm moment and a 500 N compressive force.
Table 7.6: Range of motion during left and right lateral bending movements. All units in degrees.

<table>
<thead>
<tr>
<th>Lateral Bending</th>
<th>Linear model</th>
<th>Carter and Hayes</th>
<th>Keller</th>
<th>Kopperdahl</th>
<th>Morgan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Cal</td>
<td>Cal</td>
<td>No Cal</td>
<td>Cal</td>
<td>No Cal</td>
</tr>
<tr>
<td>Left</td>
<td>2.93</td>
<td>2.32</td>
<td>2.32</td>
<td>2.32</td>
<td>2.40</td>
</tr>
<tr>
<td>Right</td>
<td>4.11</td>
<td>2.69</td>
<td>2.68</td>
<td>2.69</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Figure 7.7: Lateral bending movement to the right and left when subjecting the FSU to a 7.8 Nm and 700 N.

Table 7.7: Range of motion during left and right axial rotation movements. All units in degrees.

<table>
<thead>
<tr>
<th>Axial Rotation</th>
<th>Linear model</th>
<th>Carter and Hayes</th>
<th>Keller</th>
<th>Kopperdahl</th>
<th>Morgan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Cal</td>
<td>Cal</td>
<td>No Cal</td>
<td>Cal</td>
<td>No Cal</td>
</tr>
<tr>
<td>Left</td>
<td>2.48</td>
<td>1.31</td>
<td>1.31</td>
<td>1.73</td>
<td>1.31</td>
</tr>
<tr>
<td>Right</td>
<td>3.37</td>
<td>1.82</td>
<td>1.82</td>
<td>2.17</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Figure 7.8: Axial rotation movement to the left and right when subjecting the FSU to a 5.5 Nm and a compressive force of 720 N.

As previously verified for IDP, all ROM values are within the range of the computational models presented in the review and close to the *in vivo* models, showing once again consistency in the computational formulation.
7.3.2 Nonlinear Disc Model

This section deals with the linear and nonlinear comparability of the AF models. To avoid having a huge number of degrees of freedom, only the homogeneous model was considered in this part. Results are once again paralleled with Dreischarf et al. article [66].

7.3.2.A Stress Distribution

Figure 7.9: Stress distribution in the linear model on the left and in the nonlinear model on the right. (a) and (b) show the cortical shell, (c) and (d) show the trabecular bone. (e) shows the linear AF and (f) shows the nonlinear AF, which has been separated into its components: the hyperelastic ground substance below and the elastic collagen fibres on the right.
Visually, the stress distribution patterns in both linear and nonlinear models after applying a 460N compressive force are very similar, even showing similar scales for the cortical bone. The L5 vertebrae continues to suffer the higher stresses in its cortical shell, but a reduction in nonlinear model of the L4 stress is noticed. Trabecular bone also shows a similar distribution in both models although it should be noted that the higher stress in the linear model occurs in L5 and in the nonlinear model occurs in the AF.

The nonlinear AF had to be separated into ground substance and collagen fibres for the matter of visualization. The ground substance exhibits an impressive similar stress distribution to the linear model, having even the same scale of stress distribution. The collagen fibres show, notwithstanding, a higher stress order and have reversed regions for higher stresses. These are the zones where fibres largely deform (expand) and, since they respond only to tensile stress, there is where the higher stresses will be noted.

Macroscopically, a greater bulge of the IVD is observed in the linear than in the nonlinear model. This stiffening effect is due to the resistance offered by collagen fibres in response to the incompressible nucleus stretch.

7.3.2.B Intradiscal Pressure

In terms of intradiscal pressure, again assessed at 300 N, 460 N and 600 N, the values in the nonlinear model are always superior to the linear model, even so within the range of the computational models and close to the in vivo model. The additional pressure revealed by the nonlinear model is due to the resistance offered by the collagen fibres and the nucleus deformation is less perceptible.

<table>
<thead>
<tr>
<th>Compressive Force</th>
<th>Linear model (MPa)</th>
<th>Non Linear model (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300N</td>
<td>0.336</td>
<td>0.361</td>
</tr>
<tr>
<td>460N</td>
<td>0.515</td>
<td>0.559</td>
</tr>
<tr>
<td>600N</td>
<td>0.670</td>
<td>0.735</td>
</tr>
</tbody>
</table>

Table 7.8: Intradiscal Pressure comparison between linear and nonlinear models.

