THE MECHANICAL EVALUATION OF IMMATURE PORCINE BONE IN THE CONTEXT OF PAEDIATRIC SKELETAL INJURIES

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Declaration of Authorship

I, David Colas Åberg confirm that the work presented in my thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.”

Abstract

This thesis investigates the mechanical behaviour of immature porcine bone to enhance our understanding of paediatric skeletal injuries, particularly in the context of child abuse. Due to the limitations of using human paediatric bone, this research uses immature porcine bone as a model. The study includes a comprehensive analysis of immature porcine bone, examining its intrinsic and extrinsic properties under various loading conditions and characterising certain resultant fracture patterns.

The findings reveal age-related trends in immature porcine bone's mechanical behaviour, although direct comparisons to human infant bone were limited. Furthermore, a large scatter of the data suggests caution in interpreting the results. Despite so, the research does indicate certain non-statistical trends that immature porcine cortical bone does exhibit age-related trends in strength (flexural strength) and stiffness (flexural modulus). Bone type (femur and humerus) was identified to not predominantly be a determining factor. Loading rate did influence the mechanical response but again, the scattered data suggest caution in interpretation. Additionally, different loading types generated distinct fracture patterns in immature porcine long bones.

These results contribute to a deeper understanding of immature bone biomechanics and fracture characteristics. While acknowledging limitations in directly translating the findings to the larger arena of immature bone research, this work provides valuable insights for future studies and has the potential to aid in the development of more accurate assessments of paediatric skeletal trauma.

Impact Statement

The work completed for this Ph. D. has achieved impact inside, as well as outside of academia and has the potential for future benefits within both sectors.

The methods used and the empirical results derived from this thesis have been disseminated across the academic community throughout the duration of the Ph. D. This has included multiple presentations, lectures, and workshops on the topic of this thesis. The lectures have focused on the subject under study (the immature porcine model) and the experimental method used (mechanical testing of bone).

I have under the course of this Ph. D. been given the opportunity to tutor multiple undergraduate students (UCL Department of Mechanical Engineering) on their own projects. These separate projects have included several continuations of this Ph.D. such as the development of artificial muscle systems and the computational modelling of immature bone behaviour. Furthermore, the empirical chapters of this thesis are currently under preparation for publication in peer-reviewed journals, which would provide long-standing impact.

The work in this thesis have reached the industry through a series of practical collaborations. Specifically, the work has been shared with professionals from the Royal College of Veterinary, Great Ormond Street Hospital for Children in the UK, and the Academic Medical Center in the Netherlands. These professionals have been invaluable in their provision of material and information on paediatric trauma. Due to the primary work of this thesis entailing the mechanical evaluation of immature porcine, the results have in turn proven useful to the Royal College of Veterinary in their own research on porcine development and treatment of bone disorders in pigs. Finally, the continued research on the mechanical behaviour of immature porcine bone in the context of child has been greatly encouraged by the Great Ormond Street Hospital and Academic Medical Center. Both institutions have offered their services in providing detailed case reports for potential future case-specific experiments using the immature porcine bone model.

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Glossary

Anisotropic - Exhibiting properties with different values when measured in different directions.

Anterior – front of a body.

Appositional bone growth – A developmental process where new bone tissue is deposited on the surface of the bone, resulting in the bone thickening in width.

Axial loading – The force or forces on a structure are applied through the centre of gravity of its cross-section.

Bending – The application of forces in such a manner that the forces are applied perpendicularly to a longitudinal axis of the material, object, or structure.

Biomechanics – The study of the mechanics of living organisms

Bone Mineral Density (BMD) – The amount of bone mineral in bone tissue.

Buckle fracture – A fracture in which the bone buckles or bend but doesn't completely break all the way through.

Cartilage – A tough but flexible tissue which covers and protects the ends of long bone. Cartilage helps in holding bones together, reducing friction between joints, and supporting weight.

CATIA – A multi-platform software suite for computer-aided design. CATIA supports multiple stages of product development, including conceptualization, design, engineering, and manufacturing.

Compression – Application of forces (e.g. load) where a material, object, or structure is pressed together.

Computer Tomography (CT) – A medical imaging procedure in which multiple cross-sectional images are developed using x-rays and computer processing.

Condyle – An articular prominence of a bone. Resembles a pair of knuckles and is found at the end of a bone (particularly long bones).

Cortical bone – A type of bone material which makes up the dense outer surface of a whole bone.

Cranium – A collection of eight bones which primarily encases and protects the brain.

Deflection – The movement a material, object, or structure from its original position due to the forces and loads being applied to the it.

Diaphysis – The elongated central part of a long bone. Also referred to as the shaft.

Distal – Situated away from the torso.

Dynamic loading – A load that changes with time. An example is walking.

Epiphysis – The end part of a long bone

Ex-vivo – Outside of an organism

Femur – Thighbone; generally, the singular longest long bone in the lower extremity of a vertebrate's body.

Flexural modulus – Bending modulus; a physical property which denotes a material, object, or structure's ability to bend.

Force at fracture – The point (level of force) at which a material, object, or structure fails by fracture.

Forensic anthropology – The use of anthropological methods for analysing biological remains for medicolegal purposes.

Forensic pathology – A subspecialty of pathology where physicians investigate deaths that are not natural or are suspicious.

Forensic science – The application of science to criminal and legal matters. Forensic science is an umbrella term for multiple fields invested in using the respective field's methods in criminal and legal matters.

Force – Any action that maintains or changes the motion of an object.

Fracture – A break in continuity (e.g. bone fracture is a break in the continuity of the bone)

Fracture energy absorbed – The amount of energy a material, object, or structure can absorb before fracturing.

Fracture pattern – The morphological manner in which a bone breaks.

Bone geometry – The dimensions of a bone.

Growth plates – Areas of new bone growth. Growth plates consist of cartilage and can be found at the ends of long bones. As the bone matures, the growth plates harden into bone.

High-energy loading – Application of forces comparable in magnitude to high-energy events such as an automobile accident.

Humerus – Forelimb; long bone of the upper extremity.

Impact test – A standardised high-energy test which determines the amount of energy absorbed by a material during fracture.

In-vivo – Inside of an organism

Lateral – Extending to the side. The lateral side of a bone refers to the side that is away from the middle.

Ligament – Fibrous tissue that attaches bone to bone and helps stabilize joints.

Load – The effect of a force or series of forces.

Loading rate – Speed at which forces impact a body.

Loading type – The way forces impact a body (e.g. compression).

Maceration – The removal of soft tissue.

Macrostructure – The gross structure of a material or tissue. Visible to the naked eye.

Medial – Situated towards the median plane. The medial side of a bone refers to the side that is towards to the middle.

Metaphysis – Portion of bone between the diaphysis and epiphysis.

Microstructure – The structure of a material or tissue only visible through the use of microscopic equipment.

Oblique fracture – A slanted fracture which crosses the bone axis at an approximately 45-degree angle.

Ossification - The process of bone formation.

Paediatrics – The branch of medicine dealing with children and their diseases.

Posterior – behind a body

Proximal – Situated closer to the torso.

Quasi-static loading – The loading is applied so slowly that the material, object, or structure also deforms very slowly.

Rigidity modulus – Also known as the shear modulus. A measure of a material's elasticity during torsional loading.

Sagittal plane – Also known as the longitudinal plane. An anatomical plane which divides the body into right and left parts.

Shear – A force that acts by sliding one part of a body over another part of the body.

Spiral fracture – A bone break which occurs when a long bone is twisted with force. The fracture will appear similar to a corkscrew on an x-ray.

SPSS – Statistical software.

Static loading – A load which does not change with time. An example is gravity.

Strain – The elongation of a material or object per unit length.

Strain-rate dependency – Exhibiting properties with different values when tested at different loading rates.

Strength – The ability of a material or object to withstand an applied load without failure.

Stress – Force per unit area

Tension – Application of forces (e.g. load) where a material, object, or structure pulled apart.

Torque – A force that acts to rotate an object about an axis.

Torsion – The twisting of a material or object by applying opposite torques.

Trabecular bone – Also referred to as cancellous bone. A type of porous bone material generally found inside the bone.

Transverse fracture – A bone break where the fracture is at a right angle to the long plane of the bone.

Universal Testing Machine (UTM) – A machine which is used to test the mechanical of a given test specimen or sample.

X-ray – A form of high-energy electromagnetic radiation which when concentrated can pass through most objects. X-rays can be used to generate images of tissues and structures inside the body (e.g. bone).

Acknowledgements

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To my soulmate, my wife, Sanaz. After your own Ph.D., our wedding, and two beautiful children where shall we rank this one? It is no embellishment to say that you are my northern star. Thank you for always standing by me, encouraging me, and for always talking sense into me. I can only hope that I have made you proud.

To my dynamic duo Autrey and Aurelia, nothing puts things into perspective like you two.

The mechanical evaluation of immature porcine bone in the context of skeletal injuries

Chapter 1 Introduction

There is no exact statistic which has clearly presented the true amount of child abuse incidents in the UK (Bentley *et al.* 2018). Due to the possibility of injuries going undetected by medical professionals and a portion of cases never reaching the attention of the authorities it has been suggested that reported statistics show only the tip of a more widespread problem (Gilbert *et al*. 2009; Fallon *et al*. 2011; Radford *et al.* 2011; Harker *et al.* 2013). Records from the British Office for National Statistics reported that 6.4% of all the English and Welsh homicide victims between the year ending March 2013 to the year ending March 2015 were between the age of under to 4 years of age (ONS, 2016) Despite such recordings, self-report surveys in the aforementioned countries indicate the 'true' prevalence to be approximately 4 to 16 times higher (Gilbert *et al.* 2009; Radford, 2017).

Despite the already alarming prevalence, child abuse is considered clinically under-diagnosed (Bilo *et al.* 2010). There are numerous reasons as to why this is, but a commonly considered opinion is the difficulty in distinguishing accidental trauma from abusive trauma (Bilo *et al.* 2010). Specifically, skeletal injuries have proven a challenge due to the varying differences in the reported material properties between paediatric and adult bone (Franklyn *et al.* 2007). Adult bone is simply more accessible and as such the more studied type of bone. However, this prevents comparisons with paediatric bone. Since investigators and clinicians are additionally encumbered by the potential for inaccurate or misleading accounts from caretakers, an approach for understanding specifically the mechanical behaviour of immature bone and how that translates to fracture patterns is essential. Biomechanics has proven fundamental in informing this approach, thereby allowing researchers to significantly advance the understanding of paediatric bone behaviour (Arbogast and Maltese, 2014; Forestier-Zhang and Bishop, 2015). Such an approach forms the basis of the work presented here.

However, much remains to be explored. The field of biomechanics has seen the refinement of methods of testing and computational modelling, but further experimental testing of, specifically, physical materials is still required for the continued building of an empirical evidence base (Li *et al.* 2015). Due to the lack of human tissue, alternative models such as the immature porcine, have been suggested as a feasible substitute for certain human skeletal elements (Margulies and Thibault 2000; Coats and Margulies, 2003; Baumer *et al*. 2009; Cone *et al*. 2017; Vaughan *et al.* 2020; Saini *et al.* 2024). However, it is also important to acknowledge that the use of porcine models as proxies for human bone, particularly whole bone, has also been the subject of scrutiny. Specifically, studies have highlighted morphological and developmental differences between porcine and human bones, raising questions about the direct translatability of results (Reichert *et al.* 2009; Cheong, 2015; Cone *et al*. 2017). While acknowledging the concerns regarding the use of porcine models, this thesis argues that the immature porcine model, rather than simply the adult porcine model, remains a less explored option. This is particularly true for investigating material properties and fracture patterns, areas where significant gaps in our understanding of immature bone mechanics persist. Furthermore, while much research has focused on the differences between porcine and human bone, the focus has primarily been whole bone comparisons (Pierce *et al.* 2000; Koo *et al.* 2001; Thompson *et al.* 2015; Bertocci *et al.* 2017; Cheong *et al.* 2017; Vaughan, 2017).

Although exploring whole bone through the observation of fracture patterns, this thesis mainly focuses on the material properties of immature porcine bone to establish a fundamental understanding. Additionally, there is a lack of substantive research on the chronological changes of the material properties of immature porcine bone. Therefore, this research aims to help establish a foundational understanding of the immature porcine on a material bone level. This foundational understanding can then be used in conjunction with future experimental work, which may include the effects of muscle, sinew, and soft tissue**.** Porcines, as adult human substitutes, are common in forensic and biomechanical studies, but the science-based evaluation of the infant porcine as a human infant model is limited. As such, an exploration of the immature porcine on a material bone level could help in determining its value for future research into immature bone behaviour.

1.1 Aim

The primary aim of the research conducted for this thesis was to add to the current understanding of immature bone behaviour under stress, both on a material and extrinsic level. This thesis looked to achieve this aim through the mechanical testing of a potential human surrogate model, immature porcine bone. The research contributed to an understanding of immature porcine bone behaviour and provided a basis for a future where immature porcine bone can be further tested and compared under similar circumstances. To allow for an understanding of immature porcine bone behaviours, this thesis contains several objectives that are relevant to the overall aim. Primarily, work needs to be conducted to determine the forces required to generate a fracture on a material level. This was accompanied by an evaluation on whether the mechanical properties of immature porcine bone differs across different age groups. This is followed by a critical discussion of how the results compare to the literature on immature porcine bone behaviour, but also whether they can be interpreted in relation to studies that used human infant bone. Additionally, mechanical tests were conducted using immature porcine whole bone to observe how the fracture patterns generated compares to other porcine studies and to the literature on human infant bone.

1.2 Thesis structure

The following thesis is divided into 9 chapters including the introduction. Chapter 2 provides the background and literature review of the thesis. Regarding the reader's knowledge base prior to this thesis, chapter 2 looks to provide the requisite concepts and formulas required for interpreting the approach and technicality of the experiments conducted in this thesis. This includes a basic primer on what immature bone is, how it has been found to behave as a material, how it differentiates itself from mature bone, the explicit fracture patterns which, according to the literature, typically manifests themselves in this type of bone, and the current use of animal surrogates for forensic mechanical testing purposes. Chapter 2 clarifies the gaps in the current research on immature porcine bone and how these gaps can begin to be investigated. Chapter 3 is the problem statement where the research question and intermediate research questions for this thesis are presented. As Chapter 2 will look to clarify gaps in our current understanding, Chapter 3 presents the aim to bridging those knowledge gaps. Chapter 4 documents the materials and methods used for this thesis. This includes an overview of the preparation, and the set-up required to mechanically test the material of immature porcine bone as well as immature porcine whole bone. Chapter 4 also clarifies the method of data and statistical analysis conducted in thesis.

Chapter 5 describes experimental tests aimed at determining properties of immature porcine cortical bone under three-point bending. Chapter 5 acted as a springboard for later experiments. Described in chapter 6 concerns the mechanical evaluation of immature porcine cortical bone samples under high-energy loading rates. Here, bone material was tested under higher loading rates. Chapter 7, subsequently moved from immature porcine bone “material” to immature porcine “whole bone” and constitutes the investigation of the fracture patterns generated under three-point bending, compression, and torsion testing, but crucially for bones rather than ‘bone’ per se. Chapters 5, 6, and 7 aimed to provide a clear thread from immature porcine bone behaviour on a material level to how such traumatic forces can be illustrated on immature porcine whole bone. Chapter 8 discusses the experimental findings, their implications within the field of forensic science, and the limitations of the studies undertaken. Lastly, Chapter 9 provides a concise summary of the entire thesis, reiterates the key conclusions, and suggests avenues for future research.

Chapter 2 Background

As mentioned in Chapter 1 (1.1. Aim) The primary aim of this research project was to further understand the mechanical behaviour of immature bone under load. One important purpose for which such knowledge can be applied is in the context of skeletal injuries sustained by children. However, specifically using this type of knowledge to help determine the factors surrounding skeletal fractures in children is a complex problem (Bilo *et al.* 2010). Mechanical testing of immature bone is a fundamental step toward a more comprehensive understanding of skeletal injuries.

Due to the scarcity and ethical complications of using human paediatric bone for mechanical testing, this research project undertook a series of mechanical tests using immature porcine bones. As suggested by the literature (Laiblin and Jaeschke, 1979; Mosekilde, 1987; Raab *et al.* 1995; Aerssens *et al.* 1998; Thorwarth *et al.* 2005) and highlighted in section 2.8, immature porcine bone has shown a potential for imitating human paediatric bone and this research project aims to provide foundational immature porcine data which could be utilised for future comparative research. This chapter will discuss the theoretical and practical background and justification for the hypotheses and mechanical tests presented herein. It is also important to note that the concepts drawn together to explain the rationale for the hypotheses tested and the methodological approach used in this thesis are derived from multiple fields of study.

Due to the varying types of expertise required for understanding how immature bone can act under stress, this thesis took a multidisciplinary approach. Understanding skeletal injuries is a problem shared by several types of experts, foremost among them medical, anthropological, and engineering professionals. These experts consult multiple sources surrounding skeletal injuries in children such as clinical histories, surface-based injury characteristics, and how physical forces brings about not only visible injuries but structural (invisible to the naked eye) injuries as well. Based on the disciplines most directly related to the mechanical behaviour of immature bone and the physical manifestations of fractures, this thesis has therefore chosen an approach rooted in forensic pathology, forensic anthropology, and biomechanics. The methods derived from forensic anthropology and forensic pathology provided the theoretical and practical knowledge base for examining and investigating the externally visible bone fractures generated through the testing in Chapter 7. The methods derived from biomechanics formed the template for the experimental testing approach performed in this thesis as well as the testing of the mechanical properties exhibited during the material tests (Chapter 5 and 6). All three disciplines were found relevant for understanding the fracture patterns exhibited by immature bone as well as the mechanical behaviour of immature bone under load, specifically, in the context of paediatric skeletal injuries.

This chapter begins with an overview of how paediatric skeletal injuries are currently evaluated in consideration of potential child abuse. It is followed by an overview of the theoretical and practical underpinning of this thesis, namely the multidisciplinary use of forensic pathology, forensic anthropology, and biomechanics. The chapter will continue with a scientific primer concerning the properties of bones observed by the literature and tested in this thesis. This includes a familiarisation of what bone (focusing on immature bone) is as a material and as a whole bone (e.g., femur). This will be complimented with an overview of the long bone fracture patterns often observed and diagnosed in child abuse cases. Due to the previously referenced lack of human paediatric tissue, this chapter concludes with a critical review of previously tested animal models, placing a focus on why the immature porcine was chosen for this thesis.

2.1 The clinical presentation and detection of child abuse

As presented as a flowchart in figure 2.1, when physical abuse of children is suspected, the child is generally referred for a child protection medical evaluation, which in the UK and the rest of Europe are predominantly conducted at medical emergency departments (Naughton *et al.* 2018). As no gold standard exists for evaluating child abuse, paediatric injuries are predominantly evaluated through certain practice recommendations (Figure 2.1). This lack of a gold standard makes adherence to established protocols, and the careful documentation of each step paramount. Early detection of potential abuse, particularly fractures, is critical to preventing further harm to a child, providing evidence for legal proceedings, and informing appropriate treatment plans. This includes tools, assessments and symptoms developed and identified by larger medical organisations (College of Paediatric and Child Health, 2013).

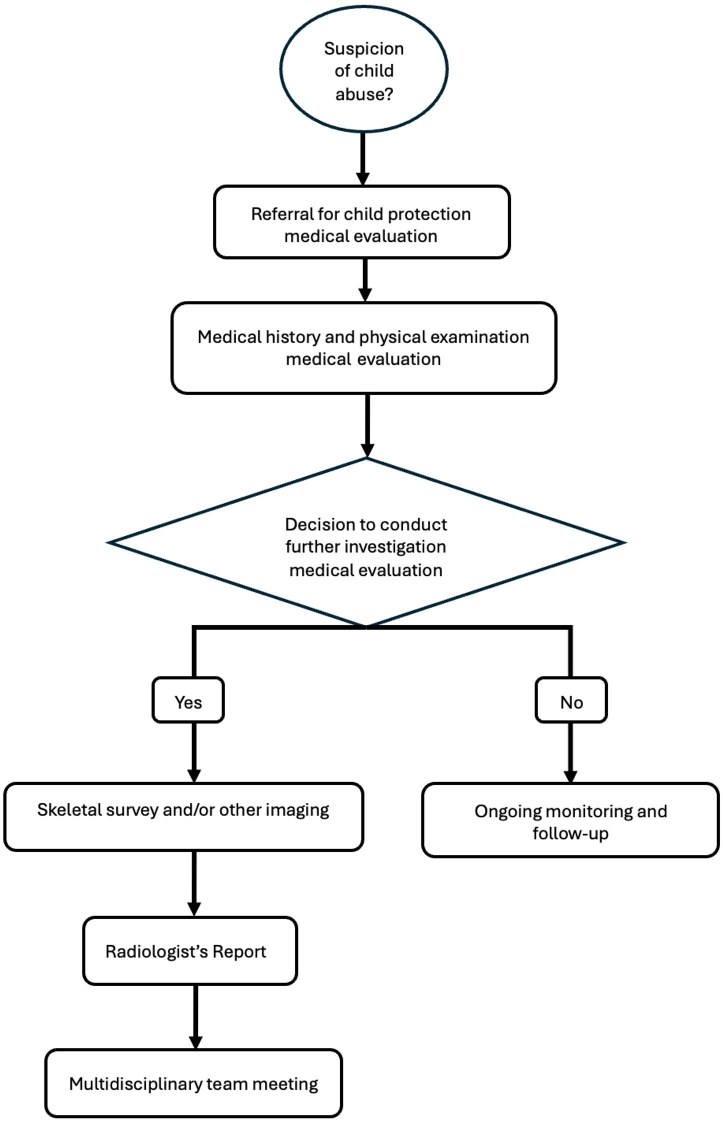

Figure 2.1: Flowchart illustrating the general decision-making process involving suspected child abuse.

The Royal College of Paediatric and Child Health (RCPCH) which acts as the primary source of paediatric injury treatment protocols in the UK, has developed a series of protocols predominantly based on case-specific situations and events (Naughton *et al.* 2018). In specific consideration of what may be suspected to be abusive fractures, the RCPCH recommends the UK-based Royal College of

Radiologists (RCR) and Society and College of Radiographers' (SCOR) protocol which in turn suggest medical imaging as an important tool for correctly identifying the injury and its accompanying characteristics (SCOR and RCR, 2017). This is particularly important as certain types of fractures are highly suggestive of abuse. For example, rib fractures, particularly posterior rib fractures, are highly specific for abuse in the absence of a clear accidental cause, such as a major car accident (Kemp *et al.* 2008). The recommendation includes that medical imaging should always involve a skeletal survey in children under two years of age and computed tomography (CT) when imaging the head of children aged 1 year and under (SCOR and RCR, 2017). Specifically, a skeletal survey is an x-ray examination of the whole body which generally involves around 20 separate x-ray images (Table 2.1). Medical imaging, such as skeletal surveys, plays a crucial role because it provides the visual evidence of fractures, helps recognise possible discrepancies in the exhibited fracture and event account, and can identify subtle fractures not visible during physical examination (See figure 2.1, skeletal survey and/or other imaging stage). This is particularly critical in young age groups as they are more vulnerable to abuse and less able to verbalise their experience.

In the event of a senior clinician (predominantly a paediatrician) suspecting that a child has been a victim of physical abuse, the skeletal survey will need to be conducted by two radiographers with documented education and training in physical abuse imaging and forensic radiography techniques. A registered children's nurse or appropriately educated health or care practitioner is also to be present during the examination (SCOR and RCR, 2017). Any referral to social care and safeguarding teams (e.g., police) is recommended to be conducted as soon as possible or within 24 hours of the clinical evaluation (SCOR and RCR, 2017). This rapid reporting is crucial for the event of initiating protective measures for the child and to preserve evidence. The RCR further recommends that a consensus report is to be completed within 24 hours of examination by two radiologists. This requirement for review by two radiologists helps to minimise bias and ensure the accurate interpretation of the imaging findings.