Figure 7.10: Image adapted containing IDP of the linear (yellow dots) and nonlinear (purple dots) models in comparison with the in vivo and computational models of [66].

As for the IDP in the physiological movements, it is inspected along with the ROM in the following section.
7.3.2.C Range of Motion

In line with what was done for the linear models, the physiological range of motion is evaluated, but this time coupled with the IDP values for the linear and nonlinear models. For an easier comparison, the results are plotted in two graphics adapted from Dreischarf et al. [66].

### Table 7.9: ROM and respective IDP for the several movements in each axis direction for the linear and nonlinear models. Units are in degrees for ROM and MPa for IDP.

<table>
<thead>
<tr>
<th>Movement</th>
<th>Linear model (°)</th>
<th>Nonlinear model (°)</th>
<th>Linear model (MPa)</th>
<th>Nonlinear model (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion</td>
<td>8.96</td>
<td>6.17</td>
<td>1.639</td>
<td>2.071</td>
</tr>
<tr>
<td>7.5Nm + 1175N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>3.24</td>
<td>2.09</td>
<td>0.282</td>
<td>0.469</td>
</tr>
<tr>
<td>7.5Nm + 500N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral bending Left</td>
<td>2.93</td>
<td>1.93</td>
<td>0.730</td>
<td>0.812</td>
</tr>
<tr>
<td>7.8Nm + 700N Right</td>
<td>4.11</td>
<td>2.34</td>
<td>0.836</td>
<td>0.972</td>
</tr>
<tr>
<td>Axial rotation Left</td>
<td>2.48</td>
<td>1.75</td>
<td>0.804</td>
<td>0.910</td>
</tr>
<tr>
<td>5.5Nm + 720N Right</td>
<td>3.37</td>
<td>1.86</td>
<td>0.821</td>
<td>0.956</td>
</tr>
</tbody>
</table>

Figure 7.11: ROM values for the linear (yellow bar) and nonlinear (purple bar) models in comparison with previous studies (Model 1-7 and in vivo). Image adapted from [66].
Figure 7.12: IDP values for the linear (yellow bar) and nonlinear (purple bar) models in comparison with previous studies (Model 1-7 and in vivo). Image adapted from [66].

Except for flexion, one can see an improvement in the ROM values for the nonlinear model. The resistance offered by considering the collagen layers led to a closeness between the FEM and the in vivo ROM values. In the flexion case, one can see that the value is identical to the other FEM studies but all far from the in vivo result. It has already been hypothesised that the loading conditions applied to mimic the experimental value is not adequate and further studies should be done to better estimate it [66].

In addition to ROM values, it is investigated how IDP values behave in such loading conditions. As predicted, the increase in stiffness by the collagen fibres led not only to a reduction in ROM but also to an increase in IDP. Except for extension, all values are higher than the measured ones in vivo. Nonetheless they remain within the range of the FEM studies. Also mentioned by the article, the in vivo IDP values emerge from the study of Wilke et al. [91], in which data came from only one subject under maximal voluntary motion. [66].

Overall, the introduction of nonlinearity factors appears to bring the output results near to those observed in vivo.


8.1 Experimental Work and Computational Validation

The experimental work followed a previous work done in Tissue Biomechanics and Biomaterials laboratory at DEM [57], with the goal of performing a mechanical compressive test until fracture of sheep vertebrae samples. The procedure was adapted from a renowned article from Buckley et al. [62] in experimental biomechanics methodologies. No cement pot was used, as commonly done in these methods. The cement pot could correct the unevenness of the load distribution on the samples’ surfaces but its stiffness would also contribute to the total stiffness of the experimental set-up and would introduce errors in the determination of the experimental Young’s Modulus of bone.

No moments nor extensions were applied to the samples, since the only goal was to perform compressions. Therefore immobilization of the sample was not a major concern, but still in the first test machine, 10 cycles of loading-unloading were executed as pre-test to accommodate the vertebrae to the compression plates. Nonetheless, this pre-test was not required in the second testing machine, since this had a feedback loop control mechanism that intrinsically did the job. The compression was taken until fracture, controlling the load rate. Fracture of the samples was always achieved before reaching the 23 kN.

Although load rate was controlled it would be desirable to control the strain rate. This way, one could determine the type of fracture, ductile or brittle, relevant to the computational validation modelling and analysis. Displacement in vertebrae could be measured in the vertebra itself, with the addition of several extensometers to minimize the errors.

Additionally, one should have in mind that increasing the load capacity, increases the errors of the load cell, making it less sensitive and calibration of the software that acquires data should be taken into account.

Short communications recently published [63] also recommend a standard sample format, to reduce geometry variability. Another possibility would be to isolate cortical from trabecular bone and perform individual tests on each.

Finally, the number and origin of samples and tests were not enough to perform a probabilistic study that would account for subject inter- and intra-variability.

Despite the mentioned sources of error, the experimental Young’s Modulus showed good agreement with literature, with a mean value of 265.20 MPa and a standard variation of 122.409 MPa. In Goldstein article [92], a survey of published mechanical properties of human trabecular bone presents the vertebral ranging from 10 to 428 MPa. The large standard deviation factor relates to the reduced number of samples and also the cortical-trabecular bone ratio.

A computational model can reproduce a real scenario to obtain results as close as those happening in reality. Modelling the loading and boundary conditions as well as the geometrical part has howbeit its limitations.

The first aspect clings to image segmentation. The number of acquired images during a CT scan is inversely proportional to the distance between slices, which influences the stair-case effect. In the sheep CT scan, a total of 403 images were acquired for the cervical segment. Although the stair-case
effect was still noticeable, the large number of slices granted it was not excessive.

The segmentation process requires the user to have prior knowledge of anatomy, as well as choosing parameters such as filter bands or morphology operations. Mimics is a powerful tool which simplifies the manipulation of the image by the user, not demanding for automatic segmentation’s parameter selection. Still an automatic segmentation for the full model is desirable, but there are yet no algorithms that appropriately do it. Hence, manual segmentation requires great sensibility to define ROIs and eliminate non-desirable artefacts.

In order to import a geometrical model into Abaqus, a pre-processing must be done by simplifying and smoothing the mesh structure. This process leads to great alterations in the surface and an overall volume reduction, so it must be carried out with cautiousness. One should bear in mind that computational validation processes depend in large scale of the geometrical model. Replicating the vertebrae cuts made in the workshop was rather difficult since they were performed by a different operator and no measurements were recorded.

Before carrying through the analysis, virtual topology was applied which allows one to have a more uniform mesh, without needing to adapt to an excessive detailed surface. The type of elements that constitute the mesh also might influence the results. Given the pressure contact type and the complex geometry, C3D4 elements were chosen. Some stress concentration peaks were found, which might have been corrected by implementing quadratic elements (C3D10), but this would jeopardize the contact interaction with the analytical surfaces.

A mesh convergence study was held to select the mesh refinement. Initially a high stress point was chosen to control the stress oscillation throughout the refinement. An average stress point cannot be chosen because this oscillation would be larger and one might not see a convergence curve.

Subsequently a displacement analysis was carried out using the material properties achieved in the experimental work. It was decided to apply a commensurable load of 18 kN to all tests, and not the load at which the elastic limit occurred for each sample. The graphics 6.8-6.11 show the comparison between experimental and computational results. Throughout these intervals, the computational models were assumed to have linear elasticity, not accounting for the visible nonlinear behaviour at the initial moments. It is perceptible that in this region, all experimental curves tend to have a greater displacement variation with smaller stress intervals. One option would be to supply an hyperelastic material property at initial moments making the computational curve to follow the experimental one, as already discussed in chapter 6.

Except for vertebra V1.2, all displacements in Abaqus model are lower than in the experimental model. However, the calculated errors were relatively low, being the highest $13.67\%$ for $V1.2$. A number of error sources were proposed and studied. The top and bottom surfaces’ area for each sample offer a small error, as seen in table 6.4. The highest error of $4.03\%$ happens again in $V1.2$, and thus might be a cause of elasticity modulus alteration.

Poisson’s ratio is neither a major parameter in Young’s Modulus alteration, rather shows how the material expands, since when $\nu$ decreases (transversal stretching is diminished), the uniaxial deformation in $z$ ($\varepsilon_z$) increases, which misleads the observer to conclude being in the presence of a
less stiff material.

The principal cause of the displacement gap between the computational and experimental models relates to the material simplification and not considering the existence of a cortical shell and trabecular bone, which have different material properties. A possible solution in posterior experiments would be to isolate the two materials and calculate their properties separately.