Finally, follow-up imaging is recommended within 11 to 14 days, and no later than 28 days after the initial skeletal survey. This provides the examiner with the chance of identifying previously unnoticeable fractures which during the healing process begin to radiographically appear. Due to an increased level of vascularity and osteogenic activity, children's bone heals more rapidly than adults (Glencross and Stuart-Macadam, 2000). Thus, if the medical examiner chooses to not pursue a second screening, there is the possibility of abusive fractures going undetected. However, if the examiner chooses to wait the recommended time for a second screening, the abusive fractures may have healed over. As described by various studies, there is a linear relationship between a child's age and the healing rate (Malone *et al.* 2011). Certain fractures such as that of the femur can heal within 3 to 4 weeks in a newborn, whereas the same injury can take 12 to 16 weeks in an adolescent (Glencross and Stuart-Macadam, 2000; Ross and Juarez, 2014). Similar differences in healing rates have also been observed to occur in the distal regions of long bones (Swischuk and Hernandez, 2004). The timing of this follow-up is therefore crucial. Performing it too early and you may miss fractures that haven't yet become radiographically apparent, while delaying it may allow some fractures to heal, potentially obscuring evidence of abuse. Documentation and notes are to be taken in record of the injury, the individuals present (clinical or affiliated with the patient), and in ensuring that the child returns for a follow-up (SCOR and RCR, 2017; See figure 2.1, the 'no' pathway, illustrating the importance of follow up even when further medical investigation is not initially conducted). In the case of a child fatality, the post-mortem procedure should be the same as in life, with a full skeletal survey (Table 2.1).

Table 2:1: Skeletal survey: standard views, including follow-up (SCOR and RCR (2017).

Smaller children (e.g. under 1 year)	
Head, chest, spine, and pelvis	Anterior-posterior (AP) and lateral skull AP chest (To include the shoulders) and both obliques (obliques to include all ribs, left and right, 1-12) AP abdomen and pelvis Lateral views to include the whole spine. (For children under one year, this may be possible with one view, for larger children and those over one-year, separate views will probably be required.)
Upper limbs	Where possible: AP of the whole arm (centred at the elbow if possible) Coned lateral elbow Coned lateral wrist Posterior-anterior (PA) hand and the wrist) Coned lateral elbow Coned lateral wrist DP hand and wrist
Lower limbs	Where possible: Whole AP lower limb, hip to ankle Coned lateral knee and ankle Coned AP ankle (mortise view) DP foot

For larger children	
Upper limbs	AP humerus (including the shoulder and elbow) AP forearm (including the elbow and wrist) Coned lateral elbow Coned lateral wrist DP hand and wrist
Lower limbs	AP femur AP tibia and fibula AP knee AP ankle Coned lateral knee Coned lateral ankle DP foot
Follow-up imaging (11-14 days, no later than 28 days after initial skeletal survey)	
Follow-up radiographs should be performed of any abnormal or suspicious areas on the initial skeletal survey plus the following view. Chest AP and both obliques (to include the shoulders and all ribs, left and right, 1-12)	

Finally, follow-up imaging is recommended within 11 to 14 days, and no later than 28 days after the initial skeletal survey. This provides the examiner with the chance of identifying previously unnoticeable fractures which during the healing process begin to radiographically appear. Due to an increased level of vascularity and osteogenic activity, children's bone heals more rapidly than adults (Glencross and Stuart-Macadam, 2000). Thus, if the medical examiner chooses to not pursue a second screening, there is the possibility of abusive fractures going undetected. However, if the examiner chooses to wait the recommended time for a second screening, the abusive fractures may have healed over. As described by various studies, there is a linear relationship between a child's age and the healing rate (Malone *et al.* 2011). Certain fractures such as that of the femur can heal within 3 to 4 weeks in a newborn, whereas the same injury can take 12 to 16 weeks in an adolescent (Glencross and Stuart-Macadam, 2000; Ross and Juarez, 2014). Similar differences in healing rates have also been observed to occur in the distal regions of long bones (Swischuk and Hernandez, 2004). Documentation and notes are to be taken in record of the injury, the individuals present (clinical or affiliated with the patient), and in ensuring that the child returns for a follow-up (SCOR and RCR, 2017). In the case of a child fatality, the post-mortem procedure should be the same as in life, with a full skeletal survey (Table 2.1).

However, the identification of what may be abusive fractures is not primarily determined by a skeletal survey but by a multitude of factors such as the medical history of the child, the account of the injury but also the determination and skill level of the clinical investigator (See figure 2.1, illustrating how the suspicion begins with the clinician, requiring a series of further steps to reach a conclusion). Experienced clinicians play a vital role in recognising patterns of injury, interpreting medical imaging findings in the context of a child's history and development, and making informed decisions about further investigations. Flaherty *et al.* (2006) identified American paediatricians to exhibit different levels of abuse suspicion when confronted with an injured child, and that the paediatrician's decisions are heavily connected to personal experiences and belief. Jones *et al.* (2008) noted that several US-based primary care doctors seldom report injuries attributed to suspected child abuse. Jones *et al.* (2008) further noted factors such as familiarity with the family, unfamiliarity with available resources and lack of experience with child protective services to be reasons behind certain paediatricians' reluctance to openly diagnose child abuse. The difficulties are compounded as attempts at non-biased detection and diagnosis of physical abuse are exceedingly limited by available predictive measures and formal screening tools (Garcia and Lawson, 2014). This becomes particularly evident when a child is medically presented with no visible signs of physical trauma, history of abuse, or current report of a life-threatening event (D'Cruz, 2004; Christian, 2015; Musters and Colaris, 2017). Several clinical studies from the USA have reported considerable variation in the diagnostic investigations used (including skeletal surveys, neuroimaging and blood investigations) and in the identification of child abuse, even after adjusting for individual patient characteristics (Harper *et al.* 2016; Lindberg *et al.* 2014; Wood *et al.* 2012). The result is often instead that the physician's decision is based on clinical experience and intuition (Garcia and Lawson, 2014).

In Bailhache *et al.'s* (2013) systematic review on the diagnostic accuracy of screening tools in detecting child abuse the authors identified all the included seven studies to have contained a methodological or quality issue. The included studies had analysed a variety of predictive factors for child abuse including individual factors such a severe retinal haemorrhage, brain ischemia (e.g. insufficient blood supply), subdural hematoma, lack of history or low-impact trauma history, medical imaging patterns, bruise location consistent with fracture site, as well as tools integrating multiple factors such as a combination of bruise region, age of child, and mechanism history or a combination of age of child, physical exam findings, and imaging results (Wells *et al.* 2002; Hettler and Greenes, 2003; Chang *et al.* 2005; Vinchon *et al.* 2005; Valvano *et al.* 2009; Pierce *et al.* 2010; Vinchon *et al.* 2010;). In fact, of these factors, only the individual variable of scalp swelling (Vinchon *et al.* 2010) and the decision tool to integrate bruising location, child age, and patient history (Pierce *et al.* 2010) were found to produce a sensitivity greater than 90%.

In conclusion, it may be opined that the clinical detection of abusive fractures in children is hampered by a lack of biomechanical knowledge of the paediatric anatomy, in-precise tools, and a reoccurring discomfort with the "cultural" aspect of child abuse. There is therefore merit in the undertaking of research which can further our understanding of immature bone behaviour and add considerations to the protocols currently employed for assessing medical situations involving injured children.

2.1.1 Incidence rate of abusive fracture in the context of age

The identification of a victim's age is recommended as the first step when investigating the manifestations of skeletal injuries (Ross and Juarez, 2014). Skeletal injuries increase exponentially once a child learns to walk, and it is due to the development of mobility in children that there is a reported inverse diagnostic relationship between victim age and the rate of abusive skeletal trauma (Bilo *et al.* 2010). As such skeletal trauma in immobile children should always be considered as possibly due to child abuse (Bilo *et al.* 2010). Specifically, children less than 2 years of age should, by recommendation be of immediate interest (Leventhal *et al.* 1993; Carty, 1997). Additional studies report that the incidence rate of non-accidental fractures peaks in children between birth and 3 years of age (Skellern *et al.* 2000; Hoskote *et al.* 2003; Loder *et al.* 2006). Non-accidental fractures have been reported to make up 49% of all trauma admission of children below 1 year of age (Loder and Bookout, 1991) but may be as high as 80% (Worlock *et al.* 1986). For children younger than the age of 3 years, Kowal-Vern *et al.* (1992) found the estimate to be 23%.

Among abused children, Loder and Bookout (1991) found that 40% of the victims were 6 months of age or less, 67% were less than 1 year of age and 80% were less than two years of age. An earlier study of 189 paediatric victims by King *et al.* (1988) found that 130 (69%) children were below the age of one year, 26 (14%) were above the age of 2 years old and 23 (12%) were between one and two years of age. Similarly, Akbarnia and colleagues (1974) and Loder *et al.* (2006) found that infants below the age of one year composed most of the children studied (e.g. 48%). These results have convinced many authors that young and immobile children are most vulnerable to child abuse and is as such the clinical driver for the predominant research conducted on accidental and non-accidental fractures (Schwend *et al.* 2000; Jayakumar *et al.* 2010; Kraft, 2011). The mechanical testing of immature porcine bone in this thesis will provide valuable data relevant to understanding fracture mechanics within the age range most susceptible to skeletal trauma.

2.2 A multidisciplinary approach

As previously stated, this thesis has made use of multiple disciplines, all of which has contributed to a better understanding how immature bone can act under load. Based on the disciplines most directly related to the mechanical behaviour of immature bone and the physical manifestations of fractures this thesis has chosen an approach rooted in forensic pathology, forensic anthropology, and material science. How and why these disciplines have contributed to the theoretical and practical underpinning of this thesis will hereby be discussed.

2.2.1 Forensic pathology

One of the disciplines consulted for this thesis is forensic pathology. Specifically, forensic pathology is equally a speciality of medicine and forensic science (Eckert, 1996). It was developed in order to study the issues related to unnatural death and the various types of traumas a living being can sustain (Eckert, 1996). The individuals and categories of death and injuries examined by pathologists will include any member of the public (child or adult) and a range of trauma contexts. Depending on jurisdiction, deaths covered involve those occurring during medical treatment, violent and suspicious deaths occurring to individuals under custody in public or private institutions, or deaths occurring to individuals working in hazardous industrial areas. The pathologist's presence and areas of involvement will include on-the-scene-investigation (managing the forensic specialists on site), post-mortem examinations (e.g., autopsy) and biological testing (e.g., blood, saliva, semen, and other forms of trace giving materials; Mitchell *et al.* 2017). Pathologists will on their authority refer further examination to toxicologists, serologists, criminalists, odontologists, or anthropologists (Eckert, 1996).

A pathologist is, by standard, a Doctor of Medicine who has had at least four years of training in pathology post-medical school, and an additional year of having handled medicolegal autopsies involving unnatural, suspicious, violent or unexpected deaths (Mitchell *et al.* 2017). It requires extensive academic and practical involvement to become a chartered forensic pathologist, but it is also the pathologist's daily interaction with specialists from multiple disciplines which makes them such resources of knowledge. The pathologist's experience with examining injuries is therefore beneficial for establishing the possible cause of injuries to living victims as well. However, the interpretation of bone trauma in children can be particularly challenging due to the unique biomechanical properties of immature bone. This thesis aims to address this challenge by providing empirical data on the fracture behaviour of immature bone, which can aid forensic pathologists in making more accurate diagnoses and contributing to better outcomes in cases of suspected child abuse.

There is additional synergy between this description of forensic pathology and the aims of this thesis. One of the contributions of the pathologist is that any injuries reported in a deceased individual can be utilised to detect and understand injuries in the living. For example, a forensic pathologist will have the experience of different bone traumas caused by accident or intent (Meilia *et al*. 2018). Whilst this thesis tested samples no longer living the aim is still, in part to, help living individuals. By testing excised bone samples, this thesis seeks to offer a clearer understanding of bone behaviour in situations where in-situ testing is not feasible.

As part of the forensic pathologists' attempts to interpret findings there might be a need to consider further questions that would be helpful to ask and have answered Hung *et al.* 2011). This is both during *in-situ* investigations and autopsy examinations where there may be a time-sensitivity in interpreting findings and further decision making (e.g., previously healed rib fractures suggesting previous abuse and need for immediate action) after an investigation or autopsy. Some post-autopsy questions might be most easily posed during follow up conversations with next of kin, or those who may have witnessed an event taking place, or a specialist who may speak to the specific possibilities surrounding a trauma (e.g., biomechanics, entomology, etc).

At whatever stage of investigation, it is important that hypotheses or viewpoints are backed up with objective evidence (Prahlow, 2010). As suggested by the research, when dealing with any external advisor who might provide such expertise as the physical forces at play in an injury, it is recommended that forensic pathologists obtain objective investigative data points, rather than accepting opinions and conclusions without further qualifications. Furthermore, it is recommended for the pathologist to seek primary sources of information leaving little room for confusion and allowing for a clear delineation of the questions and needs relevant to the investigation. Again, this highlights not only the need for a strong multi-disciplinary support system but the type of research which aims to provide increased corroboration and a reliable evidence base.

Forensic pathologists are often asked in court about the magnitude or degree of force necessary required to produce a particular trauma, as well as what impact mechanisms were involved. When providing expert witness testimony, forensic pathologists can often rely on anecdotal examples to explain the relationship between impact force and trauma and generally refer to the degree of force in a three-tier scale of ‘mild’, ‘moderate’ and ‘severe’ (Hannon, 2016). This scale categorises the appearance and severity of trauma in relation to impact force, however the ratings of ‘mild’, ‘moderate’, and ‘severe’ are ultimately subjective and lacking in empirical support.

As stated by Boyd and Boyd (2018), skeletal trauma should not be seen as separate segments, but as a continuous process. Fundamentally, any attempt to determine impact forces and/or impact mechanisms that is of forensic and/or medicolegal significance should not be left to subjectivity or conjecture (Porta, 2005). Therefore, a quantitative and impartial assessment based on scientific and biomechanical data is ultimately required when questions of injury mechanisms or impact forces are presented to the court (Porta, 2005). For example, Gläser *et al.* (2011) highlight the potential for determining the maximum striking energy an assailant can deliver. This information allows judicial authorities to compare the inflicted energy with the assailant's maximum potential energy, leading to more informed conclusions about intent. However, to achieve a standard where forensic practitioners can reliably and accurately assess trauma, there needs to be a greater emphasis on developing standardised models and methodologies. These models and methodologies should be replicable, peer-reviewed, and widely accepted within the forensic community (Roberts, 2015)

2.2.2 Forensic anthropology

Forensic anthropology is a sub-field of anthropology and consists of the use of biological, anthropological and archaeological practices in matters of legal concern (Christensen *et al.* 2014). Despite the immense knowledge base and experiences forensic pathologists draw on, both pathologists, and the scientific and forensic community has come to realise that there is a requirement for further understanding when investigating the effects certain conditions can have on the human body, particularly the skeleton (Cunha and Cattaneo, 2006; Blau, 2017; Alfsdotter, 2022). Most forensic pathologists do not have extensive training or a background in anthropology and osteology, and anthropologists may not be used to working with human remains still bearing some soft tissue (Cunha and Cattaneo, 2006). There are therefore benefits to the two professionals cooperating. The basic element grounding both professionals' approaches is similar. This includes to different extents, soft tissue, bone, and trauma. However, the medicolegal way of thinking is something that comes with experience, and it is quite different from the approach of one who is reconstructing life from an "archaeological" skeleton. The biological information that is sought from the skeleton is the same in both circumstances (sex, age, ancestry, stature, etc.); however, the consequences of the application in the classical sense of aim are not identical. Forensic anthropologists blur the aim by focusing only on applying their knowledge of the effects trauma can have on bone in a forensic setting (Crowder *et al.* 2016). It is through a juxtaposition of lab and field-specific practices that forensic anthropologists may assist forensic pathologists and even lead certain parts of an investigation. Due to this skillset, the forensic anthropologist was up until recently, primarily involved in criminal investigation involving skeletal evidence (Walker *et al.* 1997; Lewis 2007). However, as forensic anthropology has matured, the field, particularly in the US, has become more ingrained with the actions and practices of the medical examiner's offices and it is by such collaborations that the forensic anthropologists' understanding of crimes against children has expanded, a contrast to the UK and the rest of Europe, where the profession's integration within formal medicolegal systems and its application may vary considerably by country (Crowder *et al.* 2016; Passalacqua *et al.* 2023). Currently practicing forensic anthropologists will encounter cases of child abuse and this has resulted in a series of forensic anthropology publications investigating the detection of child abuse (Love and Sanchez, 2009; Baumer *et al.* 2010 Powell *et al.* 2012). Love and Sanchez (2009) proposed an alternative autopsy method for children in which the skin, subcutaneous fat, musculature, and periosteum was incised and reflected to allow for more detailed evaluations of possible bone fractures. Davy-Jow *et al.* (2012) utilised anthropomorphic data to develop a virtual reconstruction of a seven-year-old deceased body for court whilst Wei *et al.* (2017) have initiated test trials for a paediatric cranial fracture pattern classification software.

However, forensic anthropology as part of the forensic science community, is undergoing a period of transition. This change has partly been driven by a series of successful courtroom litigations of a number of testimonies relating to fingerprint and toolmark analyses (Kaye, 2010). While seemingly unrelated to forensic anthropology, these legal cases resulted in the 2009 National Academy of Sciences (NAS) report titled *Strengthening Forensic Science in the United States: A Path Forward* (U.S National Research Council, 2009). This report perceived numerous shortcomings across the different forensic fields, including a lack of statistically validation and unclear methodological processes. Although, forensic anthropology was not explicitly mentioned in the NAS 2009 report, the field has since faced similar criticism, with concerns raised about inadequate training in quantitative methods, insufficient adherence to standards, and an oversight of method-related biases (Suckling *et al.* 2016; Nakhaeizadeh *et al.* 2018; Steadman, 2018; Morgan, 2019).

Specifically, the biological and personal identification of children’s remains is in part hindered by a lack of paediatric samples and subsequently methods for correctly identifying children’s remains (Kerley, 1976; Burton *et al.* 2002; Lewis and Rutty, 2003; Lewis, 2007). Characteristics which are regularly observed to denote sex customarily develop after puberty, when hormone levels increase and sexual dimorphism becomes more discernible in the skull and pelvis (Lewis and Rutty, 2003; Lewis, 2007). In burials of potentially multiple individuals, determining the minimum number of individuals may be easier to obtain in children, as sizes vary with age and between individuals. However, depending on the case, children of a similar age may be recovered and therefore size may not be a useful distinguishable feature. Additionally, young children seldom visit the dentist or have major surgery which often nullifies the value of medical records (Lewis, 2007).

Despite these obstacles, forensic anthropology possesses a substantial knowledge base for analysing bone trauma. This stems largely from the ability of forensic anthropologists to draw on their expanding experience at crime scenes, coupled with the knowledge foundation of having studied archaeological remains (which can include children's skeletal remains). However, a limiting factor in developing standards for child identification and trauma evaluation is the lack of modern non-adult skeletal collections (Lewis, 2007). Parents or next of kind rarely consent to donating their children's bodies for medical research. This situation was further complicated by the 1999 case of the Royal Liverpool Children's Hospital (e.g. Alder Hey), where child samples were stored for research without parental consent (Great Britain Parliament, 2001). This incident sparked controversy and contributed to the legislation of the Human Tissue Act 2004 (H.M Government, 2004). Consequently, established collections of modern infant and child skeletal remains with known age, ancestry, and cause of death are rare, although some collections of paediatric skulls do exist (Shapiro and Richtsmeier, 1997).

This scarcity of child skeletal collections directly impacts trauma research. Evaluating trauma based exclusively on morphological features, as is sometimes done in a "see-and-select" approach, offers limited insights into injury mechanisms. This thesis acknowledges the limitations of relying solely on fracture morphology for trauma analysis, as highlighted by the critique of the "see-and-select" approach. While the third empirical chapter focuses on analysing fracture patterns in immature porcine bone, it is important to emphasis e that this is only one part of a broader approach to understanding child bone trauma.

The other chapters of this thesis address the limitations of the "see-and-select" approach by investigating bone biomechanics and material properties. By combining the analysis of fracture patterns with an understanding of how bone responds to forces, this research aims to provide a more comprehensive understanding of trauma mechanisms in children. For example, the research on fracture patterns in porcine bone can be complemented by biomechanical analysis to determine the specific forces and loading conditions required to produce those patterns. This integrated approach can lead to more accurate interpretations of child bone trauma and contribute to the development of standardised models and methodologies for forensic analysis.

Furthermore, this research addresses the challenges posed by the scarcity of child skeletal collections (Lewis, 2007). By utilising an immature porcine model, this thesis provides valuable data on the biomechanics and fracture behaviour of immature bone, which can contribute to the development of more accurate methods for analysing child bone trauma, even in the absence of extensive human skeletal collections.

In summary, this thesis recognises the limitations of traditional approaches in forensic anthropology and aims to bridge the gap by integrating knowledge from biomechanics and material science. By combining the analysis of fracture patterns with an understanding of bone biomechanics, this research strives to provide a more comprehensive and scientifically rigorous approach to analysing child bone trauma.
Sources and related content

2.2.3 Biomechanics

Biomechanics is a discipline that requires a focus on the properties of biological materials, such as bone, and their use and application (IRMA, 2017). It is within biomechanics and other engineering fields that the opportunity arises to study and investigate the behaviour of materials such as bone within biomechanical systems, as well as their movements and actions according to the principles of mechanical engineering (Pierce and Bertocci, 2008). Biomechanics constitutes an area of research which uses elements of physics and chemistry to investigate the magnitude of load a bone may resist before failure (Wang, 2010). Currently, the documented and published literature regarding the load bearing capacity of bone dates back approximately three decades with a varied use of investigating gait of dinosaurs, sports injuries, and orthopaedic implants using bone grafts (Lundon, 2000; Blazevich, 2002; Day *et al.* 2002). With regards to the application of biomechanics in criminal investigations, a focus has included determining the point at which a material will fail and the conditions which allow the material to fail (Kieser, 2013). Since bone is a material, the emphasis on when, where and how a failure occurs remains the same. As inferred by Berryman *et al.* (2018):

"Fracture formation in bone, especially complex fracture patterns, may seem random; however, this is not the case. There are no random fractures in bone; each fracture doggedly obeys the laws of physics. When a fracture changes direction as it propagates across the cranial vault, or when it bifurcates to form a butterfly fracture, or when it ratchets leaving secondary fractures across tubular bone or completes the fracture leaving a breakaway spur, it does so for a reason. There is nothing random in the way a fracture forms' (p. 215)."

While Berryman et al. (2018) correctly highlight the non-random nature of fracture mechanics, governed by the laws of physics, the application of these principles to human populations introduces significant complexity. The inherent variability in bone properties, influenced by factors such as age, sex, health, and genetics, creates a system with stochastic elements. Therefore, while fracture patterns exhibit repeatability within certain parameters, no two fractures are identical. This thesis has acknowledged this variability and therefore looked to employ probabilistic reasoning to account for the 'randomness' or 'chaos' introduced by individual differences. This means that while we can analyse fracture patterns based on deterministic principles, we must interpret the results within a framework that recognises the range of possible outcomes. The analysis focuses on determining the most probable scenario, acknowledging that absolute certainty is often unattainable. To achieve this, a combination of robust theory, experimental testing, statistical analysis, and expert interpretation is crucial. Further, transparency in reporting findings, and a clear explanation of the inference used is essential to maintain scientific rigor. As will be discussed further, factors such as bone geometry, micromorphology, elasticity, and density vary considerably between individuals, influencing fracture behaviour (van der Meulen *et al*. 2001; Bertocci *et al.* 2017).