To conclude this part of the discussion, a comparison between regions where compressive fractures occurred and stress concentration points in computational model was done. As it can be seen in figure 4.5 and Appendix A, the stress-strain curve displayed for bone, more specifically, trabecular bone, is not always the same. Therefore, the criteria for evaluating fracture cannot be identically applied to all samples. The ductile fractures can be evaluated by means of the distortion energy, which is the energy associated with changes in shape of the material. For isotropic materials, distortion energy is described by von Mises stress. Whenever the von Mises stress of a ductile material reaches the yield stress, it is considered to fracture at that point. On the other hand, brittle fractures are assumed to occur whenever the material is in a stress state such that its principal stresses reach the ultimate strength and rupture occurs in the specimen [93]. It should be noted that other criterion apart from stress can be chosen to evaluate fracture mechanics (such as elastic damage driven criterion to initiate cracks), but this subject remained outside the scope of this thesis. In this specific case, $V_{1.1}$ and $V_{1.2}$ showed good agreement with maximum principal stress, whereas for $V_{3}$ approached von Mises results. $V_{2}$ showed poorer agreement with computational validation, probably due to stress concentration issues. Maximum principal stresses gave better results than minimum principal stresses, although a compressive force was being applied perpendicularly to the sample. The reason might be that tensile forces in the transverse plane might have been the mechanism by which samples fractured.

This last topic was only thought after experimental work was performed, so there was no monitoring of the fracture development during the compressive tests. It is, though, an interesting question to consider in future works.

### 8.2 Computational Human Model

Similarly to the sheep model, 291 images were acquired for the human lumbar spine, being the pixel resolution sufficient for a small stair-case effect. Along with bone segmentation, soft tissues like intervertebral disc, paraspinal muscle and fat were also extracted from the CT image. Nonetheless, the attenuation of X-rays by these tissues is smaller, presenting not so fair images. For this reason, manual segmentation was applied and a greater margin of error might exist.

As in sheep, pre-processing techniques were applied with even higher caution, because this time several interactions between the FSU parts had to be assigned. Also, a partition was made with Solidworks in the IVD to separate nucleus pulposus from annulus fibrosus. The boundary between both parts was decided to be $30\%$ of the surface area, based on literature data [23], and an elliptical shape was chosen. It should be noted that this boundary is still controversial, because collagen fibres...
of NP rather spread into the AF’s ground substance and mix with the other fibre types and it varies with anatomic region. NP was defined as a fluid cavity containing an incompressible fluid, which tried to simulate water mixed with proteoglycans [94].

A convergence analysis was also conducted to each part of the assembly, saving stress from two points in the vertebrae and one at the AF. The NP, being the master interaction with the latter, was assigned with a greater seed, since no stress analysis could be performed in its type of elements.

In the CT-\(\rho\) relationships, a total of 25 types of materials were considered. A higher number could have been chosen to increase the resolution and perhaps the cortical shell could have been more evident.

Because no pure water nor phantoms were available in the system, calibration was carried out using fat and muscle. This procedure did alter the density values, revealing the contribution of water attenuation effect in commercial CT scanners. However, this attenuation was not so appreciable as expected, since calibration equation did not vary much in slope and offset from the non-calibrated density equation.

A large number of \(\rho\)-E relationships have been reported over the last years to describe trabecular bone, particularly in vertebrae. Nevertheless, these relations have shown to be highly dependent on the experimental method adopted to establish them, thusly, having low correlation values for the considered density range, as seen in table 5.2. Sensible to this fact, it was opted to study several relations, not optioning for only one.

Two types of analysis were made in this work. The first compared a linear homogeneous FSU model with two types of linear heterogeneous models: the bone density non-calibrated model and the bone density-calibrated one. The second compared a linear modelling of the AF with a nonlinear approach. The results were given in terms of stress distribution, range of motion and intradiscal pressure. Validation was possible using Dreischarf et al. [66] recent review article.

In the homogeneous model, stress tends to concentrate more in the cortical shell, being higher in L5 vertebra than in L4, and displaying an uneven stress distribution in the AF, as expected. Intradiscal pressure is more elevated in the present model than in the literature. A possible explanation might be the disc’s height, which is smaller than the reported models. The ROM values are over the physiological range for axial rotation, but for the other motions they present themselves within the computational models’ ranges.

The heterogeneous models showed more spread stress distribution, although the cortical shell is still perceptible. The range for the Young’s Modulus for these models was higher than the values assigned for the homogeneous models (470-24000 MPa vs 100 MPa for trabecular bone and 12000 MPa for cortical bone), hence the IDP were conjointly greater. The higher stiffness of the vertebrae contributed as well to a decrease of the ROM values, resembling more the values of the article.

Differences in ROM and IDP between the calibrated and non-calibrated models were not so clear, whereas in stress distribution they were only noted for Kopperdahl relation.

From the CT-\(\rho\) and \(\rho\)-E relationships, it has been shown their influence in stress analysis and distribution, so their adoption must be carefully made when applied in clinical practice, for example, in
Several *in vitro* studies on intact lumbar segments have confirmed that the disc exhibits stiffening effects under moments and compression forces resulting in a nonlinear load-displacement response. These observations invalidate the linear assumption of disc stiffness and emphasize the growing contribution of disc and motion segments in supporting external loads [17]. After researching the state of the art, it was opted to model the AF with two components: the collagen fibres, radially distributed rebar layers responding only in tension, and the ground substance, an hyperelastic material, which would uphold compression stress states. Results were compared with the previous linear model and with the review article.

In terms of stress distribution, the stress in cortical shell remained the highest, although a reduction in L4's stress was noticed. On the trabecular bone a stress transfer from L5 to the disc is highlighted, revealing the load-bearing role of the IVD. Since the stress distribution was analysed for a compressive stress state, the ground matrix in the AF played the leading role, resembling the linear response. The collagen fibres had a higher order in stress scale and its distribution showed that stress was only present at the regions where tensile stress occurs, allowing them to expand.

In IDP analysis, the resistance offered by collagen fibres contributed to the increase in IDP in comparison with the linear response. Additionally, when evaluating the physiological motion in table 7.9, the existence of preloads acting on the disc led to a bigger axial strain, which once more contributed to the fibres resistance increment. In the validation process, the question whether *in vivo* data represents several groups of population is raised, since the values were picked up from only one individual. Even so, it should be pointed out that in younger individuals, the IDP is expected to be higher [95].

Finally, the ROM showed great agreement to the *in vivo* and computational data. The stiffness improvement in the IVD led to a decrease of the lumbar segment range of motion, allowing the model to enter the physiological range. Only flexion showed a farer value from *in vivo*, but consistent with the other computational models. It was proposed that the loading conditions do not represent the real motion and further studies should be done.
Conclusions and Future Work
In this thesis, an experimental follow-up work to determine the equivalent Young’s Modulus for
the full vertebra was developed. The protocol was adapted from a cited article in biomechanical
experimental methodologies - Buckley et al. [62] - and the achieved results compared with a review
article by Goldstein [92].

The compressive tests were carried out until reaching the specimen’s ultimate force and one was
able to determine to equivalent Young’s Modulus. In future works, it would be interesting to control the
effect of strain-rate in the mechanical behaviour until fracture displayed by the vertebrae. Other types
of information could be studied, such as the ultimate tensile strength and/or applying eccentric loads
in a lumbar spine unit to study ROM, following the standard recommendations of Danesi et al. [63].

A validation computational model of the sheep cervical spine was developed alongside with the
experimental work. Although the achieved results were very satisfactory, a complete fracture biome-
chanical model would be a great challenge. A way to implement it would be through eXtended Finite
Element Method (X-FEM), where cracks would be initiated due to a chosen fracture criterion (such as
Mohr’s or von Mises criterion) and propagated through the analysis. The tensile tests in conjunction
with the compressive ones would facilitate this analysis.

The most problematic lumbar spine segment, L4-L5, was analysed varying the material properties
in bone and comparing linear with nonlinear behaviour of the intervertebral disc. The different res-
ponses were analysed through stress distribution, intradiscal pressure and range of motion within the
physiological range and compared with previous studies summarized in Dreischarf et al. [66] review
article.

A lot of detail with the FSU model was taken, notwithstanding, the ligaments between the posterior
elements of adjacent vertebrae and the capsular joints between the articular facets were not consid-
ered. The inclusion of these parts would improve the response and add the evaluation of facet joint
force study.

Having all those variables gathered, the next instinctual step is to study pathological conditions, for
example nucleus degeneration or herniation, and/or the available surgical options and spinal devices
impact in the biomechanical response of this lumbar segment.