2.3 The need for experimental research

The aim of forensic science is to provide robust empirical evidence which will accurately and appropriately help the judiciary arm achieve justice for the victims of crime (USNRC, 2009). It is an aim which is increasingly achieved using technical and scientific evidence (Broeders, 2006). However, it is also due to this increase of current and new forms of technical and scientific evidence that the proper understanding and weighing of the evidence is becoming increasingly more important. It is pivotal for forensic practitioners who are required to be adept at critically analysing results, data, circumstantial information, legal propositions and cases as a whole (Houck and Siegel 2015). However, the various contexts and natures of each different forensic investigation also informs the difficulty in developing generalised protocols and methodologies that can be applied in the course of multiple and varying forensic investigations. Standard operating procedures are not possible for each specific investigation as the context and impinging variables change, and the interpretation of the applicable procedures to each new case is essential (Kafadar, 2015; Houck and Siegel 2015). It is instead in the development of an empirical knowledge base that best practices in evidence collection, analysis, and presentation are developed and refined (Saks and Koehler, 2008; Saks, 2010; Morgan *et al.* 2009; Leonetti 2024). However, the need for a robust evidence base does not stop here. It is through this knowledge that secondary level studies, who mimic the forensic context of specific cases, can be conducted, greatly providing to the individual elements associated with specific situations (Morgan and Bull 2007; Morgan *et al.* 2009).

In the context of paediatric bone trauma, much of the existing research relies on injury databases and case studies where injuries are substantiated through observational means, such as witness testimony (Love and Martinez, 2018). While valuable, these observational studies have limitations. For example, the differentiation between accidental and non-accidental fractures can be challenging as the fractures exclusively does not speak to malice or intent (Bilo *et al*. 2010). Furthermore, there is a lack of quantitative data on bone development, strength, and fracture mechanisms in children (Diaz and Petersen, 2014). This limits the ability to verify diagnoses and understand the specific conditions under which different fracture patterns occur. Building upon the identified limitations of current forensic practices, particularly the need for quantitative data and standardised methodologies as highlighted in sections 2.2.1, 2.2.2, and 2.2.3, this thesis has employed experimental research to address the gaps in our understanding of paediatric skeletal injuries. An objective of forensic science (e.g., forensic pathology) is to provide robust empirical evidence, a goal increasingly achieved through technical and scientific evidence (Broeders, 2006; USNRC, 2009). However, as Houck and Siegel (2015) point out, the variability of forensic investigations makes generalised protocols difficult, emphasising the need for a strong empirical knowledge base. Therefore, experimental research is crucial for generating quantitative data on bone strength, fracture mechanisms, and the relationship between loading conditions and fracture morphology. By conducting mechanical tests on immature porcine bone, this research generates data that can validate and refine observations made in previous studies, contributing to a more nuanced understanding of child bone trauma. This method also allows for the controlled environment necessary to create data that can be statistically analysed and reproduced.

This thesis addresses the existing limitations by employing experimental research to investigate immature bone trauma. While the third empirical chapter involves comparing fracture patterns in porcine bone to those observed in other studies, it is important to emphasise that this is also done within the context of the other experimental studies within this thesis which are not observational. By considering both experimental and observational data in conjunction, a more holistic and nuanced understanding of child bone trauma can be achieved. By conducting mechanical tests on immature porcine bone, this research generates quantitative data on bone strength, fracture mechanisms, and the relationship between loading conditions and fracture morphology. These findings can contribute to validating or refining the observations made in previous studies, leading to a more nuanced understanding of child bone trauma.

In conclusion, this thesis highlights the crucial role of experimental research in enhancing our understanding of paediatric bone trauma. By generating empirical data and providing a more controlled environment for investigating fracture patterns, this research has contributed to a more robust evidence base for forensic analysis and ultimately improve the accuracy and reliability of biomechanical research involving immature bone in the context of child abuse.

2.4 Material and biomechanical properties of bone

Having established the crucial role of experimental research in addressing the knowledge gaps surrounding paediatric bone trauma, it is now essential to delve into the fundamental material and biomechanical properties of bone itself. A thorough understanding of these properties is paramount for interpreting the results of experimental investigations and for developing accurate models of bone behaviour. Therefore, this sub-chapter provides a comprehensive overview of bone composition, structure, and biomechanics, laying the groundwork for the analysis and discussion of experimental findings presented in subsequent chapters.

Bone is a hard connective tissue which makes up all of the skeletal systems in vertebrate animals (Wang *et al.* 2010). Bone is a dynamic structure composed of both living tissues, such as bone cells, fat cells, and blood vessels (Currey, 2002). Bone is also composed of non-living materials, such as water and minerals (Wang *et al.* 2010). Whole bones are multi-purpose structures which provides support, shape, and a framework for the body (Currey, 2002). Depending on location and function, bones may act as surfaces for the attachment of muscles, levers which enable movement, and as protection for vital organs (Frazer, 1946). In addition to these structural and mechanical functions, bone also contributes to the body’s physiology (Wang, 2010). This includes the storage of calcium, a mineral essential for the activity of nerve and muscle cells. Even the interior of bone serves a function (Wang, 2010). Specifically, the soft inner core of bone contains bone marrow, which is the site where red blood cells, certain white blood cells, and blood platelets are formed (Wang, 2010).

However, ‘bone’ is also multi-purpose term for bone material (e.g., cortical and trabecular) and the structure bone (e.g., long bones; Martin and Burr, 1989; Martin *et al.* 1998; Muscolino, 2006). While references to the word bone may generally be attributed to certain shapes and functions such as the long bones, the material bone is comprised of a complex and hierarchical structure which in unison provides bone with both brittle and elastic properties (Rho *et al.* 1998). Specifically, bone material is a relatively hard and lightweight composite material comprised of the type-I protein collagen, various non-collagenous proteins, lipids, water, and calcium phosphate in the chemical arrangement termed calcium hydroxyapatite (Delmas *et al.* 1984; Boskey 2007). Bone material consists of an arrangement of material structures which on different architectural levels work in concert to perform mechanical, biological, and chemical functions. This includes the provision of structural support, protection and storage of healing cells, and mineral ion homeostasis (Weiner and Traub, 1992; Landis, 1995; Rho *et al.* 1998).

Bone is a material with a hierarchical structure that gives it both strength and flexibility. Its composition of collagen, minerals, and water creates a complex interplay that influences how bone responds to external forces. Understanding these properties is crucial for interpreting the results of mechanical tests. For this thesis, mechanical testing techniques (which in this context also constitutes material testing techniques) such as bending tests to quantify bone's response to stress and strain. These tests, grounded in material science principles, provide valuable insights into bone's behaviour under different loading conditions. By focusing on the macroscopic level, which is often the first point of observation in forensic and clinical assessments, our findings can be directly applied to real-world scenarios. Through this material science approach, this thesis aimed to create a bridge from which future studies on the microscopic observations of immature bone can be connected to the macroscopic observations made in this thesis. This ultimately will have contributed to a more comprehensive understanding of bone behaviour with implications for moving the research forward in the biomechanical, medicolegal, and anthropological fields.

2.4.1 The structure and shape of long bones

At the macrostructural level, bone material is separated into cortical and trabecular bone (Currey, 2002; Figure 2.2). Generally, cortical bone makes up the shell while trabecular bone makes up the interior (Currey, 2002). Although both cortical and trabecular bone are easily distinguished by their degree of porosity or density, the true differentiation comes from histological evaluation of the tissue's microstructure. However, in compact coarse-trabecular bone the structure is unclear, and it is difficult to distinguish between the two types of bone with any clarity (Enlow 1963;1968). Compact coarse-trabecular is produced by cortical bone wrapping around the struts of cancellous bone, without replacement or remodelling of the old trabecular bone (Wang, 2010). The microstructure produced by the compaction of trabecular bone is composed of irregular, sinuous convolutions of lamellae - a thin layer of tissue (Wang, 2010). In contrast, the microstructure of cortical bone is composed of regular, cylindrically shaped lamellae. Therefore, reliable differentiation can only be achieved by microscopic methods (Martin *et al.* 1998).

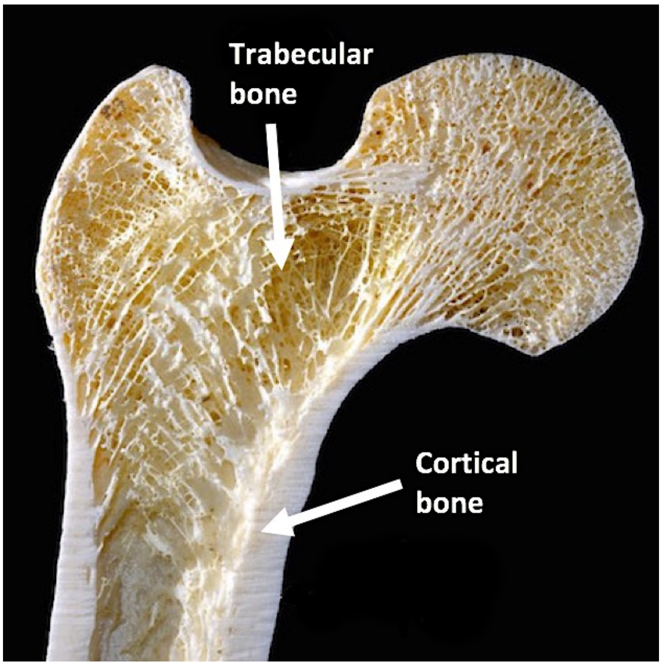

Figure 2.2: Cortical and trabecular bone (human femur; Yale, 2019).

In consideration of this thesis' focus on long bones, it is important to emphasise that long bones predominantly serve as load bearers, levers, and enablers of mobility in the skeletal system. As such long bones are often involved in acts of motion and exertion, transferring and absorbing forces which may arise due to both natural and unnatural exertions. As the name suggests long bones are defined by their specific shape which in turn is categorized by the three parts, the diaphysis, the epiphysis, and metaphysis. A representation of a long bone, specifically the femur can be observed in figure 2.3.

The diaphysis (i.e. shaft) is the tubular midsection that runs between the proximal and distal ends of the long bone. The shaft is primarily composed of an outer shell of cortical bone and an inner medullar cavity of bone marrow. Besides load bearing functions, the diaphysis also acts as attachment point for muscles (Drake, 2010; Bilo *et al.* 2010). The wider sections at each end of the long bone are referred to as the epiphysis. The outer shell is completely composed of cortical bone whilst the inside consists of trabecular bone. The epiphysis functions as a producer of red blood cells as well as a distributor of pressure across the joints (Drake, 2010; Bilo *et al.* 2010). The area between the diaphysis and the epiphysis. The metaphysis transfers load and stress from the joints at the epiphyses into the longer and stronger diaphysis. It is also the area which contain the growth plate, a layer of hyaline (transparent) cartilage which acts as the site for longitudinal bone growth during childhood (Drake, 2010; Bilo *et al.* 2010).

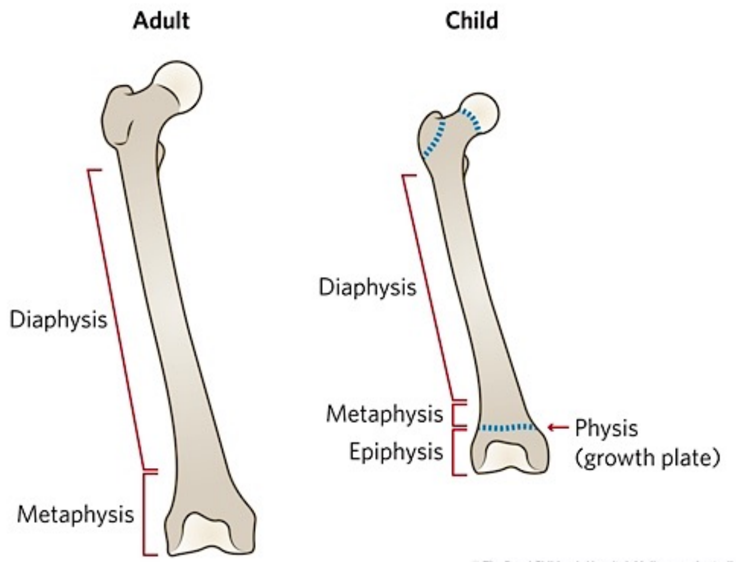

Figure 2.3: Diagram of adult and paediatric femur (RCH, 2019).

2.4.2 Bone development

Bone development begins in the embryo with the formation of cartilage which in turn is gradually replaced with bone in a process referred to as endochondral ossification (Boskey and Coleman, 2010; Figure 2.4). During endochondral ossification, developmental signals within the growth plates, cartilaginous tissue found at the various sites in bone (e.g. the distal and proximal ends of long bones) will regulate the variation and maturation of the resident cells (e.g. chondrocytes; Zuscik *et al.* 2008; Figure 2.4).

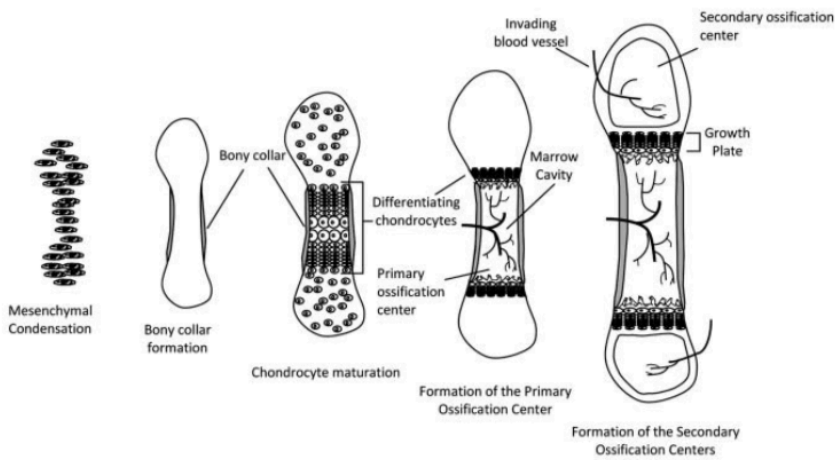

Figure 2.4: Endochondral ossification of long bones (Boskey and Coleman, 2010).

The chondrocytes will in turn proliferate, deposit a matrix and become surrounded by a mineralized matrix, and subsequently undergo apoptosis (programmed cell death). This is followed by the blood vessels invading the tissue forming a marrow cavity, and the calcified cartilage being replaced by bone, hence why bone grows in length at the ends (Boskey and Coleman, 2010). When bone grows in width (appositional bone growth) it does so exclusively through what is referred to as periosteal expansion; that is, new bone forming on an already existing surface. In most species, the growth plates close at puberty, essentially stopping the growth of certain bones (e.g. long bones) completely (Boskey and Coleman, 2010; Figure 2.5). However, appositional bone growth continues throughout life, which in turn explains the changes bone may exhibit in shape and mechanical properties if subjected to physical resistances or certain conditions such as Rickets (Rauch, 2005).

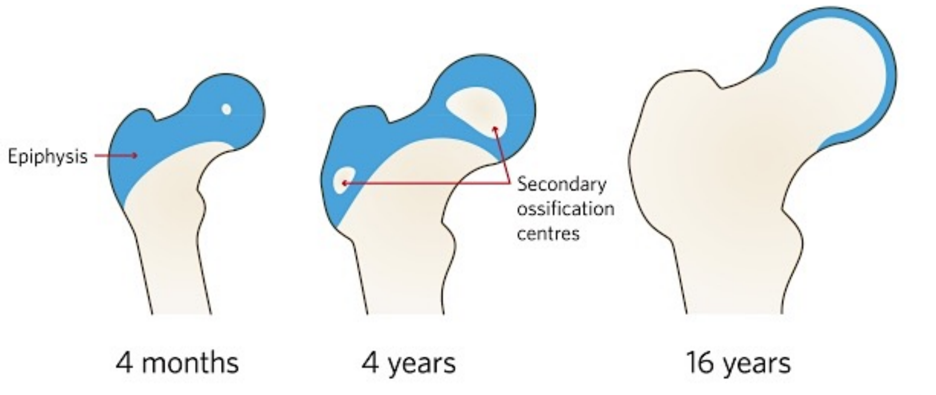

Figure 2.5: The proximal femoral epiphyseal secondary ossification centre at approximately 4 months, 4 years, and 16 years. The chronological replacement of cartilaginous tissue (blue) with bone (RCH, 2019).

During early growth, the infant skeleton may be considered quite pliable. This suggests that a significant force is required to generate skeletal trauma in children (Humphries, 2011). For example, during the development of long bones, the diaphysis is capped by cartilaginous growth plates and a bony epiphysis which when subjected to physical forces can generate a fracture (Ross and Juarez, 2014; Bilo *et al.* 2010).

2.4.3 The biomechanics of bones

The relationship between the mechanical behaviour of the entirety of an object and its material properties is complex. Despite several technical advancements in experimental techniques, there are still considerable gaps in our knowledge of the mechanical properties of certain biological materials, including bone (Sharir *et al.* 2008). Particularly, the mechanical testing of paediatric bone is difficult to conduct due to the availability of paediatric tissue (H.M Government, 2004). Parents or next of kin rarely choose to donate their children’s bodies for medical research. Various attempts have been made to address these challenges, particularly in the development of methodological frameworks and alternative models (Great Britain Parliament, 2001; H.M Government, 2004). The aim of this thesis is the investigation of the mechanical properties of immature porcine bone. Therefore, a brief background on the basic science underlying the mechanical behaviour of bone is required.

2.4.4 A biomechanical view on bone behaviour

To resist fracture, a bone must be stiff enough to withstand the applied forces, but also flexible enough to allow the energy to be absorbed during impact (Currey, 2001). Optimal fracture resistance is therefore best provided when there is a balance between stiffness and elasticity. The biomechanical properties that determine the behaviour of bone can be divided into intrinsic biomechanical properties, which reflect the behaviour of bone material, and the extrinsic mechanical properties, which pertain to the mechanical behaviour of whole bones (Table 2.2; Turner, 2001). The intrinsic properties of bone are exclusively bone tissue and independent of the size, shape, and mass of the whole bone (Bouxsein, 2005). The extrinsic properties of bone are also dependent on the intrinsic properties (it makes up the whole bone) but also the geometry of the whole bone (Bouxsein, 2005). Since bone behaves differently whether affected intrinsically or extrinsically a potential load or force may affect the bone mechanically different. This is an important distinction when testing bone as a material but also as a whole bone (which this thesis will do; See Chapter 3). To clarify the differences between intrinsic and extrinsic bone properties a more detailed section will follow (Figure 2.6).

Table 2:2: Summary of biomechanical properties and the intrinsic and extrinsic measures in bone (Forestier-Zhang and Bishop, 2015).

Biomechanical property	Definition	Intrinsic(material) measure	Extrinsic (whole bone) measure
Strength	The amount of force required to cause a fracture	Strength (e.g. stress)	Strength (e.g. stress)
Stiffness	The resistance to elastic deformation. The rigidity of an object.	Intrinsic stiffness	Extrinsic stiffness
Toughness	The amount of energy an object can absorb before breaking.	Energy absorbed	Energy absorbed
Ductility	The total deformation an object undergoes before fracturing.	Strain	Strain

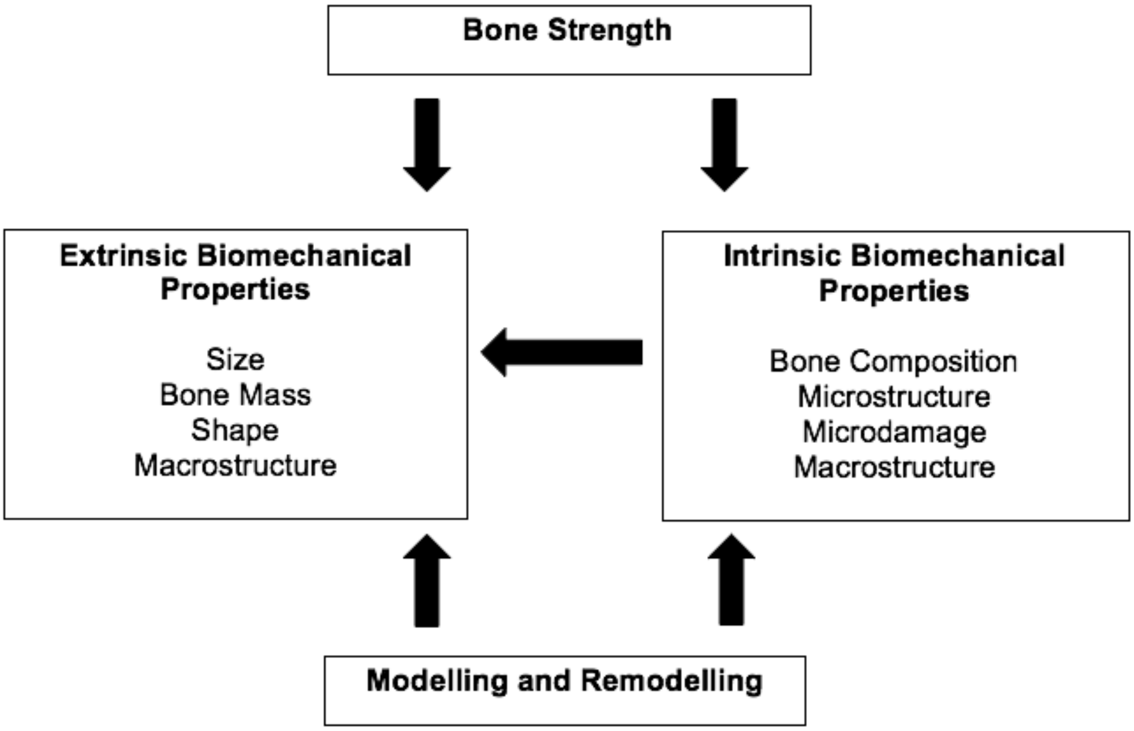

Figure 2.6: Determinants of the mechanical behaviour of bone (reformatted from Forestier-Zhang and Bishop, 2015).

2.4.5 Intrinsic biomechanical properties

The intrinsic biomechanical properties of bone material can be quantified through the biomechanical testing of samples of a standardised geometry (Forestier-Zhang and Bishop, 2015). If a sample is subjected to a force, the results would produce a relationship between force and deformation (e.g., the samples change in size and shape due to the applied force; Forestier-Zhang and Bishop, 2015). This relationship is generally represented by the stress-strain curve which is a normalised version of the force-deformation curve and a material property (Figure 2.7). Specifically, stress constitutes the load applied divided by the cross-sectional area of the sample. The deformation of a sample, namely the extent the sample's shape changes due to the applied load, is divided by its original length to calculate strain.

Intrinsic stiffness

Stiffness is the resistance of an elastic body to deform (Forestier-Zhang and Bishop, 2015). Elastic deformation is a temporary change in shape or size that reverses back to its original shape and size once the load has been removed. The opposite property is flexibility. An example is glass which as a stiff material fractures as soon as elastic deformation occurs. In contrast, rubber deforms elastically (but not indefinitely) when loaded with the same force(s) (Sharir *et al.* 2008).]

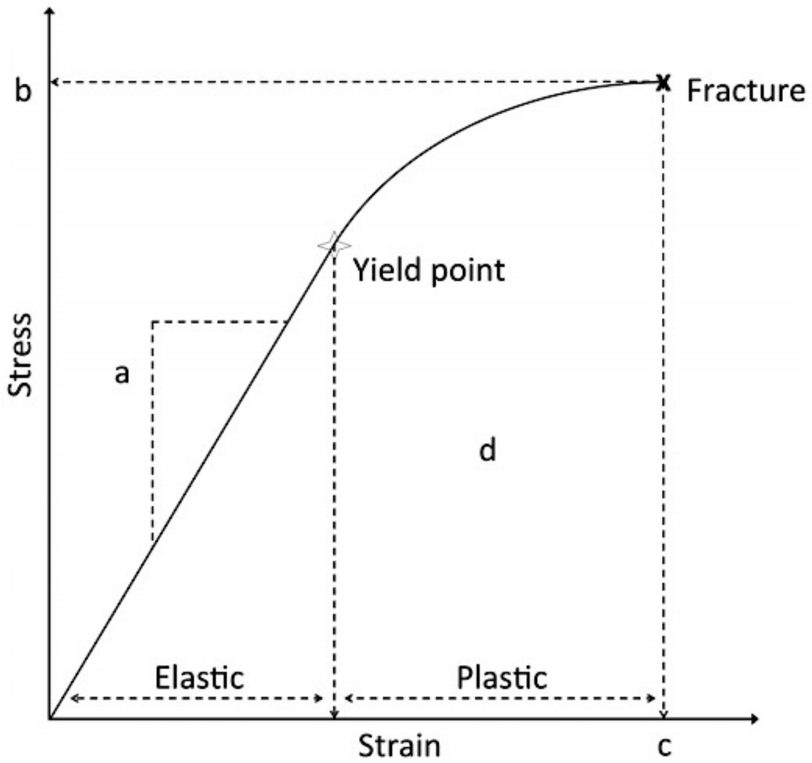

Figure 2.7: Stress-strain curve representative of the mechanical behaviour of bone (Forestier-Zhang and Bishop, 2015).

Young's modulus of elasticity is a measure of intrinsic stiffness and is defined as the gradient of the stress-strain curve in the linear region (stress divided by strain) (Area a; Figure 2.7). It correlates highly with mineralisation of the collagen matrix (Currey, 2001; Bouxsein, 2005). The hydroxyapatite crystals situated in between collagen fibrils of the organic matrix resist elastic deformation, thus increasing Young's modulus of elasticity (Bouxsein, 2003).

Intrinsic strength (stress)

Ultimate stress is the maximum force a material can withstand before fracturing (Turner, 2006). It denotes the intrinsic (material) strength of bone where 'strength' is defined mechanically (Forestier-Zhang and Bishop, 2015). Intrinsic stress is calculated as the height of the stress-strain curve at the point of failure (Point b; Figure 2.7). The stress or strength of a material is not uniformly distributed in a specimen and is dependent of the direction of the load. As such the distribution of stress will differ depending on whether the sample is subjected to bending or compression loads.

Intrinsic ductility (strain)

Ductility refers to a material's ability to deform in order to accommodate a loading force and prevent a fracture (Forestier-Zhang and Bishop, 2015). Intrinsic ductility is characterised by strain which is the total deformation a material undergoes before failure. Strain is calculated as the total length of the stress-strain curve along the x-axis (Point c; Figure 2.7). The intrinsic ductility of bone is determined by both elastic (reversible) and plastic (irreversible) deformation. When the amount of stress applied on a bone material reaches a certain point, referred to as the yield point, bone can no longer deform elastically. On the stress-strain curve, the yield point can be observed where the line begins to curve. Beyond the yield point, energy from loading is absorbed permanently irrespective of the removal of the force. The effect is an irreversible deformation referred to as plastic deformation or post-yield strain (Morgan and Bouxsein, 2005).

Intrinsic toughness (energy absorbed)

Toughness is characterised by the amount of energy a material can absorb before failure. Intrinsic toughness of bone, also referred to as 'modulus of toughness' or 'energy absorbed' is calculated as the accumulated area under the stress-stress curve (Area d in Figure 2.7; Turner, 2002). Intrinsic toughness is associated with both plasticity and ductility. Physiological micro cracking (e.g. micro fractures) and micro damage may occur in plastic (irreversible) deformation to absorb and dissipate energy (Bouxsein, 2003).

2.4.6 Extrinsic biomechanical properties

While this thesis primarily focuses on the material properties of bone, it is important to consider how those properties influence the extrinsic behaviour of the whole bone, especially in the context of fracture analysis. Whole bone biomechanical testing, using methods like three-point bending, compression, and torsion tests (Viguet-Carrin *et al*. 2006), provides insights into how bones respond to different loading conditions.

Similar to material testing, whole bone tests generate stress-strain curves (Turner, 2002; Morgan and Bouxsein, 2005). However, interpreting these curves for whole bones requires considering factors beyond just the material itself, such as bone geometry and loading conditions.

Extrinsic stiffness

A stiffer bone, represented by a steeper slope in the elastic region of the stress-strain curve (Area a; Figure 2.7), can resist deformation under load (Forestier-Zhang and Bishop, 2015). However, excessive stiffness can make the bone more susceptible to fracture when subjected to high forces.

Extrinsic strength

The ultimate load a bone can withstand before fracturing (Point b; Figure 2.6) is a critical factor in fracture analysis (Forestier-Zhang and Bishop, 2015). This is influenced by both the material strength and the bone's geometry.

Energy absorption

The area under the stress-strain curve (Area c; Figure 2.7) represents the energy absorbed by the bone before fracture (Turner, 2002). Bones that can absorb more energy are generally more resistant to fracture.

Extrinsic ductility

The maximum deformation before fracture (Point c; Figure 2.7) reflects the bone's ductility (Forestier-Zhang and Bishop, 2015). More ductile bones can undergo greater deformation before breaking, which can be important in impact scenarios.

These biomechanical factors, in conjunction with the intrinsic material properties, play a crucial role in determining how a bone will fracture under different loading conditions. Analysing fracture patterns requires considering both the material properties and the whole bone mechanics to understand the interplay of forces and deformation leading to fracture.

This understanding of whole bone biomechanics will inform the analysis of fracture characteristics in the third experiment of this thesis, where immature porcine bones are subjected to three-point bending, compression, and torsion tests. By considering these biomechanical factors, a deeper understanding can be gained of how bone fractures occur in real-world scenarios, which has implications for forensic and clinical applications. Having established a foundation in the general biomechanical principles of bone, the discussion now turns specifically to the unique characteristics of paediatric bone and the implications of these differences.

2.4.7 Immature vs. mature bone

Bone has been noted to exhibit distinct mechanical properties during its developmental stages. Understanding these differences is crucial for comprehending bone health, healing, and response to mechanical loads, which can vary significantly based on the biological age of the tissue. Furthermore, these distinctions are critical within the context of this thesis, which has chosen to test immature porcine bone instead of mature porcine bone.

In general, immature bone typically demonstrates lower mechanical strength compared to mature bone and this effect has been observed across multiple mechanical tests (e.g., Three-point bending, compression, torsion; Szabo and

Rimnac, 2022). Studies have indicated that the modulus of elasticity, bending strength, and ultimate tensile stress are notably diminished in immature bone (Ambrose *et al.* 2018; López Valdés *et al.* 2024). For instance, Ambrose *et al.* (2018) reported elastic moduli ranging from approximately 0.45 to 1.98 GPa in infants, contrasting sharply with the values reported for older children, which range from approximately 10.3 to 21.6 GPa (Berteau *et al.* 2014). Additional studies by Hirsch and Evans (1965) and Vinz (1969; 1970), among them testing samples from newborn to 85 years of age, have provided evidence suggesting an increase in ultimate tensile stress and elastic modulus throughout skeletal development including a particular increase in ultimate tensile stress in the samples aged newborn to 13 years of age. Currey and Butler (1975) performed 3-point bending tests on femoral mid-shaft specimens harvested from 18 subjects in the age range of 2 to 48 years. The results indicated that the bending strength of children's cortical bones under the age of five was in the range of 150 to 180 MPa compared to the adult specimens at the higher value of 180 to 210 MPa. Similarly, Öhman *et al.* (2011 found that under compression, adult bones have higher values in elastic modulus and compressive yield stress.

This lower performance has been attributed to the structural characteristics of immature bone, which possesses a higher proportion of collagen and immature cross-links (Berteau *et al.* 2015). Specifically, immature bone exhibits a higher immature/mature cross-link ratio that contributes to its ability to plastically deform under stress, as evidenced in comparative studies (Lefèvre *et al.* 2020; Berteau *et al.* 2015). This plastic behaviour indicates an adaptive mechanism that enables young bones to withstand various mechanical loads during formative years (Currey and Butler, 1975). These differences are critical to consider when utilising immature bone instead of mature bone, as the degree of plastic deformation will influence the resultant fracture (Berteau *et al.* 2015; Klein-Nulend *et al*. 2012).

As bone matures, significant changes occur within its matrix. The conversion of immature collagen cross-links to more stable, mature forms is critical in increasing bone toughness and reducing susceptibility to fractures (Wang *et al.* 2002). Mature bone, characterised by a more organised collagen matrix and higher mineral density, displays greater stiffness because of these alterations (Ng *et al.* 2015). Certain enzymatic cross-links have been observed to enhance the material integrity of mature bone, contributing to its enhanced mechanical properties (Barth *et al*. 2011).

Notably, plastic behaviour in immature bones is linked with their propensity for greenstick fractures, common in paediatric populations, where the bone bends rather than breaks (Berteau *et al.* (2015). This is a phenomenon that is not observed to the same extent in mature bone. This difference underlines the necessity for paediatric medical interventions that acknowledge the unique biomechanical characteristics of immature bone (Lefèvre *et al.* 2020). Moreover, it highlights the importance of understanding these variations when interpreting fracture patterns in the context of potential child abuse, where accurate assessment is crucial.

In conclusion, the mechanical properties of bone evolve significantly with maturity. Immature bone presents lower elasticity and strength due to its distinct collagen cross-link profile and mineralisation status, resulting in increased plasticity under mechanical stress (Lefèvre *et al.* 2020; Berteau *et al.* 2015). In contrast, mature bone has been observed to exhibit enhanced toughness linked to a greater concentration of stable collagen cross-links and mineral content. This is indicated by its adapted ability to endure higher mechanical loads and prevent fractures (Wang *et al.* 2002). Understanding these properties and relationships is integral for this thesis as it highlights why immature porcine bone and not adult porcine is potentially a better alternative and warranting mechanical testing. The lack of specific data on immature porcine bone limits our ability to fully characterise the mechanical behaviour of immature porcine bone. Therefore, this thesis aims to bridge this gap by conducting a systematic testing of the immature porcine bone under controlled experimental conditions. By generating empirical data specific to our model system, we seek to establish a robust foundation for understanding the biomechanical behaviour of immature porcine bone and for interpreting the fracture patterns generated in our experiments. With an understanding of the mechanical properties of bone, and how those properties are affected by strain and plastic deformation, it is now useful to examine how those properties influence long bone fractures

2.5 Long bone fractures

Diaphyseal fractures have been observed to occur four times more frequently than metaphyseal fractures with the humerus, femur, and tibia being most frequent anatomical sites for such injuries (King *et al.* 1988). Non-accidental long bone fractures have been noted to make up approximately 28 to 53% of all fractures in children under the age of 1 year (Loder and Bookout, 1991; Kowal-Vern *et al.* 1992; Carty, 1993). However, the clinical diagnosis of non-accidental long bone fractures varies significantly and a clue to the difficulty in accurately diagnosing long bone fractures (Loder and Bookout, 1991; Kowal-Vern *et al.* 1992; Carty, 1993).

2.5.1 Fracture morphology

While the general fracture morphologies described below occur across all ages, the unique mechanical behaviour of immature bone necessitates a specific terminology and consideration of distinct characteristics when describing paediatric fractures. This section will present a brief overview of common fracture patterns observed in long bones, with a focus on their manifestation in children. It will be followed by a review of published research specifically on humeral and femoral fractures, one of the focuses of this thesis, within the context of paediatric injury.

Bowing (bending) fractures

Bending fractures are incomplete fractures which predominantly occur in long bones (Figure 2.8; Bilo *et al.* 2010). The mechanism of injury may be observed in scenarios in which the bone bends past its limit of plastic deformation (which can be over 45º) and despite issuing the ‘crackling’ sound of a fracture, reverts back to its original position (Noonan and Price, 1998). Thus, bending or ‘bowing’ fractures may occur without evidence of extreme angular deformity. Radiographically, bending fractures may be difficult to identify with the only detectable trace being the somewhat unnatural shape of the bone (Rydholm and Nilsson, 1979). The change may be so slight that bowing fracture have been referenced as not actually constituting a fracture (Musters and Colaris, 2017). Further reports have observed bowing fractures to display no visible haemorrhaging, periosteal new bone formation, or remodelling (Musters and Colaris, 2017).

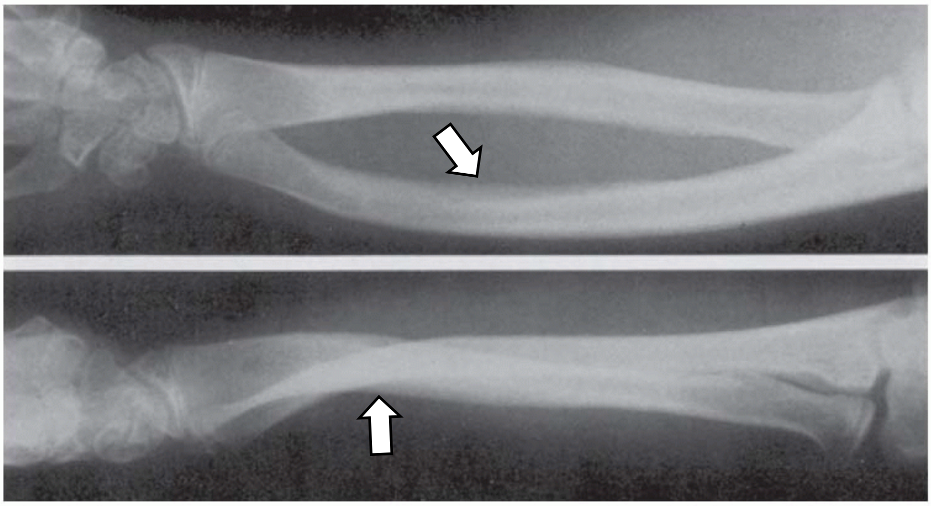

Figure 2.8: Traumatic bowing of the ulna in a child (Rang, 2005).

Greenstick fractures

If a bone is angulated beyond the limit of spontaneous recovery, a greenstick fracture may occur (Figure 2.9). A greenstick fracture is an incomplete fracture which occurs on the tension side of the bone whilst the cortex and periosteum at the compression side remains intact (Bilo *et al.* 2010). Symptoms beside the fracture include the plastic deformation of the compression side. As is common in most fractures, the moment of failure is exhibited by considerable displacement followed by an elastic recoil of the soft tissues which somewhat recalibrates the position (Ogden, 2000; Rang, 2005; Jones, 2009).

Figure 2.9: Greenstick fracture in a child (Rang, 2005).

Transverse fractures

A transverse fracture occurs more or less perpendicular to the long axis of the bone (Bilo *et al.* 2010) (Figure 2.10). Transverse fractures are often the effect of failure under tensile and bending loads (Jones, 2009; Ogden). As is the effect under bending forces, one portion of the bone is subjected to compression forces while the other is subjected to tensile forces (Turner and Burr, 1993; Levine, 2002). Transverse fractures are often observed in scenarios involving direct impacts such as through an intentional blow or fall on a protruding surface (e.g. stair or table edge; Pierce *et al.* 2004).

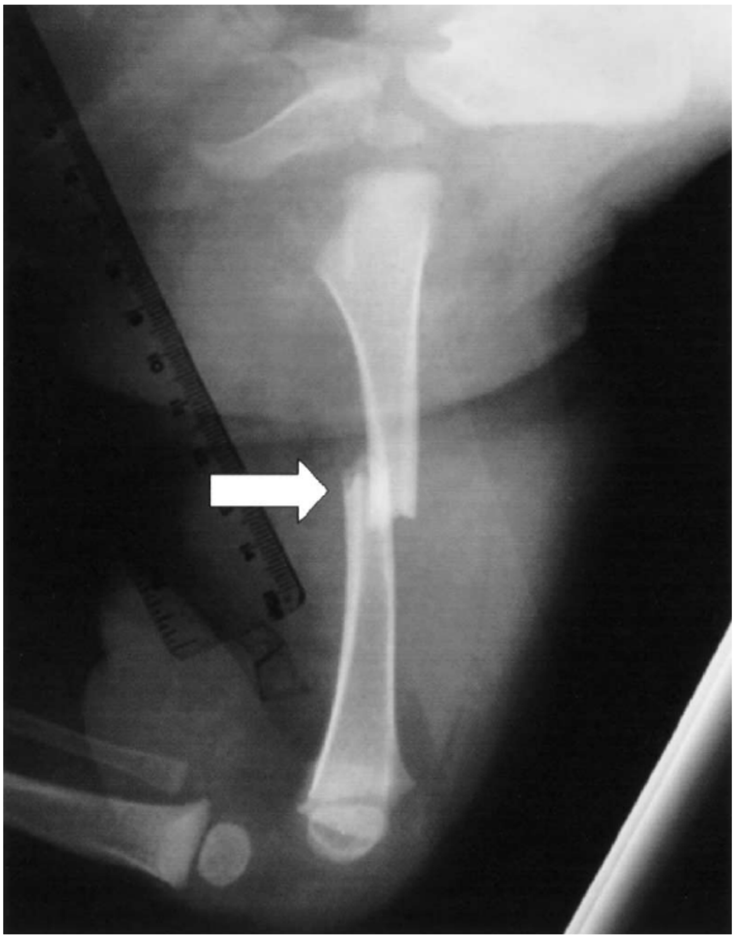

Figure 2.10: Transverse fracture (Pierce et al. 2004).

Oblique fractures

Oblique fractures propagate through axial overload at approximately a 30º to 45º angle of the longitudinal axis of the bone (Rang*,* 2005) (Figure 2.11). The fracture is typically a result of a combined load comprising of a longitudinal compression force with the addition of rotation. In real scenarios, oblique fractures may be observed as the result of falls from heights, followed by a twist as the knee impacts the ground surface (Pierce *et al.* 2004). Long oblique fractures are predominantly the product of torsional loads whilst shorter oblique fractures are the result of bending or compressional loads (Rogers, 1992; Levine, 2002).

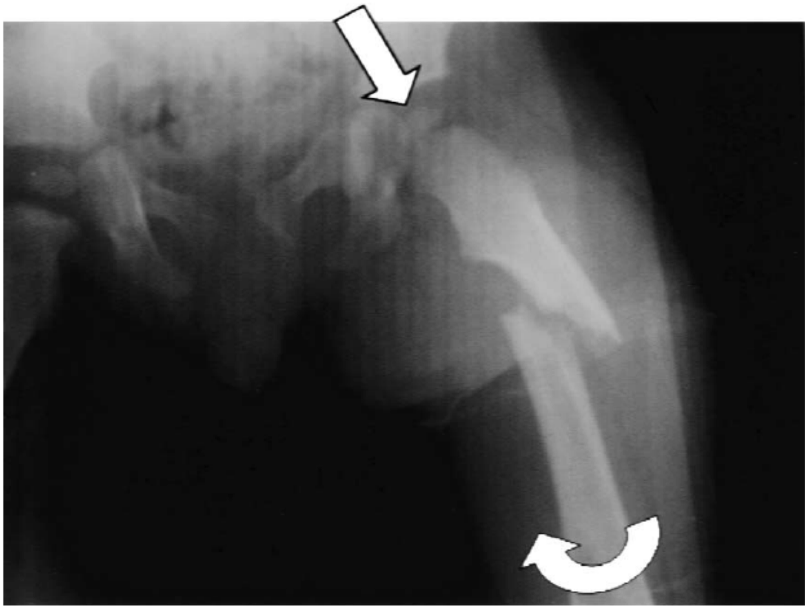

Figure 2.11: Oblique fracture (Pierce et al. 2004).

Spiral fractures

A spiral fracture is characterised by the fracture line circling around the central axis of the bone (Figure 2.12). Mechanically, spiral fractures are the result of a pure torque moment applied along the longitudinal axis of a bone (Pierce *et al*. 2004). The torsional forces places the bone under shear and tension stresses and its ability to withstand the aforementioned stresses is the determinant for whether a fracture occurs (Bilo *et al.* 2010).

Figure 2.12: Spiral fracture (Pierce et al. 2004).

Torsion generates a state of pure shear between parallel transverse planes. Simultaneously, tensile and compressive stresses are present, reaching maximum at a 45° angle to the longitudinal axis (Turner and Burr, 1993; Pierce and Bertocci, 2008). Since bone generally fails in tension rather than compression in such situations, spiral fractures have come to be strongly associated with torsional forces (Pathria, 2002). However, as observed in the experimental research by Kress *et al.* (1995), the prevalence of spiral fractures as a result of torsional loads has not been as substantial when mechanically testing immature bone (Kress *et al.* 1995; Pierce *et al.* 2000).

2.5.2 A review of research on humeral fractures

It has been suggested by several authors that humeral fractures are most frequently observed in abused children (King *et al.* 1988; Strait *et al.* 1995). In children less than 15 months of age the reported percentage of abusive humeral fractures ranges from 67% to 100% (Worlock *et al.* 1986; Thomas *et al.* 1991; Leventhal *et al.* 1993). The most frequent area of humeral injury is the diaphyseal and metaphyseal regions (King *et al.* 1988; Strait *et al.* 1995). Humeral transverse fractures have been observed to be caused by direct-impact force whilst spiral and oblique fracture have been reported in cases involving mechanisms of torque and twisting (Bilo *et al.* 2010). In a report aimed at investigating the specificity of humeral fractures in abuse, Williams and Hardcastle (2005) concluded that while humeral fractures cannot be seen as pathognomonic for child abuse the discovery of humeral fractures in young children should always warrant closer inspection of the event history and previous information. Based on the studies included within the report, Williams and Hardcastle (2005) concluded that there is no golden standard in which to diagnose and define abusive humeral fractures. Despite the lack of an exact method of measurement, the reported research does indicate that there is a high degree of humeral fractures in cases of child abuse. Specifically, spiral and oblique fractures in children 3 years of age and younger were observed to have been the result of intentional violence (Strait *et al.* 1995).

Fractures of the humerus may be sustained in the event of birth trauma, direct or indirect applications of force, or as the result of disease (Bilo *et al.* 2010). Humeral diaphyseal fractures have been observed during breech births or cases where the child has fallen on his or hers extended arm (Reed *et al.* 1994; Bilo *et al.* 2010). Direct impact forces to the diaphysis have been observed to result in transverse fractures which in mobile children have been reported in events of traffic accidents, sports, and play (Landin, 1983; Samardzic *et al.* 1990; Dalldorf and Bryan, 1994).

Humeral fractures to the distal end above the elbow joint (condylar region) have been attributed to both accidents and abuse (Strait *et al.* 1995). Emery *et al.* (2016) reported that specifically three most common elbow fractures in the paediatric orthopaedic literature to be supracondylar (50-70%), lateral condylar (17-34%; Figure 2.13), and medial epicondylar fractures (10%).

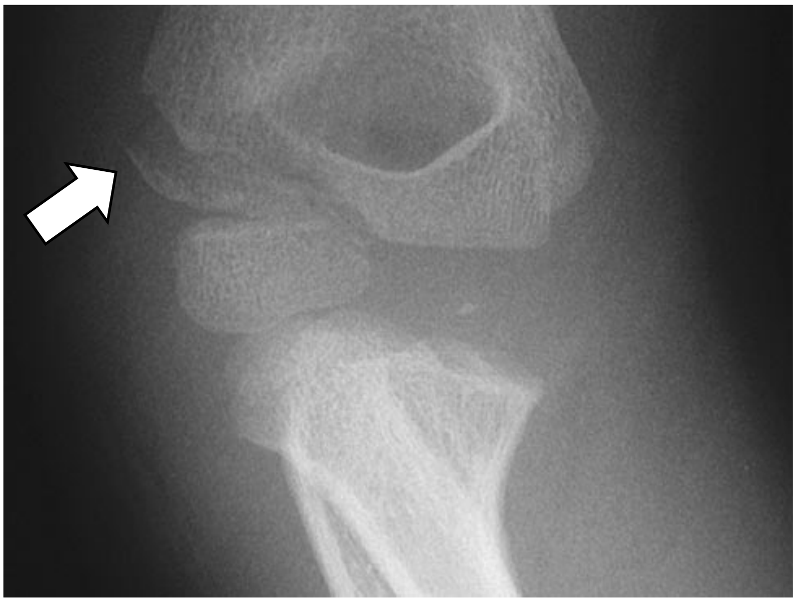

Figure 2.13: Lateral condyle fracture of the right humerus (Shaath et al. 2019).