Low back pain is a common condition affecting Western society and its prevalence has increased
over the last years. A strong correlation between LBP and disc degeneration has been found, ur-
ging a deeper understanding of the latter. Combining experimental and computational models allows
therefore to weigh the great impact of this problem and increase the knowledge about it.
References


Results from Experimental Work
Figure A.1: Stress-strain curve and linear fit for Vertebra 1 slice 2, starting at strain zero, from the optimal chosen linear region.

Figure A.2: Stress-strain curve and linear fit for Vertebra 2, starting at strain zero, from the optimal chosen linear region.
Figure A.3: Stress-strain curve and linear fit for Vertebra 3, starting at strain zero, from the optimal chosen linear region.
CT-Density Relations
B.1 Non Calibrated Relation

The measured CT numbers are assumed to be the correct ones ($CTH = CT$), therefore

\[
CT_{\text{air}} = -981 \text{HU} \quad \rho_{\text{air}} = 0 \text{gcm}^{-3}
\]

\[
CT_{\text{highest(bone)}} = 1442 \text{HU} \quad \rho_{\text{bone}} = 1.92 \text{gcm}^{-3}
\]

\[
\rho = a + bCTH = a + bCT
\]

\[
\begin{cases}
0 = a + b \times (-981) \\
1.92 = a + b \times 1442
\end{cases}
\]

\[
\rho = 0.777350392 + 7.924061081 \times 10^{-4} \times CT \quad \text{[gcm}^{-3}] \tag{B.1}
\]

B.2 Calibrated Relation

The measured CT numbers are different from the correct ones ($CTH \neq CT$). For a kilovoltage peak (KVP) of 135 keV, the correspondent effective energy ($E_{\text{eff}}$) is approximately 70 keV. The CT values for each tissue at this energy is presented in the table, given by [83]:

<table>
<thead>
<tr>
<th>Energy (keV)</th>
<th>Water (cm$^{-1}$)</th>
<th>W(H)</th>
<th>Muscle (cm$^{-1}$)</th>
<th>M(H)</th>
<th>Fat (cm$^{-1}$)</th>
<th>F(H)</th>
<th>Air (cm$^{-1}$)</th>
<th>A(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.206</td>
<td>0</td>
<td>0.213</td>
<td>34</td>
<td>0.18</td>
<td>-126</td>
<td>0.00002259375</td>
<td>-999</td>
</tr>
<tr>
<td>70</td>
<td><strong>0.193</strong></td>
<td>0</td>
<td><strong>0.199</strong></td>
<td><strong>31</strong></td>
<td><strong>0.172</strong></td>
<td><strong>-109</strong></td>
<td><strong>0.00002259375</strong></td>
<td><strong>-1001</strong></td>
</tr>
<tr>
<td>75</td>
<td>0.188</td>
<td>0</td>
<td>0.194</td>
<td>32</td>
<td>0.168</td>
<td>-106</td>
<td>0.00002200271</td>
<td>-997</td>
</tr>
<tr>
<td>80</td>
<td>0.184</td>
<td>0</td>
<td>0.19</td>
<td>33</td>
<td>0.165</td>
<td>-103</td>
<td>0.00002200271</td>
<td>-1000</td>
</tr>
</tbody>
</table>

\[
CT_{\text{air}} = -981 \text{HU} \quad \rho_{\text{air}} = 0 \text{gcm}^{-3}
\]

\[
CT_{\text{fat}} = -97 \text{HU}
\]

\[
CT_{\text{muscle}} = 53 \text{HU}
\]

\[
CT_{\text{bone}} = 1442 \text{HU} \quad \rho_{\text{bone}} = 1.92 \text{gcm}^{-3}
\]

With a curve-fitting, one obtains the $CTH - CT$ relation:

![Calibration with 3 Materials](image)

*Figure B.1: Curve fitting of CT scale with air, fat and muscle ROIs.*
Substituting the CTH values by the respective correction \(CTH = 1.0019CT - 17.361\), one can obtain \(a\) and \(b\) by:

\[
\begin{align*}
0 &= a + b(1.0019 \times (-981) - 17.361) \\
1.92 &= a + b(1.0019 \times 1442 - 17.361)
\end{align*}
\]

\[
\begin{align*}
a &= 0.791081265 \\
b &= 7.909033917 \times 10^{-4}
\end{align*}
\]

\[
\rho = 0.791081265 + 7.909033917 \times 10^{-4}CT\text{[gcm}^{-3}\text{]} \tag{B.2}
\]