In accidents, the anamnesis usually indicates the child having fallen on his or her extended arm (e.g. hand in dorsiflexion and elbow in hyperextension) or directly on to the bent elbow. In such situations, the mechanism of injury has been reported to be axial compression (Chacha, 1970; Fowles and Kassab, 1980). In data derived from Strait *et al.* (1995), 20% of the supracondylar fractures in children 15 months or younger of age the trauma were found to be abusive. Because the distal humerus is primarily cartilage at early age, the radiographic representation is often subtle (Broder, 2011; Figure 2.14). Furthermore, Bilo *et al.* (2010) reported that if birth trauma can be excluded then fractures of the distal epiphysis in children are almost exclusively the result of child abuse.

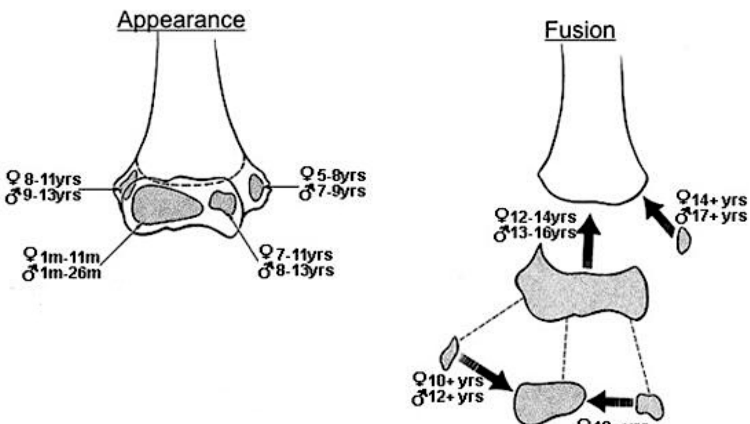

Figure 2.14: Chronological depiction of the fusion of the distal humeral condyles (Shaath et al. 2019).

2.5.3 A review of research on femoral fractures

Femoral shaft fractures may only make up between 28 and 45% of all isolated long bone fractures (King *et al.* 1988; Loder and Bookout, 1991; Carty, 1993), but the risk that a femoral fracture is the result of abuse has been reported to be as high as 92% (Rex and Kay, 2000). Detecting whether a femoral diaphyseal fracture is abusive is encumbered by the fact that similar injuries have been observed to be sustained in children who fall while learning to walk (Thomas *et al.* 1991). Controversially, Schwend *et al.* (2000) took it a step further, stating that femoral fractures are rarely a symptom of child abuse, which is contrary to the mainstream opinion that femoral fractures are highly suggestive of abuse (Carty, 1993; Thomas *et al.* 1991). In Schwend *et al.* (2000), isolated femoral diaphyseal fractures in 139 children under the age of 4 years were studied, and it was concluded that 91% of the children were likely to have suffered the fracture as a result of an accident. It is a figure which is significantly higher than any other published study. However, Schwend *et al.* (2000) also chose to include motor vehicle accidents and only femoral shaft fractures in the study which would inherently tilt the result towards fractures that are accidental in nature. Furthermore, the lack of follow-ups on the final outcome of the reported injuries means that the true statistic is not necessarily reflected.

There has been some contention as to whether spiral fractures of the femoral diaphysis are specific for child abuse. As reiterated by several authors, the specificity of a spiral fracture can never be attributed to child abuse solely based on the fracture type alone (Leventhal *et al.* 1993; Scherl *et al.* 2000). Instead, what can be concluded is the mechanism of injury which in the context of spiral fractures are the result of applied torque. However, since torque-producing movements can be intentionally generated it has been advised, when involving non-mobile children, to carefully consider the possibility of abuse (Bilo *et al.* 2010). In mobile children forces of torque may be observed in certain accidental scenarios such as a running child slipping and falling while the foot is in a more or less stationary position. (Beals and Tufts, 1983, Kleinman, 1998, Reisdorff *et al.* 1993, Schwend *et al.* 2000). Spiral fractures of the femoral diaphysis may also occur in falls in which the knee and hip are more or less stationary while the lower leg moves in relation to the stationary joints (Bilo *et al.* 2010).

2.6 Animal models as an alternative to paediatric bone

As previously mentioned, (Chapter 2.1.2.), there is lack of paediatric samples of which to mechanically test. However, this is a limitation which has inspired researchers to seek out new ways to test. One such example is the use of animal models which, to the extent of what is possible, provides the option of mechanical testing of bone. Similarly, this thesis has taken an animal model approach to experimentation and demonstrates through a review of the literature, which animal models are better suited for paediatric bone testing. The specific approach undertaken in this work is justified in Chapter 3. However, to gain a more complete understanding to that decision, the following section contains a more complete review of animal models. This is so that the strengths and weaknesses of the different animal models can be more fully assessed. The last decades have resulted in several comparative studies on the selection and use of animal models for orthopaedics and biomaterial research (Laiblin and Jaeschke, 1979; Pintar *et al.* 2000; Martini *et al.* 2001; Franklyn *et al.* 2007; Baumer *et al.* 2009; Crandall *et al.* 2011; Cheong, 2015). However, the research has pertained primarily to the adult population with little focus on the features and properties of immature bone. Due to a lack of paediatric tissue, the use of immature animal bone has become an important alternative (Franklyn *et al.* 2007; Baumer *et al.* 2009; Cheong, 2015). The aim of this thesis is the mechanical evaluation of the animal bone as a suitable surrogate for paediatric bone and as such this section will first discuss the factor of biofidelity when choosing an animal model. The section will be concluded with a detail of the most common animal models used in the biomechanical testing of immature bone as well as an argument for why the immature porcine is suggestively the most appropriate model to use in this this thesis.

2.7 Biofidelity

A primary gauge for an animal model is biofidelity (Vaughan, 2017). Biofidelity is the ability of an acquired surrogate to approximate the behaviour of a living human being under comparable loading conditions (Crandall *et al.* 2011). To best determine biofidelity, forensic investigators and material scientists may use measured variables that include kinetic parameters such as force and moment, and kinematic parameters such as displacement and acceleration (Crandall *et al.* 2011). However, an underpinning factor in considering a human surrogate and specifically an animal model is that the choice of model is entirely dependent on the research question being addressed (Pierce *et al.* 2008). Despite such necessary considerations, there is an agreement over certain characteristics which allows for orthopaedic and biomaterial research to be conducted. These characteristics, in lieu of the aim of this thesis, includes bone physiology, specifically in regards whole bone architecture and material characteristics (Martini *et al.* 2001; Liebschner, 2004; Pearce *et al.* 2007; Reichert *et al.* 2009).

2.8 Choosing an animal model

In the absence of human tissue, animal surrogates provide an alternative option for studying a humans' pathophysiological response to physical trauma. For example, porcine models were originally chosen as human substitute because it could be positioned in a manner similar to a seated human (Roach, 1999). Despite the multitude of experimental tests involving the comparison of human and 'animal' models, differences in anatomy and physiology will inherently complicate the interpretation of any similarities (Franklyn *et al.* 2007). Most animals used in biomechanical research are quadrupeds and as such structural differences in comparison with biped humans, inter-species variations, and differently tested anatomical sites hinders any direct comparison between studies (Franklyn *et al.* 2007; Crandall *et al.* 2011). Even apes, who are quadrupedal animals which sometimes walk bipedally exhibit a large amount of structural differences to humans. When an ape moves in a quadrupedal manner, the trunk slopes forward from the hip at an angle approximately 30° from the vertical. However, if an ape assumes a bipedal manner of motion, the trunk slopes forward from the hip at an angle approximately 70° angle from the vertical (Alexander, 2004). In relation to humans, the difference may be severe causing vertical ground reaction forces which alter the manner skeletal systems are loaded (Franklyn *et al.* 2007).

Despite these differences, there are mechanical similarities between humans and animals. For example, the goat has been used as a surrogate due to possessing a vertical neck position similar to humans (Pintar et al. 2000). A study by Martini et al. (2001) analysed the then use of mammal models, found the most common choices to be rats (36%) and mice (26%) with more human size animals such as dogs at 9% and pigs and sheep at 2% each. Animals like humans also have specific developmental stages throughout their life and it is in part these similarities and dissimilarities which enable certain animals to be used for immature bone research. Maturation obviously varies by specifics as observed in Table 2.3.

Additional complications with animal research are the interpretation of animal results in relation to humans. This has led to the scaling and extrapolation of results from animals to humans despite differences in physiology and anatomy. Dimensional analysis techniques have been used successfully in estimating human response based on animal data. However, it is important to remember that assumptions of geometric similarity and loading factors are in the end just assumptions (Crandall *et al.* 2011). The following section will in more detail discuss those animals models which are more commonly discussed and used as paediatric bone proxies. The section will run through the strengths and weakness of each different animal models.

Table 2:3: Developmental comparison between human, pig, and cow (Franklyn et al. 2007).

Stages of life	**Human**	**Pig/piglet**	**Cow**
Weaned	6-12 months	6 weeks	3 months
Childhood	10 years	6 months	12 months
Physically mature	18 years	1.5 years	2 years
Middle age	40 years	4 years	10 years
Elderly	70 years	5 years	20 years
Lifespan	80 years	6 years	25 years

2.8.1 Canine bone

Perhaps due to being a companion animal, the dog is one most of extensively studied animals (Martini *et al.* 2001). As a surrogate, the dog's primary advantage is its similarity in bone composition (bone mineral content, hydroxyproline, extractable proteins and IGF-1 content) to humans. Some differences to humans in the bone microstructure have been described including the remodelling of the canine bone which primarily takes place in the middle, along the length of the diaphysis, with the peripheral of the bone containing lamellar bone (Pearce *et al.* 2007). This is in contrast to humans who possess extensive Haversian remodelling throughout the cortical bone in adults. In addition, canines experience a higher rate of bone remodelling compared to humans (Reichert *et al.* 2009).

Nevertheless, canine bone is closely representative of human bone as a large proportion of dog bone remodels to secondary bone by skeletal maturity, which is in contrast to rats, rabbits, and sheep who predominantly exhibit a primary plexiform bone structure (an interwoven combination of collagen fibres with osteocytes distributed randomly, resulting in a brick-like appearance; Cheong, 2015). This is important to consider when fatigue testing, because it changes the mechanical strength of specimen (Liebschner, 2004; Martini *et al.* 2001). This makes canines the most preferred animal of choice among the different large animals (Martini *et al.* 2001; Liebschner, 2004; Pearce *et al.* 2007; Reichert *et al.* 2009).

2.8.2 Ovine bone

It has been reported that adult sheep offer the advantage of possessing a body weight similar to adult humans and having long bones of dimensions suitable for testing human implants and prostheses which is not possible in smaller animals such as rabbits and canines (Newman *et al.* 1995). Ovine bones have a similar macrostructure to human bones, but histologically, the bone structure of sheep is different from that of humans. In sheep, bone consists predominantly of the primary bone structure in comparison with the largely secondary bone structure of humans

(Eitel *et al.* 1981; Sumner-Smith, 2002). Secondary bone remodelling in ovines does not take place until the approximate age of between 7 and 9 years, while at the age of 3 to 4 years ovines have a plexiform bone structure comprising of a combination of woven and lamellar bones within which the vascular plexuses are sandwiched (Newman *et al.* 1995; Liebschner, 2004). Mature ovines have a significantly higher trabecular bone density and subsequently greater bone strength when compared to humans (Nafei *et al.* 2000; Liebschner, 2004). However, differences may change with location. Some researchers argue that sheep are still a valuable model for human bone turnover and remodelling activity, despite defining several differences in bone structure (Chavassieux *et al.* 2007; Pastoureau *et al.* 1989; den Boer *et al.* 1999). Sheep also exhibit a larger amount of bone ingrowth than humans which is probably due to the greater amount of cancellous bone in the distal femur of sheep compared to humans (Willie *et al.* 2004).

2.8.3 Porcine bone

Pigs have, for the last decades, been a popular choice in a series of studies including orthopaedic, biomechanical, and forensic research on bone diseases, bone fractures, and bone necrosis (Friedman, 1999; Thompson, 2004; Baumer *et al.* 2009; Saini *et al*. 2024). Pigs are considered to be closely representative of human bone in regard to bone anatomy, morphology, healing capacity, remodelling, mineral density and concentration (Aerssens *et al.* 1998; Thorwarth *et al.* 2005). Similarities have also been identified in the femoral cross-sectional diameter and areas as well as the lamellar bone structure (Raab *et al.* 1991; Mosekilde, 1987; Laiblin and Jaeschke, 1979). However, it is crucial to acknowledge that while these similarities exist, they do not equate to a perfect equivalence, and significant limitations must be considered.

Limitations such as the commercial pig's dense trabecular bone network, large growth rate, body weight, and reportedly combatant temperament have made them at points a less preferential choice. Cone *et al.* (2017) highlighted the complexities of age-related comparisons between porcine and human bone. It was noted that pig strains reach sexual maturity between 4.5 and 6 months, often considered "early adolescence," while skeletal age in humans is typically assessed via left-hand radiographs, a method not directly transferable to porcine anatomy. Furthermore, physes closure in Yorkshire pig hind limbs occurs between 12 and 20 months, defining "adolescence" in this species. Cone *et al*. (2017) also emphasise the significant variability between porcine strains, with commercial breeds selected for rapid growth contrasting with minipig lines bred for limited growth. While smaller breeds overcome initial differences with human bone, they are cost prohibitive and difficult to source (Martini *et al.* 2001; Pearce *et al.* 2007).

A noted key challenge in utilising immature porcine bone as a surrogate for human infants lies in the fundamental differences in early mobility. Infant porcines exhibit precocial ambulation (almost immediately mobile), engaging in immediate weight-bearing, while human infants are altricial, with delayed independent movement. These variations in loading history significantly impact bone adaptive responses and mechanical properties. Bonney and Goodman (2021) addressed this issue by employing porcine bone from individuals less than 36 hours old, thereby minimising, but not eliminating, the effects of load-induced adaptations and growth rate, and providing a more controlled comparative baseline. This difference in loading, due to quadrupedal versus bipedal gait, is a foundational biomechanical difference that must be considered when interpreting results. As highlighted further by Bonney and Goodman (2021) significant differences in mechanical properties, such as hardness, exist between porcine and human bone, further emphasizing the need for caution.

In consideration of immature bone, the immature porcine does not pose as much of an issue as an animal model in comparison with more mature porcines. Regarding specifically cranial bone, Margulies and Thibault (2000) tested both immature cranial human and immature porcine bone specimens under three-point bending reported "that the elastic modulus, rupture modulus, and energy absorbed to failure of infant (2 to 3 days old) cranial (parietal) bone are similar to that of the human infant in three-point bending". While Margulies and Thibault (2000) attempted to correlate the mechanical behaviour of human infant cranial bone to porcine infant cranial bone, the ages and number of the specimens tested were limited. In a continuation on the aforementioned research Baumer *et al.* (2009) conducted three-point bending tests on 34 cranial bone specimens in the age range of 3 to 21 days of age. Similarly, Saini et al. (2024) observed that the flexural properties (derived from three-point bending tests) of the 12-day old porcine cranial samples were found to be closest to that of an 11-month-old human cranial sample. Whilst the results of specifically Baumer *et al*. (2009) and Saini *et al.* (2024) might suggest that days of porcine age may correlate with human months during the infancy period, the lack of analysis between Margulies and Thibault (2000), Baumer *et al.* (2009), Saini *et al.* (2024) makes the possible trend of days equal months unclear. Furthermore, it is important to note that results gained from cranial bone testing, may not be directly transferable to long bone testing, due to the different structures of the bones.

Despite the uncertainties concerning the adult pig as a suitable human surrogate, the immature porcine offers an alternative due to size and temperament but with the potential detriment of being immediately mobile at birth. However, this is a noted concern for all animal models. At present, the detailed evaluation of the immature porcine as suitable surrogate for human children is limited. As far as the author is aware only Margulies and Thibault (2000), Baumer *et al.* (2009), Vaughan (2017), and Saini *et al.* (2024) have tested the intrinsic mechanical properties of immature porcine bone material. Furthermore, both Margulies and Thibault (2000), Baumer *et al.* (2009), and Saini *et al.* (2024) were conducted using cranial bone, which consists of a layer of diploë (spongy bone), lined on either side by a layer of compact bone. Vaughan (2017) subjected immature porcine femoral samples to tensile testing which in turn may not be correlated to other types of mechanical tests.

In consideration of immature porcine whole bone research, Koo *et al* (2001) subjected immature porcine femora and humerii in the age range of 6 to 68 days of age to three-point bending. Both the left and right humerii and femora from 12 domestic pigs harvested and subjected to a perpendicular applied load to the long axis of the bones at a rate of 0.1mm/s for the humerii and 1mm/s for the femora. However, since the aim of Koo *et al.* (2001) was not the investigation of the material properties of animal surrogates but the usefulness of non-invasive use of dual-energy x-rays, the results in concern to human data or age were not expounded. However, the results from Koo *et al.* (2001) does indicate that there is a difference in bending strength between left and right specimens as well as the humerus and femur.

Further mechanical studies using immature porcine bone to generate fracture morphologies were conducted by Pierce *et al.* (2000). Three-point bending and torsional loading conditions were used in an attempt to generate transverse and spiral fractures respectively in 22 porcine femora of the age range of 3 to 12 months. Specifically, 15 femora were tested under three-point bending where the load was applied at a loading rate of 1mm/s resulting in transverse fractures in all the bones. Torsional tests were conducted for the remaining femora at a force applied rate of 1º per second. Of the first 5 femora tested, only one resulted in a spiral fracture while the remaining samples resulted in a fracture at the growth plate. This was followed by an adjustment of the test set-up so that only half the area between the growth plates of the bones were exposed. In an approach using applied loads at various rates and a similar test setup to Pierce *et al.* (2000), Bertocci *et al.* (2017) were able to generate continuous transverse and oblique fractures under bending and spiral fractures under torsion. Whilst Bertocci *et al.* (2017) employed immature porcine femora of 3 months of age, the results did suggest that fracture morphology is dependent upon loading conditions.

Additional immature porcine tests were performed by Vaughan (2017) who demonstrated in torsion and four-point bending tests on immature porcine long bones (age range 1 to 24 days of age) that there are age and rate of loading effects influencing the fracture morphology. In regard to the mechanical testing of whole immature porcine bone, the published literature has presented fracture morphologies observed in both accidental and non-accidental child injury cases. Despite the continuous use of whole immature porcine bone in mechanical testing, few considerations have been made concerning the impact of age. The varying geometry of bones, despite the same anatomical bone, prohibits to a certain extent the establishment of an accurate age series. However, consideration to the continuous development immature bone undergoes permits certain questions such as whether bone fusion in regard to age has an effect on the fracture morphologies generated.

2.8.4 Model selection

In consideration of the various animal models which have been used as a surrogate for paediatric bone the immature porcine has shown the greatest degree of consistency and potential of mechanical and fracture behaviour in relation to human infant bone. Whilst the physical geometry of adult porcine long bones is shorter and sturdier than human long bones, these differences are less apparent when concerning the immature porcine long bones. However, it is important to acknowledge that the immature porcine model, while valuable, is not a perfect representation of human paediatric bone. Key limitations include differences in bone morphology, growth patterns, and loading conditions due to the quadrupedal gait of pigs compared to the bipedal gait of humans. Additionally, variability across porcine strains can introduce further complexity. Notably, there is a lack of comprehensive studies on the mechanical testing of immature porcine long bones, making this research particularly valuable in expanding the understanding of porcine bone development and its potential as a human proxy.

Both Margulies and Thibault (2000) and Baumer *et al.* (2009) observed quantifiable age-related similarities between immature porcine and paediatric cranial bone. However, while Margulies and Thibault (2000) found similarities in the mechanical behaviour of infant porcine and human cranial bone, their limited sample size may restrict the generalizability of these findings to a broader population. A possible risk and limitation of the porcine model remains immediate mobility upon birth which may result in variable results (Cone *et al.* 2017). This risk becomes greater if looking to mechanically test long bones, which this thesis does. While this thesis aim is to evaluate mechanical and fracture-related changes with age, risks can be limited by using animal models as young as possible.

Regarding fracture patterns, Pierce *et al.* (2000), Vaughan (2017), and Bertocci *et al.* (2017) have shown a consistent ability to generate fracture patterns similar to those observed in accidental and non-accidental child fracture incidents. Cheong (2015) was also able to generate human observed fracture patterns using immature ovine long bones, but the tests were primarily conducted using tibiae which has not been as extensively tested as the immature porcine femur and humerus. Furthermore, the greater availability of both the immature porcine and ovine make them more suitable options than the canine in terms of ease of procurement, ethics, and testing. Despite similarities in availability and fracture morphology across animal types, immature porcine bone provides the better platform for mechanical testing and comparative purposes across both age, material, and fracture analysis. The immature porcine has therefore been deemed as the more appropriate choice of model for this thesis.

2.9 Age selection

A definite correlation between human cortical bone and immature porcine cortical bone has as of yet not been agreed upon. Kilborn *et al.* (2002) suggested that by calculating the ratio between physis closure and lifespan, an age estimate of when the animal stops growing can be calculated. This information is useful for coming up with an approximate age correlation between humans and animals. Nevertheless, several age correlations regarding porcine and human have been presented. Dobbing suggested a correlation of one week of porcine life being equal to one month of human life, based on brain development. Thibault and Margulies (2000) used 2 to 3 days old porcines to represent human infants under one month of age and one-year old porcines to depict a child of approximately four years of age. In a subsequent study, Prange and Margulies (2002) used 5-day old porcines as a substitute for a human infant 1 month or less in age, and 4-week-old porcines to represent a child between 1 to 3 years of age. Age-related rates of change in the bending rigidity of the infant human and infant porcine skulls were observed by Baumer *et al.* (2009) to signify months in humans to be the equivalent of days in porcines. A more current study by Bertocci *et al.* (2017) chose 3-month-old porcine femora to represent a 3-year-old child based on ossification, and metaphyseal and epiphyseal morphology.

Comparisons of these age correlations with the relationships in Table 2.3 indicates that there is a reasonable agreement between research studies and the information derived from industry sources. However, certain disagreements still exist. According to Table 2.3 and the current literature, a 1-year-old porcine would be the equivalent of a 15-year-old adolescent when considering the different ratios between the age groups. In another study, Prange *et al.* 1999 found that a 1-year-old porcine displays similar properties to an adult human, which may be considered more accurate since an adolescent of 15 years of age is developmentally close to adulthood. Based on the literature's current estimation of porcine bone development in comparison to human the data there appears to be no consensus, and it may be suggested that should the immature porcine be continued to be used as a suitable surrogate a more in depth mechanical evaluation concerning the age correlation between porcines and humans in regard to the mechanical properties should be conducted. However, while establishing a precise correlation between human infant and porcine infant bone ages is a crucial long-term goal, the immediate focus should be on conducting further research to determine whether there is a significant change in mechanical properties with age in immature porcine bone itself. This fundamental understanding will lay the groundwork for future comparative studies and refine the use of the porcine model in research.

2.10 Conclusion

This chapter has reviewed the lack of testable paediatric tissue but also the potential for animal surrogates. It has been suggested that immature porcine bone is comparable to paediatric bone but that further research is required. This specifically includes research concerning the intrinsic and extrinsic mechanical differences or similarities between the two species. Further research is also required concerning age-related similarities and how the factor of age influences mechanical and fracture behaviour. To contribute to a deeper understanding of immature bone behaviour, which may have implications for the study of non-accidental fractures in children, this thesis will set out to mechanically evaluate a paediatric proxy model in the form of the immature porcine. This includes testing for the important factor of age which is the underpinning of the unique mechanical behaviour of immature bone. As such the following chapter will in more detail address the aim and specific goals of this thesis.

Chapter 3 Problem Statement

As informed by Chapter 1 and Chapter 2 of this thesis, a gap in the literature on the mechanical behaviour of immature bone has been observed. Attempting, even if partially, to investigate the factors which underlie this mechanical behaviour can add to our overall understanding of immature bone and ultimately how forces may affect such bone. Specifically, but not exclusively, gaps in the research remain regarding:

1. An evidence-based understanding of how applied forces can affect immature bone behaviour. This includes a lack of systematic testing on the effects of stress on immature bone material as well as the type of fracture patterns which can be generated in whole immature bone in a controlled environment.

2. Additionally, there is a lack of literature on the effect age may have on immature bone under load and any resultant fracture characteristics. There is a need for this type of empirically derived data because it can inform decision-making. This data would add to trauma analysis research by looking for any variation in the characteristics or behaviour of bone by age. If different ages of bone act differently in baseline tests, this needs to be accounted for in forensic interpretation.

A further theme of this thesis is the use of porcine bone as an experimental substitute for human paediatric bone. The ideal context here would be availability of human bone which would allow direct experimental comparison of immature porcine bone. However, human paediatric bone is of course extremely restricted and in short supply. It was not possible to get access to paediatric bone for this thesis. However, to justify the potential use of porcine bone as a substitute, this thesis thoroughly reviewed the research that has been conducted using human bone (see section 3.1) and where possible compared the empirical results to published research (see sections 5.6, 6.5, and 7.4).

To discuss the progress of this thesis towards transferable knowledge for practice, it is useful to discuss the potential forensic pathology context. At present the British child protection framework is extensive but not all encompassing. There is still an uncertainty on the prevalence of non-accidental fractures, and it has been suggested that multiple cases go unreported due to a lack of empirically based research on accidental and non-accidental fractures (Flaherty *et al.* 2014; Mitchell *et al.* 2020). The responsibility of the physician, paediatrician, radiologist, and forensic professional is to accurately determine the nature of the fracture and therefore help substantiate the event. One area of concern is the manifestation of skeletal injuries in infants from birth to 3 years of age (Skellern *et al.* 2000; Hoskote *et al.* 2003; Loder *et al.* 2006). It is a period where children undergo accelerated growth and changes in anatomy (Altai *et al.* 2018). It is therefore an age where fractures can have multiple meanings as well as manners of manifestation (Altai *et al.* 2018). Currently, there is a need to augment the research on the fracture mechanics of immature bone and how those mechanics induce certain types of fracture patterns. There is also a lack of comparative data as to whether fracture morphologies differ between paediatric age groups. These challenges remain despite the literature suggesting room for improvement for physicians in both child abuse training and the availability of medical intervention tools (Leventhal *et al.* 1993; Flaherty *et al.* 2006; Parrish *et al.* 2017).

Note that this thesis does not presume to provide a defining method for accurately differentiating between accidental and non-accidental fractures in children. Moving towards a method for making such a distinction would require an extensive sample of medical case files containing an underlying knowledge of intent and detailed information in terms of forensic science evidence, and all the physical variables at the time of injury. Such robust data is simply not available, not least for ethical reasons and issues relating to child protection, but also because those involved in an incident cannot be aware of all the physical factors at play. Since the focus of the research is to assist in understanding how bone behaves whilst in tissue, the author must also be transparent in how controlled, elaborate, and similar the testing environment can be to an actual (injury) event. It would also be unwise to suggest any automated method that would take the expertise of the physician out of the loop. Instead, this thesis argues that a more complete understanding of the biomechanical responses of immature bone under load contributes to the ongoing research into more accurate diagnoses and decision-making. Such an understanding can only be established through scientific evidence, which can be gathered through the application of empirically based testing in environments as controlled as possible.

The decision-making process undertaken by clinicians and professionals includes asking and answering certain questions, which look to better link the presented injury with the implied context of the injury. This will involve studying the witness statements and the child's medical history. Practitioners use a set range of questions to understand the link between the witness accounts of an injury, and the observed injury (Christian, 2015; Naughton et al. 2018). This is often achieved by looking at the nature of injuries. For fractures this includes details on the fracture morphology (Bilo et al. 2010; Lindberg et al. 2012). However, the ground truth of fracture morphologies can only be standardised through the continued collection of empirical data, which can only be achieved through the appropriate testing of bone behaviour in controlled environments. For immature bone, this includes further evaluating how a certain type of load (the manner in which multiple forces, a load, may act on a body) or rate of loading (the speed by which multiple forces, a load, is applied to a body) may fracture a bone. It further demands asking whether this behaviour differs between different ages and whether loading type is exclusive to a specific type of fracture type. Thus, the current thesis set out to test the mechanical behaviour of immature bone, adding the results to the first step in a long process of standardising clinician and professional decision-making (Figure 3.1)

Figure 3.1: The step-by-step process to supported decision-making in a clinical setting. The current thesis lies within the blue box representing the beginning of the process.

To achieve this, the thesis has looked at three aspects of immature bone: mechanical properties, loading rate, and fracture morphology. Current advances in biomechanical research have so far suggested that loading type and loading rate have specific effects on immature bone. Bertocci *et al.* (2017) and Cheong *et al.* (2017) subjected whole immature long bones to three-point bending and four-point bending respectively and observed that fracture morphologies may be determined by loading rate. Pierce *et al.* (2005) found the combination of bone mineral density and bone geometry to be predictors of force at fracture in immature femora under three-point bending. Such studies have added significant value to our current understanding of the mechanical behaviour of immature bone. However, much remains to be explored regarding immature bone behaviour. The starting point in this thesis was therefore the exploration of bone biomechanics - the effect of various loading types and loading rates on a material level.

There are also outstanding questions regarding how bone material varies across age groups when subjected to similar forces, and whether different mechanisms of loading would yield a different fracture pattern depending on age. Age related differences in the properties of bones are obviously of direct relevance to the paediatric context explored in this thesis. Using immature canine bone, Torzilli *et al.* (1982) observed that growing bone contained fewer osteons, lower density, and lower mineral content, and suggested that immature bone - while highly deformable - has a lower resistance to stress compared to older bone. Similar statements have been echoed by Carrier (1983), Currey and Pond (1989), and Brear et al. (1990).

It is worth noting, however, that variances such as bone geometry (e.g. whole bone or machined samples), anatomical site and species of bone can have an effect. This also something which has not been fully explored to date. Kriewall *et al.* (1981) and Margulies and Thibault (2000) found evidence indicating that it is the structural differentiation of developing bone and not any changes in material properties which account for increases in energy absorbed and elastic modulus with age. Carrier (1983) noted that long bones of juvenile and sub-adult mammals are relatively thicker to compensate for being composed of a weaker material thus retaining structural stiffness and maintaining mechanical integrity. In conducting research into the biomechanics of immature bone, the long bones are a vital area of study. Specifically, the diaphyseal area of long bones is one of the most commonly identified sites of fracture in cases involving both child abuse and accidental scenarios (King et al. 1988; Leventhal *et al.* 1993; Loder *et al.* 2006). As such, long bone fractures are not considered strong indicators of child abuse alone. Caffey (1946) and Carty (1993) reported that *spiral*, *oblique*, and *transverse* fractures of the long bones constitute the most prevalent fracture patterns observed in non-accidental fracture cases. The collection of data on fracture patterns produced under controlled mechanical testing adds to the existing body of knowledge regarding immature bone while building a comprehensive knowledge base of immature bone trauma.

3.1 Animal models and porcine bone

In order to explore the mechanical properties, loading rate and fracture morphology, an experimental approach was deemed necessary, and as such, real bone specimens were required. As human infant bone specimens are very scarce, a suitable surrogate animal model was considered as an option. In regard to biofidelity and commonality of use, the pig (porcine) is one of the most regularly utilised surrogates for paediatric tissue and research has been conducted in order to determine the similarities between specifically human children and immature porcine.

Based on brain development, Dobbing and Sands (1979) proposed a correlation of one week of a porcine's life to being the equivalent of one month of human life. Subsequent research has assumed similar correlations between porcine and human paediatric ages. Margulies and Thibault (2000) used two to three-day old porcines to represent human infants one month old and one-year old porcines to represent children approximately four years of age. In later studies and based on cranial bone samples, Prange and Margulies (2002) used five-day old and four-week-old porcines to respectively represent human infants less than one-month of age and a toddler between one and three-years of age. Baumer *et al.* (2009) proposed one day in a porcine's life could equate to one month in a human. Therefore, if using the specific and concerned human age spectrum of new-born to 3 years of age the equivalent porcine age spectrum would be new-born to 36 days of age. While porcines, typically used as substitutes for adult humans, are a common material for forensic and biomechanical research, the evidence-based evaluation of the immature porcine as a human infant model requires further exploring.

It has been noted by researchers that the porcine's physiology is similar to humans as it has growing and adult skeletal phases similar in rates of mineralization, remodelling and healing (Cheong 2015). The porcine's femoral cross-sectional geometry is also a good representative of adult bones (Pearce *et al.* 2007), but it must be noted that porcine bones are also compared to humans much shorter in length (Reichert *et al.* 2009). What has been documented as the main limitation of using porcines as an animal model for humans is its rapid growth rate and excessive weight (Martini *et al.* 2001; Pearce et al. 2007). human children have therefore only been noted to share certain similarities whilst the porcine is very young (Baumer *et al.* 2009). For bridging the weight discrepancy, smaller breeds such as miniature porcines have been suggested as an alternative, but they can be costly and difficult to attain (Martini *et al.* 2001; Pearce *et al.* 2007) and have a nonrepresentative length to cross section ratio. Considering the porcine's limitations it bears mentioning that no animal model will be a perfect representation of human bone. It also bears mentioning that just as the miniature pig has certain benefits to being used as an animal model, the immature and 'regular' sized pig carries the same benefits as well as a geometry less similar to a mature porcine. As of yet, this thesis has been the first attempt to test the material properties of cortical bone from immature porcines at different ages (See Chapter 2, section 2.5.1). Since there is a lack of academic consensus as to whether immature porcine bone ages in any manner similar to human infant bone, examining different stages of development appeared sensible.

Due to the immature porcine's potential and prevalence in forensic and medical research further study was deemed necessary. It is therefore in the interest of furthering knowledge that biomechanical research into the material properties of immature porcine bone is continued and tested to further provide to the potential veracity of the animal as a paediatric substitute. Should the research which has already been conducted for this thesis, but also future research be conducted in verifying the immature porcine an empirically suited animal surrogate for children, the results would allow for a stronger argument of their relevance to paediatric fracture cases. If not, the research would at least provide a platform for where alternative surrogate models other than the immature porcine may need to be more extensively explored. For this reason, where appropriate, data and results have been compared with other studies using human bone and/or substitutes. This includes Currey and Butler (1975) whose seminal work tested the mechanical properties of paediatric bone. Additional research has been conducted on immature porcine bone, albeit on different anatomical sites (Dobbing, 1974; Margulies and Thibault, 2000;

Coats and Margulies, 2003; Franklyn, 2007; Baumer et al. 2009). Since the methods between all of these studies and this thesis differs comparisons can only be made tentatively. However, it is still the assumption that certain similarities or differences will be able to be highlighted and used for focus for future research.

3.2 Aim

While the use of porcine animal models to explore immature bone and its behaviour is a recognised method with encouraging evidence of suitability, it remains a developing research area. Furthermore, previous research indicates the potential for immature porcine bone to serve as a human infant proxy model (Margulies and Thibault 2000; Baumer *et al.* 2009; Powell *et al.* 2013; Bertocci *et al.* 2017). Few studies have explored how the material properties and fracture patterns in immature porcine bone differ according to age. This thesis aims to address these gaps by investigating the mechanical behaviour of immature porcine bone with the following research question:

What is the mechanical behaviour of immature porcine bone and how can understanding this assist with forensic enquiry?

This overarching research question is answered through three specific questions:

1. What are the basic underlying mechanical properties of immature porcine bone as a material?

2. As a material, how does immature porcine bone act under high impact loads?

3. What type of fracture patterns can be generated through the mechanical testing of whole immature porcine long bones?

In order to answer these three questions a series of experiments was planned and conducted using immature porcine bone material and whole bones. These experiments have been detailed in three experimental chapters, across which the following activities were undertaken:

I. Selection and preparation of comparative immature porcine bone, from which both bone material and whole bones could be mechanically tested;
II. the design and construction of a platform which allowed for the repeated mechanical testing of immature porcine bone;
III. the evaluation of the mechanical behaviour of immature porcine bone across age;
IV. the mechanical evaluation of immature porcine bone material under both lower and higher loading rates;
V. the evaluation of the mechanical behaviour of immature porcine bone depending on bone type, and;
VI. the generation of fracture morphologies under multiple loading types such as bending, compression, and torsion.

The findings of these empirical studies are discussed in the context of how they might assist in building evidence for understanding the behaviour of bones in a paediatric context.

In summary, this thesis has addressed the gaps in knowledge about immature bone behaviour and fracture response. Due to the limited availability of paediatric tissue, immature porcine bone is investigated as an alternative model. The research examines the mechanical properties and fracture characteristics of immature porcine bone, addressing how it reacts under various loading rates and types. This chapter outlines the research aim, questions, and the experimental approach. Chapter 4 will detail the methods for collecting, preparing, and testing immature porcine bone.

Chapter 4 Method

The development of a robust evidence base is in part achieved through detailed and transparent planning. These underpinnings enable the execution of repeatable experimentation (Morgan, 2009). To answer the research questions posed in Chapter 3, work was first undertaken to acquire and process immature porcine bone material. Further work included designing the experiments that could test the specific hypotheses detailed in Chapter 3 and later explored in the experimental chapters (See Chapter 5, 6 and 7). This chapter is a detailed account of these processes as well as the justification for the methodological choices made. Therefore, this chapter will act as a pre-cursor to the three experimental chapters that will follow. A detailed list of the bone tissue (amount, age, date of birth, anatomical bone, and anatomical side) used in this project can be found in the Appendices.

4.1 Material and test preparation

4.1.1 Porcine acquisition

For this thesis, 30 immature pigs (newborn to 40 days of age) were acquired in cooperation with the Royal Veterinary College (RVC) and J.J. Genetics. One of the reasons for the popularity of porcine bone as an animal model is its availability. At present, porcine bone can be found and purchased in the majority of areas where a licensed abattoir or butcher is located. However, the manner in which pigs are advertised and sold by abattoirs and butchers in England did not adequately meet the criteria of this thesis. Pig stocks are by standard bred or purchased (by the proprietor) and sold (to the customer) according to weight (H.M Government, 1984). This is due to the predominant area of need for pig being the food industry where quantity by weight is most relevant. Since one aim of this thesis is the mechanical evaluation of immature porcine bone across different ages, it was discovered through initial meetings with several local abattoirs and butchers that only the weight and not the pig's exact age could be determined.

To ensure for the controlled criteria of age, specimen quality, and uniform physical characteristics (e.g. breed and sex), a specialty breeder (J.J. Genetics) was contracted in cooperation with the Royal Veterinary College in England. To ensure uniformity, all pigs were selected to be males and of the English Large White breed. Certain breeds, such as the mini pig, have been recommended by the literature as a suitable animal model (size and ease of handling) but the breed is difficult to obtain in quantity (Pearce *et al.* 2007; Gutierrez *et al.* 2015). The English Large White was chosen due to the availability of the breed and therefore potential future testing (British Pig Association, 2019). The choice for the sex of the animal model was chosen according to the criteria of uniformity between the test specimens. Using dissection data from 341 male and female Large White carcasses, Fortin *et al.* (1987) found a small but quantitatively untested difference between males and females. Note that Fortin *et al.* (1987)'s observations were based on the fat depth at the shoulder, mid back, and loin sites and not the skeleton. Hence, since there was likely to be a limited sample size and there is a lack of research as to the skeletal differences between the Large White sexes, it was decided to stick to one sex only. Female pigs have been noted as being fiscally more expensive due to their ability to breed. As such, due to available resources only male Large White pigs were selected for this thesis.

Prior to maceration, all pigs were externally inspected for trauma at the RVC facilities. The inspection was carried out by the author and operating technicians with a specific focus on physical trauma (e.g., bruises or lesions) which might suggest any pre-existing fracture. For reasons of transportation and the age of the youngest specimens, all the pigs younger than 10 days of age were destroyed at the breeding facility whilst the pigs 10 days of age and older were destroyed at the RVC facilities.

In the UK, animals sold and purchased through breeders and livestock markets are protected by the Welfare of Animals at Markets Order (1990) and The Welfare of Horses at Markets (and Other Places of Sale) Order (1990). The order enforces provisions for animal handling by trained and competent stockmen, and for facilities, equipment and procedures that allow for dealing with pigs. EU Directive 93/119/EC in turn protects animals at the time of slaughter or destruction and is implemented in Great Britain by the Welfare of Animals (Slaughter or Killing) Regulations 1995 (as amended). Animals bred or kept for the production of meat, skin, fur or other products must be spared any avoidable excitement, pain or suffering during movement, lairage (the keeping of animals in stalls, pens, covered areas or fields), restraint, stunning, slaughter and killing. As such, all the animals selected for this study were, prior to death, handled by licensed stockman at the RVC facility and destroyed in accordance with UK and EU legislation (H.M Government, 1990; 1995; EU Parliament, 1993).

4.1.2 Maceration

For ease of testing and the possible mechanical analysis of immature porcine femora and humerii, each of the tested humerii and femora needed to be surgically disarticulated and removed of all soft tissue prior to testing. The maceration (i.e. defleshing) of soft tissue from a fleshed subject is a common practice in fields where there is a need for analysing or testing of skeletal remains (Mairs *et al.* 2004; Steadman *et al.* 2006). For specifically forensic purposes, forensic anthropologists and pathologists generally remove the soft tissues of the deceased in order to expose the bone for trauma analysis and any skeletal characteristics which may aid in the building of a biological profile (Mairs *et al.* 2004; Wang *et al.* 2010; Leeper, 2016). For mechanical tests involving bone, the integrity of the test rests foremost on the simple assumption that you are only testing the performance of a specific material. Therefore, if the aim of the research is to test the mechanical behaviour of bone, then any secondary tissue (e.g. soft tissue) needs to be removed.

Maceration is the technical term for the removal of soft tissue (Couse and Conno, 2015). However, maceration can be conducted in a variety of manners. This includes using heat, chemicals, insects, or surgical instruments to remove soft tissue (Davis and Payne, 1992; Hendry, 1999; Gobalet, 2003; Mairs, *et al.* 2004; Couse and Conno, 2015; King and Birch, 2015). In choosing the optimal maceration technique for this thesis, the most important outcomes were the integrity of the bone throughout and the removal of the soft tissue. As used in King and Birch (2015), seven techniques involving manual, natural, and chemical maceration were tested using adult porcine ribs. Applying an eight-point scoring system, the quality of the macerated bones was judged according to evidence of porosity, flaking, retention of physical markers (e.g. stab marks, fractures etc.), resulting durability of the bone, the ease of performing the technique, health and safety of the employed technique, duration of the procedure, cost, and the ease of procuring and transporting the required equipment. For a fuller review of maceration methods, the reader is referred to King and Birch (2015).

In brief, King and Birch (2015) suggest microwave maceration and manual maceration to be the two most effective techniques; the authors note that microwave maceration was found to be quick, effective, and clean, although disadvantages included the difficulty of transporting the material and a microwave large enough to contain larger bones. Manual maceration, which only involves the use of latex gloves, forceps and a scalpel for the removal of soft tissue, was observed to be inexpensive but time consuming, physically demanding, and required experience (King and Birch 2015). Couse and Conno (2015) recommend manual maceration for researchers experienced in osteology, but noted that microwave maceration was, in comparison with manual maceration, observed as having a lesser impact on the macerated bones' porosity, flaking, and durability of bone. However, Lee *et al.* (2010) tested the effectiveness of maceration using a microwave, boiling bones, detergents, Hydrogen peroxide, and Ethylenediaminetetraacetic acid (EDTA) but found the methods more harmful to the bones than manual maceration. It is worth noting that the criteria used by King and Birch (2015) for bone integrity (porosity, flaking, and durability) was primarily based on visual observation. The highest possible score for bone durability was designated as "*normal bone texture and quality. Strong, will not fracture without exceptional force. Bone can withstand normal handling and storage* (p.130)".

It is understandable that under the constraints of a forensic investigation, the chosen maceration technique has to be quick as well as efficient, although such time constraints did not apply to this thesis project. Additional studies involving the effects of microwaves on the material and histological properties of bone indicated an increase in bone demineralization and decalcification (Tinling *et al.* 2004; Imaizumi *et al.* 2013). While microwave maceration might prove the better technique, as of yet no mechanical tests have verified the difference between pre- and post-irradiated skeletal tissue. While manual maceration requires expertise, it was deemed best by the author (who is trained in osteological techniques) in conjunction with the added benefit of having several trained RVC technicians available, that manual maceration was the appropriate technique of choice.

4.1.3 Freezing and cold storing bone

Experiments involving the procurement of organic tissue followed by observation and physical testing are rarely conducted in one sitting. To allow for convenience and planning, fresh bone samples are generally frozen up until the day of the experiment (Margulies and Thibault, 2000; Baumer *et al.* 2009; Cheong, 2015). However, in planning for a mechanical test involving bone which has been previously frozen, the structural effect of freezing a bone has to be considered.

Biological and biochemical processes slow or completely stop when exposed to low temperatures (Janaway *et al.* 2009). Most enzymes in normothermic animals have been shown to exhibit a 1.5 to 2-fold decrease in metabolic rate for every 10º C decrease in temperature (Rubinsky, 2003). As for the effect of freezing on the mechanical properties of bone, the literature has shown contrasting evidence. Some studies have reported a small decrease in tensile strength after freezing and thawing (Abramov, 1975; Cowin, 2001). Pelker *et al.* (1984) observed that the freezing of rat femurs under -20º C, -70º C, and -196º C did not adversely affect bone under torsion and compression. Glezies *et al.* (1998) also reported that 3 months of frozen storage at -18º C was non-affective on sheep vertebrae subjected to compression loads. Moreno and Forriol (2002) observed that sheep femurs frozen at - 20° C and thawed after 60 days were stronger under three-point bending than fresh unfrozen samples. Moreno and Forriol (2002) further observed a higher mineral content in the frozen specimens; the authors suggest that this may be due to higher levels of phosphorus which when combined with calcium creates the compound hydroxyapatite, the strength component of bone. Itoman and Nakamura (1991) noted similar increases in rat bone stiffness after freezing the bone samples in -80º C but also noted decreased bone stiffness in the samples decalcified. Kaye *et al.* (2012) utilised Reference Point Indentation to directly compare the fracture toughness in bovine femurs before being frozen at -20º C for up to 20 days and the subsequent effects after thawing. While Kaye *et al.* (2012) observed a small decrease in bone degradation and strength, the effects of degradation may have been due to the woven bone layers in the bovine femoral and tibial bones. Woven bone has a higher water content as opposed to lamellar bone layers and freezing may have had the effect of increasing the normal water crystallization level (Weinstein *et al.* 1994).

In contrast to the documented effects of freezing on bone, research by Weaver (1966) observed the freezing human tibia at -20° C for 48 hours to have no effect on the hardness of the cortical bone. Sedlin (1965) in turn used bending to test human femoral cortical bone and similarly found that freezing at -20° C did not have a statistically significant effect on ultimate stress, modulus of elasticity, or energy absorbed until failure. Guidoni *et al.* (2010) found, through nanoindentation, that freezing at -15° C did not affect the elastic modulus of cortical bovine bone.

As can be observed from the aforementioned studies, little consensus exists as to the effects of cold storing bone. If the criteria of a study (as in this thesis) is the replication of conditions to in-vivo, the research criteria would dictate the use of fresh bone. Unfortunately, the need to freeze bone is the only realistic choice (Hale and Ross, 2016). Specifically, experimental research on bone tissue is often conducted according to the simple fact that the acquisition, movement, and preparation of bone tissue, acquisition of equipment, and preparation cannot be conducted simultaneously. As immature bone is composed of larger proportions of woven bone, the effects of freezing on immature bone is uncertain and requiring of further research (Clarke, 2008; Boskey and Coleman, 2010). However, due the time restraints in actively shipping and preparing the samples and experimental equipment, the bones acquired for this thesis were frozen. An overview of the literature indicates temperature, time of storage, and type of bone as factors to consider when preparing for an experiment. Specifically, storage under -20º C has been documented to generate the least amount of possible trauma to bone (Sedlin, 1965; Weaver, 1966; van Haaren *et al.* 2008; Goh *et al.* 2010). Potential frost bite trauma can in turn be further limited by wrapping the bone in saline soaked gauzes (Margulies and Thibault, 2000; Baumer *et al.* 2009).

4.1.4 Testing conditions

Bone is an organic tissue and therefore as much time spent preparing bone for testing must also be spent maintaining bone during testing. Specifically, temperature and bone hydration have been noted as important factors to consider during testing (Oksztulska-Kolanek *et al.* 2016). Turner and Burr (2001) recommended 37º C as the most optimal test temperature which is in line with most recordings of the average human body temperature being 37º C (Obermeyer *et al.* 2017). A series of studies have also observed testing at a 'room' temperature of 23º C to increase the Young's modulus by circa 2 to 4% compared to tests at 37º C (Bonfield and Tully, 1982).

Further recommendations for testing bone includes adequate bone hydration which Turner (2001) suggests is best accomplished by keeping the bone sample in a 0.9% Sodium Chloride (NaCl) solution at room temperature for the night prior to the experiment. Broz *et al.* (1993) and Currey (1988) noted that the mechanical ductility of dry cortical bone is, by most observations, able to recover once rewetted in saline solution. After the submerging bone samples for approximately 3 hours, both Broz *et al.* (1993) and Currey (1988) found the bone samples' original weight, Young's modulus, displacement, and energy absorbed to have recovered. Kourtis *et al*.(2014) in turn recommended slowly thawing the bone, while wrapped in saline-soaked gauzes, at room temperature on the day before testing. Further recommendations for the importance of keeping bone hydrated prior and during testing was noted by Zhang *et al.* (2018a) and Li *et al.* (2013) who maintained that specimen hydration is crucial as fresh and fresh-frozen bone has shown a requirement for saline in order to maintain cell viability.

4.1.5 Material conditions

Based on the consulted literature, a final protocol for the material preparation for this thesis' experimental studies was devised and implemented. The steps undertaken were the following:

1. Thirty Large White immature porcines (age range newborn to 40 days) were acquired from the Royal College of Veterinary (RVC; located in Potters Bar, England) on 4th November 2017.

2. The ethical destruction of the immature porcines was conducted by licenced handlers at the RVC facilities (4th November 2017).

Each of the porcine's femoral and humeral bones were disarticulated at each bone's adjoining joints and manually macerated of all soft tissue using a scalpel, a pair of scissors, and tweezers (4th November 2017; Figure 4.1 and 4.2).

Figure 4.1: Manual maceration process.

Figure 4.2: Macerated immature porcine femora and humerii.

Post-maceration, each bone was CT-imaged (SkyScan 1176) at the RVC's facilities and evaluated (by the author and a licensed veterinarian radiologist) for potential pre-experiment injuries (4th November 2017; Figure 4.3).

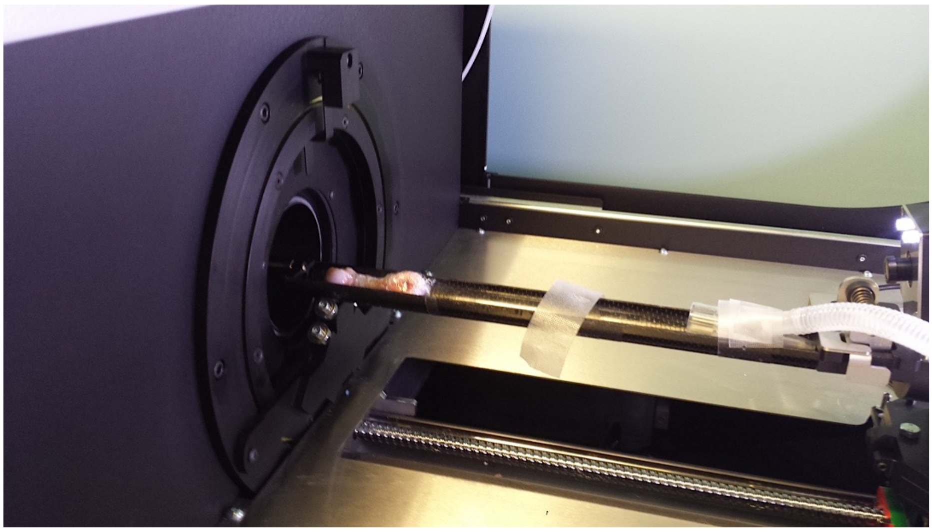

Figure 4.3: CT-imaging post manual maceration.

1. As each experiment (see Chapters 5 through 7) was scheduled and conducted off-site at University College London's (UCL) facilities (Central London, England), each bone was separately prepared for storage and shipping. Specifically, all bones were wrapped in saline soaked gauzes, placed in polyethylene bags and stored in ice packed polystyrene boxes for next-day shipping.
2. On 5th November 2017, all bones were shipped by car to the UCL Department of Archaeology (Central London, England) and immediately upon arrival, moved from the ice-packed polystyrene boxes and into frozen storage at a temperature of - 20° C.
3. All bones were freeze stored between 9 and 12 months until the date of experimentation (September through November 2018).

The details for how the specific samples were created for each experiment are noted in their respective chapters (See Chapter 5 through 7).

4.2 Biomechanical testing – general considerations

After sample preparation, all the porcine bones were subjected to a series of mechanical tests in line with the original research questions and aims of the thesis. This includes using mechanical tests which, firstly, simulate the type of loads bone is commonly subjected to in life, and secondly, allows for the building of a further understanding of the mechanical behaviour of immature bone (the overall thesis aim; see Chapter 3). In essence, these tests describe how bone materials behave under different type of load conditions. As these were biomechanical tests, specifically designed to identify how bone behaves under stress, it is important to give an overview of these along with explaining the types of tests available and commonly used in the literature. There are a number of different ways in which bone can be subjected to stress and load- for example bending, compressing and impact to failure. Each type of test that is used later in this thesis is summarised below to give a clear account of what the test measures and how the resulting data can be interpreted in relation to the bone material, bone, age, and type of load (Research questions 1,2, and 3; see Chapter 3).

4.2.1 Types of biomechanical testing

Bone is an anisotropic material and as such dependent on the direction of any applied load (Turner *et al.* 1999; Hoffmeister *et al.* 2000; Swadener *et al.* 2001; Yeni *et al.* 2004). It is because the type of loading has such a great influence on the mechanical behaviour of bone that the basic classification for biomechanical testing is determined by load direction. This generally includes the five loading types: compression, tension, torsion, shear and bending - all of which can be evaluated by an appropriate test (Oksztulska-Kolanek *et al.* 2016). In addition to the mechanical behaviour of bone depending on loading type, bone is also strain-rate dependent which indicates that bone behaviour is determined by the rate of the applied load (Currey, 1975; Currey, 1989; Shaffler, 1989; Margulies and Thibault, 2000; Ferreira *et al.* 2006; Hansen, 2008; Vaughan, 2017; Cheong *et al.* 2017).

4.2.2 Bending Test

There are two common types of bending configuration: three-point bending and four-point bending (Figure 4.4). Specimens used for bending tests are generally in the shape of prismatic beams, which are beams with a constant cross-sectional shape along their length or whole bones (generally long bones) which are irregular in shape.

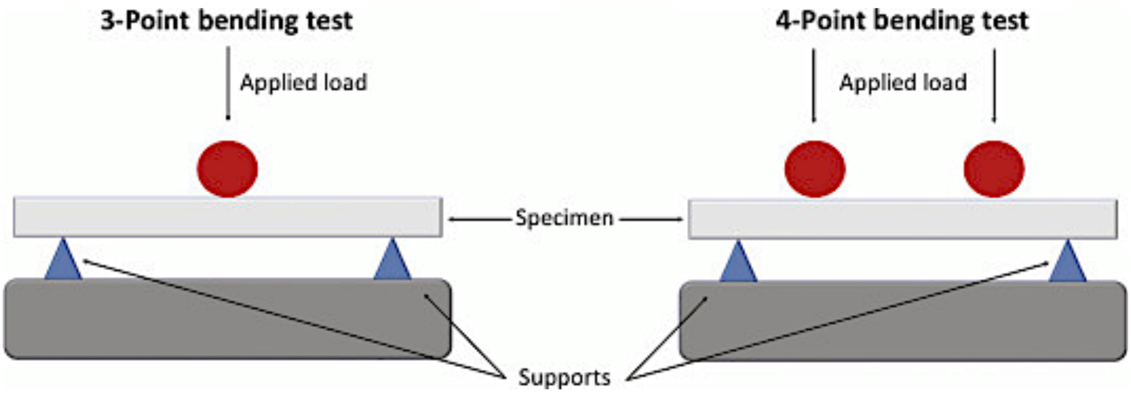

Figure 4.4: General specimen and loading configuration for three-point point bending (left) and four-point bending configuration (right; Narayan , 2019).

Due to the relative simplicity in specimen preparation and experimental setup in comparison with other mechanical tests, bending tests are widely used for the testing of biological tissues and materials (Wang *et al.* 2010). However, since bending specimens are also subjected to tension, compression, and shear forces during testing, the flexural modulus (e.g., equivalent of Young's modulus in bending) and flexural strength (e.g., bending strength) are usually not equivalent to the elastic modulus (e.g., Young's modulus) and strength measured and recorded during other mechanical tests (Wang *et al.* 2010).

An attempt to reduce shear during bending tests requires using a specimen or bone longer in length than the cross-sectional dimension of the specimen or bone (Wang *et al.* 2010). This reduction in shear is crucial because shear forces can lead to distortions and stress concentrations that may affect the accuracy of bending measurements, potentially masking the true flexural properties of the material. In three-point bending tests, the specimen is positioned onto two supports while a single-pronged impact head is applied to the opposite surface at a point precisely in the middle between the two supports (Figure 4.4). In a three-point bending regime, maximal load generally occurs at the point of the applied load (namely the impact head), and as such the bone will ultimately fracture at this location (Sharir *et al.* 2008). The alternate four-point bending configuration is similar but with the exception that the load is applied by two impacting heads placed at equal distances from the mid-point of the specimen or bone (Figure 4.4). The predominant advantage of four-point bending is that the segment of the bone between the two impact heads is subjected to a uniform moment, creating a pure bending moment (Sharir *et al.* 2008; Wang *et al.* 2010). However, the advantage of the three-point bending test is the plainness and ease of configuration. While the configuration's disadvantage is creating high shear stress near the midsection of the bone, four point-bending tests requires equal force at each loading point, which is difficult to achieve in the case of whole bone testing (Turner, 2001). As such, the three-point bending test is more frequently used to evaluate the biomechanical properties of the long bones (Turner, 1993; 2001; Sharir *et al.* 2008; Wang *et al.* 2010). It is also worth noting that due to its popularity three-point bending enables more comparative results between studies, something severely lacking in the already scarce literature on immature bone.

4.2.3 Compression test

A compression test constitutes the perpendicular application of a load onto a whole bone in such a manner that the whole bone is compressed (Wang *et al.* 2010). While a substantial portion of the published literature has investigated the mechanical properties of cortical bone and trabecular bone under compression, available studies concerning compression or axial loading tests on whole long bones has been conducted with limited success (Linde *et al.* 1988; Røhl *et al.* 1991; An and Draughn, 1999; Bayraktar *et al.* 2004; El Masri *et al.* 2012). This is likely due to the irregular and non-linear structural geometry of whole long bones, which - when subjected to axial loads - mostly undergoes bending rather than pure compression (Bilo *et al.* 2010). When a fracture is caused by compression, the bone generally fails because the compression load is not completely travelling along the central axis of the bone (Carter, 1984). In such a scenario, the compression load will cause the bone to bow which results in tension on one side, ultimately determining the nature of the failure (Bilo *et al.* 2010).

Currently, multiple publications detailing the axial compression properties of long bones (real or composite) have reported difficulties in producing reproducible bone alignment without a high variability in the testing set-up (Cristofolini and Viceconti, 2000; Heiner and Brown, 2001; Aguel, 2004; Heiner, 2008; Gardner *et al.* 2010). Within the axial compression studies conducted, the tested bone tended to be potted in a natural position only for the femoral tests; Cristofolini and Viceconti (2000), Heiner and Brown (2001), and Heiner (2008) held the femoral diaphysis at 11º from the vertical in the direction of adduction (proximal to distal), while Gardner *et al.* (2010) adducted their femoral specimen at 10º from the vertical, although this change was not observed to have had any noticeable differences in the results. Aguel (2004), which remains the only study to have conducted compression tests on immature porcine femora, was found to have potted each of the distal ends and aligned the entire bone by using a straight edge rule to ensure that the midline of the femoral shaft was properly aligned. Whilst the reported mechanical responses have differed depending on the study, the exhibited fracture patterns have been consistent with clinical reports (See Chapter 2, section 2.2 for more information).

4.2.4 Torsion test

A torsion test constitutes a mechanical testing method for determining the shear properties of a material or whole bone (Wang *et al.* 2010). In the preparation of a torsion test, the bone is prepared by firmly embedding the epiphyseal ends of the tested bone in rectangular or cylindrical blocks of plastic material which are fitted into the grips of the torsion testing machine (Sharir *et al.* 2010). Using one of several available testing devices, torque (a twisting motion) is applied to one of these grips while the other is kept firm, and then the load and angular deformation are recorded (Theobald *et al.* 2012; Vaughan, 2017). This setup allows the approximate determination of the shear modulus of bone.

Torsion based biomechanical tests can be unreliable and difficult to successfully and consistently administer. As has been reported by several studies, one of the challenges in the torsional testing of whole bones is the difficulty of excluding other eccentric loading forces due the irregular geometry of bone and therefore lack of a plane of symmetry (Cristofolini and Viceconti, 2000; Gray *et al.* 2008; Cheong, 2015). To address this the aforementioned studies utilised a system of cross-rails and hinges to eliminate undesired loads generated during the testing (Cristofolini and Viceconti, 2000; Gray *et al.* 2008; Cheong, 2015). Varghese *et al.* (2011) did not include a similar set-up for the torsion testing of their bone specimens and found the results to vary from Gray et al. (2008). This is despite Varghese *et al.* (2010) testing at the same loading rate as Gray *et al.* (2008). Theobald (2012) and Vaughan (2017) also conducted torsion tests at similar loading rates and found the mechanical responses to differ but not the resultant fracture patterns.

4.2.5 Impact test

The tests reported so far can be done at a range of different load levels, but do not necessarily focus on high impact scenarios. Common causes for accidental paediatric fractures are falls, high-energy trauma during sports, and vehicular accidents. These stresses are primarily described by the levels of high energy involved and not indicative of the same level of force generally generated during day-to-day activities such as running or jumping (Currey, 1959). In building a better mechanical understanding for bone behaviour under high-energy impacts, the mechanical parameter of toughness (e.g. energy absorbed) is a key bone property (Abdel-Wahab and Silberschmidt, 2011; Panagiotopoulos *et al.* 2005; Poundarik *et al.* 2012). Investigating the energy absorbed allows for comparison across various loading conditions and thus offers an effective means of characterizing fracture thresholds for a wide range of scenarios (Bertocci *et al.* 2017). The Charpy impact test is a standardised high-energy test which can be used in determining the amount of energy absorbed by the test sample during fracture (Abdel-Wahab and Silberschmidt, 2011). Although the analysis of the impact resistance of bone is crucial in understanding the behaviour of bone under immediate impact, the literature is limited on the testing of bone and non-existent regarding immature bone (Dubey *et* *al.* 2016). A Charpy impact test is the striking of a notched or unnotched rectangular sample with a controlled weight pendulum which is swung from a set height (Figure 4.5).

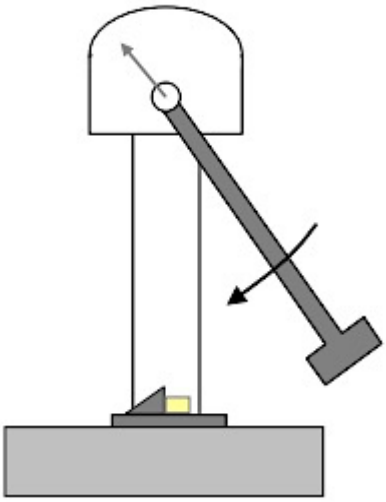

Figure 4.5: Representation of a pendulum (Charpy) impact test (Wang et al. 2010).

The test requires a rectangular sample which under standard should be 55 mm in length, 10 mm in width, and 2 mm in depth (Hughes, 2009; Jawaid *et al.* 2018). However, smaller and more alternatively measured rectangular samples have been successfully impacted and approved by the literature, should Charpy impact tests be conducted with a material such as bone where exact sizes are not possible (Wang *et al.* 2010). In a manner similar to a three-point bending test, a Charpy impact test requires the sample to be placed on two back supporting struts with the impact pendulum striking across the depth-measured face of the sample (Figure 4.6).

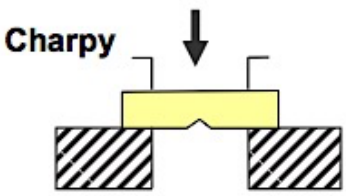

Figure 4.6: Specimen orientation during Charpy impact test (Wang et al. 2010).

After striking the sample, the pendulum continues to travel upward to a height which is lower than the release height. The energy absorbed in fracturing the sample is calculated from the differences in height and is a measure of the impact energy

(Sokolov and Alexander, 1997). Whilst the benefit of testing samples with a universal testing machine is the sheer number of properties that can be measured, Charpy impact tests still provide an effective manner in which to generate greater high-energy loads. The drawback is that measurement is limited to energy absorbed: but as standard mechanical tests provide the parameters necessary to calculate energy absorbed, comparisons can still be made between tests and studies.

The need for high-energy impact testing such as the Charpy test becomes especially relevant when considering the nature of trauma. Various types of fractures (e.g. transverse fractures) can be generated through a direct blow. As an example of the amount of force generated from a direct blow, Smith *et al.* (2000) observed that a straight rear-hand punch from a novice boxer equals around 2381 N (± 116 N); they also reported the punching force for a lead hand at 160 N (± 97). In a similar series of studies on the impact force generated by a punch, Karpitowski *et al.* (1994) recorded the maximal striking force to be 2697 N which is comparable to Busko *et al.* (2016) who recorded female and male participants to rear hand punch at 2490.3 N (± 846.2 N) and 2490.3 N (± 8462.2 N) respectively. As such, despite the limitations of a Charpy impact test, the mechanical test is an easily available option should a more up to date universal testing machine not be available.

4.3 Fracture morphology evaluation

The mechanical properties of bone will vary in fracture response depending on the direction of both the cortical and trabecular bones (Hoffmeister *et al.* 2000; Turner, 1999; Wang *et al.* 2010). Therefore, the loading type (such as bending, compression, and torsion) has a considerable influence on the fracture characteristics exhibited by both bone material and whole bones. In the clinical evaluation of child fractures, the anisotropic behaviour (the mechanical behaviour differs depending on the direction of the load) of immature bone is important since the evaluation of fracture patterns can in several ways determine the injury mechanisms. If successfully determined, it can prove crucial in verifying additional aspects surrounding a child's injury such as the injury story. This thesis primarily consists of the experimental testing of immature porcine long bones in three-point bending, compression, and torsion. Therefore, the following section is a presentation and discussion on the fracture patterns consistently observed and experimentally produced in three-point bending, compression, and torsion.

4.3.1 Three-point bending

Only the fracture patterns for transverse, oblique, and spiral fracture morphologies have been replicated using immature bone in a controlled environment under three-point bending. Pierce *et al.* (2000) was able to generate consistent transverse fracture patterns in 12 immature porcine femora under lower loading rates. Similar results were observed by Bertocci *et al.* (2017) who identified only transverse and oblique fractures when subjecting three-month old porcine femora to a loading rate of 50 mm/s. However, Foreman *et al.* (2012) observed that fractures in the paediatric popular rarely initiate at the mid-diaphysis at high-energy loading rates, instead producing oblique or comminuted fractures initiated off-centre. Oyang *et al.* (2003) conducted three-point bending tests at both low and high-energy rates; the tests were terminated when the force-time output dropped to zero, producing no fracture pattern comparable to Bertocci *et al.* (2017).

4.3.2 Compression test

At this point in time only Aguel (2004) has conducted axial compression tests on immature bone, specifically immature porcine femora (age unspecified by the author). The study found that at a loading rate of 1 mm/s all the specimens failed at the condylar growth plate with the condyles sliding to the posterior of the femur (Aguel, 2004). Compression loads have been observed to generate condylar fractures also referred to as Salter-Harris fractures (Mann and Rajmaira, 1990); this is consistent with the clinical literature. Salter-Harris fractures have been reported as primarily associated with accidental scenarios in infancy and mobile children (Mann and Rajmaira, 1990). Mann and Rajmaira (1990) have gone as far as having attributed the Salter-Harris fractures to 30% of all trauma-related fractures. However, greenstick, transverse and oblique fractures have also been observed in these scenarios (Bilo *et al.* 2010).

The effect of bowing in bone (and specifically immature bones) is generated by a force that causes tension of the convex side of the bone and compression of the opposite (concave) side. The process is generally that the bone fails on the tension side first generating a greenstick fracture if the applied loading stops, and a transverse fracture if it does not (Bilo *et al.* 2010). Should the bone fail on the concave side, other types of fractures such as the buckle fractures (an incomplete fracture appearing almost as a swelling of the bone) will occur. While oblique fractures are generally the partial result of a compression load, the fracture morphology are often the full effect of compression loads acting in concert with torque or bowing. (Pierce *et al.* 2004).

4.3.3 Torsion test

Spiral fractures have been consistently produced in the majority of the available literature (Cristofolini and Viceconti, 2000; Taylor *et al.* 2003; Aguel, 2004; Wullschleger, 2010; Varghese *et al.* 2011; Bertocci *et al.* 2017; Cheong *et al.* 2017; Vaughan, 2017). Only Pierce *et al.* (2000), who tested immature porcine femora, observed several failures at the growth plates under torsion. Kress *et al.* (1995) - who observed spiral fractures during three-point bend testing - suggested a possible correlation with torsional loads. Cheong (2015) noted that by exposing the bone specimens to one degree of freedom during torsion testing, axial loading is introduced. This suggests that the addition of axial loading during torsion testing is a possible determining factor in the continued development of spiral fractures (Cheong, 2015). Despite the majority of studies successfully generating spiral fractures, the underlying mechanics which induces these during torsion remains largely unknown and the findings from Pierce *et al.* 2000 remain unexplored.

4.4 Experimental and equipment conditions

Based on the physical conditions required for the testing of immature porcine cortical bone and immature porcine whole long bones, a protocol was devised and

implemented for this thesis. While the mechanical testing of immature porcine bone is covered in Chapters 5, 6, and 7, there were several equipment preparations required for actually conducting the experiments. These preparations were specific for the material three-point bend configuration (Chapter 5), the impact test configuration (Chapter 6), and whole bone test configurations (Chapter 7). These equipment preparations will be described and detailed in the following sections.
In explaining these preparations, the first section below (section 4.4.1) will explain the general design and construction of the customised equipment required for the experiments covered in this thesis. The next section (section 4.4.2) will explain the specific equipment construction conducted for the three-point bending material tests detailed in Chapter 5. The following section (4.4.3) will detail the process for the construction of the customised equipment used for the high-impact tests covered in Chapter 6. Finally, section 4.4.4 will cover the steps undertaken in designing and constructing the customised equipment required for testing whole bones in three-point bending, compression, and torsion (Detailed in Chapter 7).

4.4.1 Consideration for the methods

In this study, the mechanical testing of immature bone under both three-point bending and high-impact loading was conducted using bespoke, non-standardised rigs and sample preparations. Given the unique challenges associated with testing immature bone, including limited material availability and specific mechanical properties distinct from mature bone, the author adopted an approach that deviated from American Society for Testing and Materials (ASTM) standards to accommodate these constraints. This sub-chapter outlines the methodological considerations underlying these decisions while acknowledging the potential limitations these choices may impose for future replication and cross-study comparisons.

Justification for deviation from ASTM standards

Adherence to ASTM standards, such as ASTM D790 (2022) for bending and ASTM D695-23 (2024) for compression, is typically essential for ensuring consistency and comparability in mechanical testing. However, in this research, strict adherence to these standards for specimen preparation and equipment specifications was not feasible due to the inherent constraints posed by the limited availability of immature bone samples. Specifically, the small and fragile nature of these samples presented significant challenges. ASTM standards, which are generally designed around standardised, prismatic, or cylindrical material samples (Zysset *et al.* 2007), could not be strictly applied without risking the structural integrity of the specimens. Therefore, to maximize the utilisation of the available material and to enable a statistically robust sample size, nonstandard specimen dimensions were employed.

Furthermore, the available mechanical testing equipment was not designed to accommodate the unique dimensions and material properties of immature bone. Consequently, standard ASTM testing rigs and fixtures were unsuitable. To address this, custom-built rigs were developed, particularly for the three-point bending, Charpy impact, and whole bone tests, ensuring that the equipment could effectively accommodate the specific characteristics of the immature bone samples.

These adaptations were crucial in enabling effective mechanical testing despite the logistical constraints. It is important to note that while ASTM standards exist for testing orthopaedic devices, such as screws, plates, and implants using synthetic bone or bone-like materials (ASTM F1839 (2021), ASTM F543 (2024), ASTM F382 (2024), ASTM F2028 (2024), ASTM D790 (2022), and ASTM F2694 (2024)), there is no specific ASTM standard dedicated to the mechanical testing of biological, organic bone tissue itself. These standards are primarily designed to create a consistent testing environment for medical devices using synthetic substitutes that mimic bone properties, reflecting the inherent challenges of standardizing tests on biological bone. Nevertheless, it is acknowledged that the use of nonstandard equipment and specimen dimensions may limit the direct comparability of the results obtained in this study with those from other studies that rigorously follow ASTM standards.

Potential Limitations and Implications for repeatability

While the methodological adaptations employed in this study were essential for data collection given the inherent constraints, it is crucial to acknowledge the limitations they introduce. This is for future researchers to carefully consider. Firstly, the use of custom-built rigs and nonstandard specimen dimensions presents challenges to replicability and consistency. Researchers attempting to reproduce these findings may encounter variations in setup, specimen dimensions, and load distributions, potentially leading to differences in observed mechanical properties. This methodological variation could significantly hinder consistent cross-study comparisons.

Secondly, the deviation from established ASTM standards inherently limits the direct comparability of this study's results with those obtained from studies utilising standardised equipment and specimen sizes. Consequently, researchers must exercise caution when interpreting the results, particularly when comparing data such as flexural modulus and impact energy with values derived from ASTM-compliant setups.

Finally, the smaller specimen dimensions and the use of bespoke rigs may introduce potential influences on certain mechanical properties. Factors unique to this setup, such as non-uniform stress distribution or atypical load distribution profiles, could affect the observed outcomes. While efforts were made to minimise these influences, they remain an inherent limitation of the chosen methods, and their potential impact should be carefully considered when interpreting the results. The limitations of this study, including the use of custom rigs and non-standardised dimensions, highlight the need for further research. Future studies should focus on validating these findings, and on exploring how the results translate to forensic investigations.

One of the core issues encountered with the flexural (three-point bend) tests was the need to deviate from an ideal “depth to span ratio”. This relates to the dimensions of the specimens (principally their depth) with respect to the distance between the support points. There was very little option for any alternatives, and the decision was taken to go ahead principally on the assumption that absolute values of properties, although of key value to the present (and future) studies, were less important than a confirmation of an age related trend, in those values, and that such a trend would still be visible in the results, even if absolute values were deemed unreliable. That said, steps were taken to address the non-ideal depth to span ratio, principally in the form of mathematically derived corrections, which are fully described in Chapter 5. The ultimate outcome of these corrections is discussed in Chapter 8 but was positive and provided the author with plausible absolute data in additions to the trends seen.

4.4.2 Design and construction

1. In order to construct the equipment used for the experiments detailed in Chapter 5, 6, and 7, 3D designs containing equipment dimensions, material, and threading was formulated using a computer-aided-design software (CATIA, version 5).
2. All 3D designs were converted into 2D drawings for construction.
3. The construction of the mechanical equipment used in this thesis was conducted in cooperation with the mechanical workshop at the UCL Department of Mechanical Engineering (August to November 2018).
4. Construction of the equipment was conducted using a CNC machining mill.

4.4.3 Three-point bending tests (material)

1. A miniature test rig was designed and developed in August 2018 at the UCL Department of Mechanical Engineering (Figure 4.7; measurements length * width * height was 5 cm * 3 cm * 3 cm). Specifically, the design was created using two steel blocks which could be adjusted to the definite span length of each sample using a threaded bolt.

A customised impact head was fashioned so that impact to the samples could be applied parallel and fixed, and with no force being absorbed by the bending or motion of the rig blocks (Figure 4.7; August 2018).

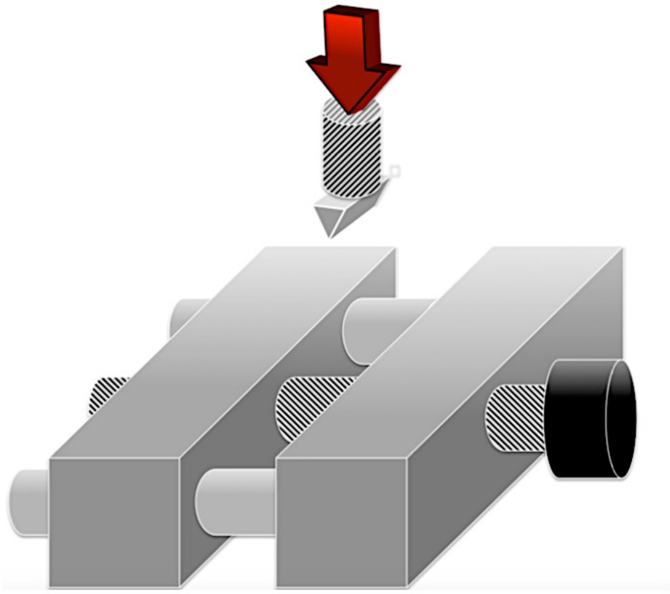

Figure 4.7: Three-point bending (material) design. Loading conditions are presented in red.

4.4.4 High-energy impact tests

1. Impact testing was conducted using a Zwick 5102 Pendulum Impact Tester (Zwick). However, the Zwick impact tester was found incapable of accommodating the sample sizes used for the high-energy impact tests.
2. Miniature holders were therefore fashioned to accommodate and hold the small samples prior to impact (September 2018; Figure 4.8; measurements of holders in Length from longest point * Width * Height was 5 cm * 2 cm * 3 cm).
3. To allow for the pendulum and hammer to both impact the samples and move freely between the miniature holders, a new pendulum rod measuring 225 mm (+ 5.9 with impacting hammer) and hammer (.1087 kg; .211 kg with the rod) was designed, constructed and fitted onto the existing Zwick measurement system (September 2018; Figure 4.8). These measurements were in turn used in Chapter 6 to calculate a loading speed of 2.93 m/s.

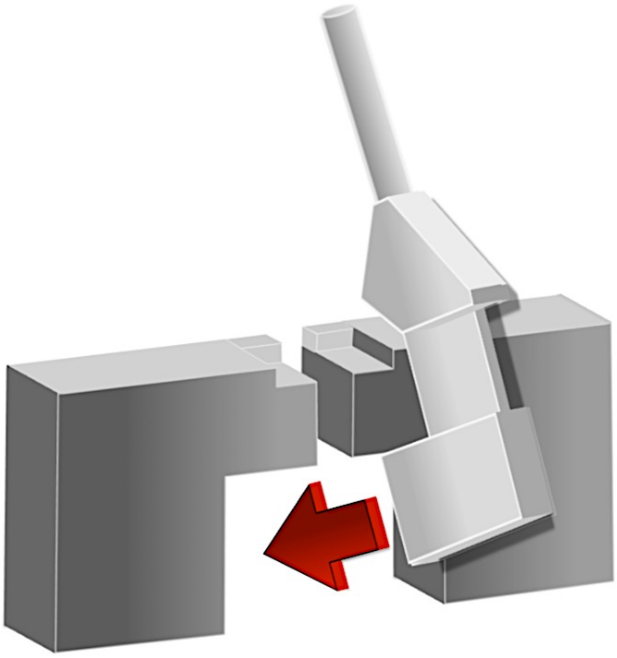

Figure 4.8: Customised impact test holders along with the hammer.

4.4.5 Whole bone tests

1. Given the non-uniform shape and size of whole long bones, supportive holders were designed at the UCL Department of Mechanical Engineering (October 2018; Figure 4.9). Specifically, these holders were designed to provide a hard exterior for gripping but also a hollow interior, which could be filled with bone cement, a mouldable material used for the mechanical fixation of the bone specimens. Two models of holders were designed and manufactured in consideration of the mean distal and proximal measurements of all the tested whole bone specimens (4 mm * 4 mm; 8 mm * 8 mm).

Each whole bone specimen tested in this thesis was embedded in a holder. The specific test set-up for the three-point bending and compression experiments are detailed in Chapter 7.

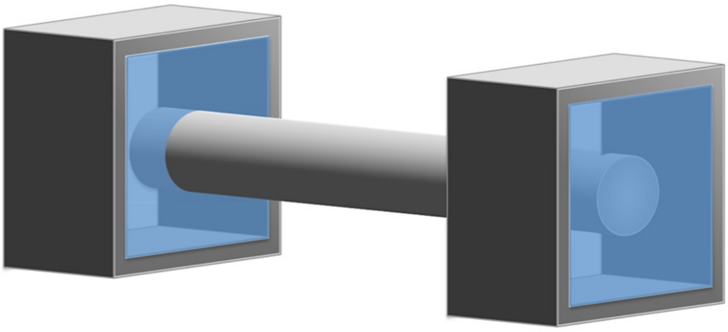

Figure 4.9: A cylindrical sample embedded for three-point bending, compression, and torsion testing.

2. To allow for controlled torsional motion during torsion testing (Chapter 7), a specialised torsion rig was designed and constructed at the UCL Department of Mechanical Engineering (November 2018; Figure 4.10). The torsion rig was specifically designed to fit and secure both holder sizes (The measured size of the rig by length * width * height was 25 cm * 12cm * 15cm).

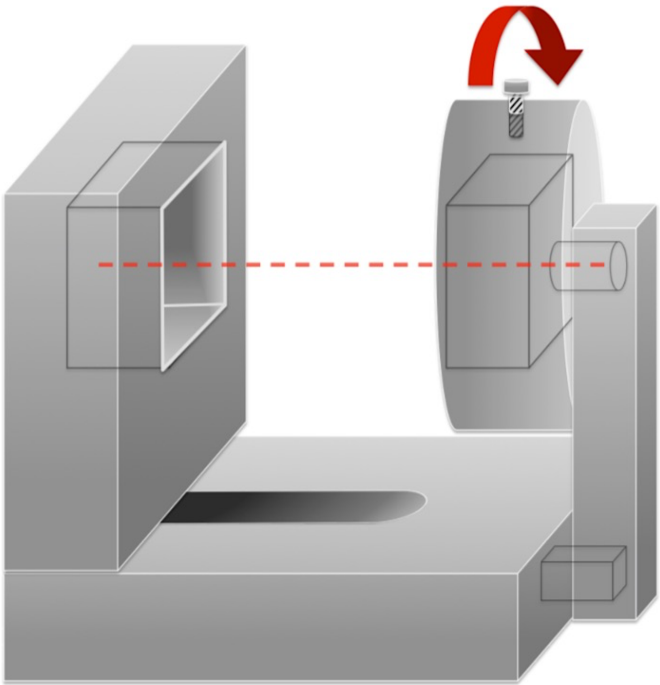

Figure 4.10: The designed and developed custom-based torsion rig.

1. The torsion design and rig consisted of one cylindrical attachment which allowed for free rotation.

3. A second attachment was designed and positioned to hold the opposite end of the bone in a fixed position, hence eliminating any potential translational motion supplied by the cylindrical attachment.
4. The cylindrical attachment was then connected to an Instron 3300 Series universal testing machine using a cable wire. Through the motion of the testing machine in tension, a controlled torsional movement was in turn generated through the cylindrical attachment, and inserted bone. The information was recorded and analysed according to the data quantification detailed in Chapter 7.

4.5 Conclusion

The material and methods described in this chapter have been specifically reviewed and chosen for the purpose of evaluating the mechanical behaviour of immature porcine bone. The experimental data produced by these tests will add to the current body of research surrounding immature bone behaviour under mechanical load. However, it is noted the method does involve the creation of custom testing rigs for mechanically testing bone samples of a unique size. These decisions were made in order to maximise the amount of available testing samples but also at the expense of following more standardised test protocols and sample dimensions. This chapter has primarily concerned the preparation, construction, and reasoning behind choice of method. It will therefore inform the following three subsequent chapters (5,6, and 7) which are the experiments conducted for this thesis.

Chapter 5 Mechanical Properties of Immature Porcine Bone Under Three-Point Bending

Research Question 1: What are the basic underlying mechanical properties of immature porcine bone as a material?

5.1 Introduction

A comprehensive understanding of immature bone fracture mechanisms necessitates a multi-level approach, encompassing both intrinsic and extrinsic bone properties. This chapter adopts a foundational approach, beginning with the mechanical evaluation of bone material, which will serve as a stepping stone towards understanding whole bone behaviour. Bone can be understood across multiple levels, beginning with collagen fibrils, progressing to material bone classifications like cortical and trabecular bone, and culminating in identifiable whole bones such as the femur and humerus (Suresh, 2001; Wu *et al.* 2012). As discussed in section 2.4, bone fulfils a variety of functions, and these functions are in turn a product of the physical geometry of bone as well as its material properties. An example is the specific design of long bones which are not only geometrically different from flat bones but structurally different as well (Weiner and Wagner, 1998). Specifically, bone has evolved to fulfil a variety of functions which in conjunction with the geometry of whole bones are fine-tuned and functional; however, the relationship between the material bone and the individual whole bone is as of yet not fully understood, especially for developing bone where the mechanical behaviour of the material differs from the mechanical behaviour of adult material bone (Weiner and Wagner, 1998).

The effect of applied loads to developing bone is, as detailed by the literature, an ongoing research focus and is dependent on factors such as the age of the bone, the bone's anatomical site and geometry, and the rate of applied loading (Cheong *et al.* 2017). Case reports and experimental tests contain several examples of where whole immature bone have fractured, and others have not despite the same observed injury mechanisms (Zhang-Forestier and Bishop, 2015). One proposed explanation is a difference in 'bone strength' which by engineering standards is the ability of a bone to resist an applied load without failure. However, the mechanical definition of 'bone strength' cannot be used entirely to explain why a bone fractures (Currey, 2002). Whole bones are not only varying in size and shape, but structurally composed of a complex composite material whose mechanical behaviour is different depending on the situation (Currey, 2002; Jepsen, 2003). As such any venture into the analysis of immature bone should begin at the most detailed level possible and proceed upwards in scale. This thesis will therefore begin with the mechanical testing of developing bone on a material level before proceeding to whole bones.

A comprehensive understanding of immature bone mechanics benefits from a detailed analysis of bone mineral density (BMD) and microstructural characteristics alongside mechanical testing. It would allow for a more thorough understanding of the structure-function relationships influencing bone behaviour. Understanding the structure-function relationship is crucial because it allows us to link the mechanical behaviour of bone to its underlying structure. For example, changes in bone microstructure can affect its stiffness and strength, which in turn influences how it responds to mechanical loading. Due to the tiny size of many of the porcine whole bones used in this study, and the need to ensure enough repeat specimens could be obtained for variety of mechanical tests (three-point bend, impact, and a number of pure compression tests), the decision was taken early on to employ CT scanning as both a precautionary measure (i.e., to provide a detailed digital record of each bone used in the study) and a means of determining BMD. A number of studies were consulted where a measure of BMD had been obtained from CT scan data, some of which appeared to offer promise for the present study (Schreiber *et al.* 2014; Hendrickson *et al.* 2018). After completion of the X-ray CT tomography and all the mechanical testing, the x-ray methodology for BMD determination was attempted but showed absolutely no statistically significant variation in BMD (as applied to the cortical sections of the bones) with age, although it readily confirmed the thickness of the cortex of the mid-section thickened with age (See Discussion, section 5.6).

This disappointing outcome was most probably a function of too low a resolution of the x-ray tomography employed, although upon further investigation, it became apparent from the literature, that BMDS measurements of an x-ray CT scan are more likely to be the measure of bone density per, rather than mineral content (Lee *et al.* 2013; Patel and Lee, 2016; Alawi *et al.* 2021). In hindsight, if the fractured test specimens had been kept and not discarded after testing was completed, a true BMD determination could possibly have been attempted (although the specimen dimension may have been insufficient for a statistically robust assessment). Notwithstanding this, the lack of BMD data and any related trends, does not, it itself, invalidate the mechanical test data observed but only (arguably) cloud the interpretation of the trends seen.

From a forensic and paediatric perspective, interest in immature behaviour is always important but special consideration can be given children under the age of 3 years (Khoriati *et al.* 2016). It is a period in which children become mobile yet remain heavily in the care of adults. It is during such a period in which potential fractures can have multiple meanings (Worlock *et al.* 1986; Lyons *et al.* 1999). Documented mechanisms of injury include accidental falls from heights or the violent manipulation of arms and legs, all of which can showcase the same type of injury patterns (Bertocci *et al.* 2017; Bilo *et al.* 2010).

It is important to note that this study has focused on the mechanical behaviour of immature bone, and while the findings are relevant to forensic contexts, they cannot directly distinguish between accidental and non-accidental injuries. The interpretation of injury mechanisms in such cases requires a comprehensive assessment of various factors beyond mechanical testing alone. Specifically, the femur and humerus are two injury sites common in both accidental and non-accidental scenarios (Bilo *et al.* 2010). King *et al.* (1988) observed that overall, fractures of the diaphysis occur four times more frequently than metaphyseal injuries, with the femur and humerus being the most common injury sites. While it has been noted that femoral shaft fractures consist of only 28% to 45% of all isolated long bone fractures, the risk that a femoral fracture is the result of abuse has been reported to be as high as 92% (Loder and Bookout, 1991; Carty, 1993; Rex and Kay, 2000). Humeral fractures have in turn been reported to carry a risk of being abusive in 48% of diagnosed cases involving a fracture (Kemp *et al.* 2008). Given this information, the mechanical evaluation of cortical bone, the primary type of bone found in the shaft of long bone, suggests it as a good starting point.

An obstacle in the experimental analysis of paediatric fractures is the lack of human tissue. To this author's knowledge, Currey and Butler (1975) remains the only study to have mechanically tested the material properties of human infant bone material under 3 years of age. Currey and Butler (1975) conducted three-point bending tests on machined femoral specimens between 2 and 48 years of age. Whilst Currey and Butler (1975) observed a general decrease in flexural stress with age and an increase in elastic modulus with age, the sample size was limited. In consideration of the scarcity of human infant tissue the use of animal models offers an alternative source for tissue. As has already been mentioned (Chapter 2, section 2.13.3), the pig (porcine) is an animal commonly used in forensic and biomechanical research and particularly the immature porcine has shown material properties comparative to human infant bone (Margulies and Thibault, 2000; Coats and Margulies, 2003; Baumer *et al.* 2009). However, proposed age correlations between human and porcine bone have differed depending on the types of tissues and skeletal sites tested (Dobbing, 1974; Margulies and Thibault, 2000; Coats and Margulies, 2003; Franklyn, 2007; Baumer *et al.* 2009).

In the evaluation of the mechanical behaviour of immature bone, age remains a constant factor of consideration. Several studies have reported that cortical bone becomes more brittle and weaker with age (Currey and Butler, 1975: Burstein *et al.* 1976; Mcalden *et al.* 1993; Tommasini *et al.* 2007). Similar age-related effects have been reported regarding trabecular bone (Nagaraja *et al.* 2007). In a nanoindentation study of mice in the age range of 3 to 77 to weeks where the samples were grouped into age categories of less than 12 weeks, 13 to 50 weeks, and 50 to 77 weeks, the elastic modulus was found to decrease with age. Furthermore, evidence that paediatric bone exhibits fracture patterns different to adults has also been thoroughly documented (Bilo *et al.* 2010; Ogden 2000). However, whilst distinct differences have been observed between immature and adult bone, the continued changes which occur in bone during development also makes potential differences between the earlier age groups an area of interest (Altai *et al.* 2018).

5.2 Chapter aims

In consideration of all the possible factors which can determine the mechanical properties of immature bone, the aim of this chapter is to investigate one of those potential factors, age. More specifically, this entailed the mechanical evaluation of immature cortical bone under three-point bending at different ages but also the observation of potential differences between cortical bone from different anatomical sites. In consideration of certain types of bones, the humerus and femur are both long bones, but they do differ in function (Currey, 2002). The effect of these functions on a material level have not been fully explored and raises questions regarding mechanical behaviour and physical exposure (e.g. Wolff's Law; Woo *et al.* 1981). Wolff's Law states that bone adapts its structure to resist the forces placed upon it, meaning that increased mechanical stress leads to increased bone density and strength. Therefore, variations in functional loading could have significant impacts on bone material properties. Since bone has been reported to be a strain-rate dependent material, the mechanical behaviour of bone will differ depending on the magnitude of the applied load (Cristofolini *et al.* 2010; Cheong *et al.* 2017). For example, Raftopoulos *et al.* (1993) documented the Young's modulus to increase by 10% when the loading rate is raised by 3 orders. However, little information has been published on the strain-rate dependency of immature bone and none on how loading rate may affect the mechanical behaviour of immature bone material across age (Cheong *et al.* 2017).

Based on the literature the following hypotheses are proposed:

Hypothesis 1 — Immature porcine cortical bone samples tested under three-point bending at the loading rates of 5 mm/min, 50 mm/min, and 100 mm/min will show a significant age-related difference for the mechanical properties: force at fracture, flexural strength, and flexural modulus.

Hypothesis 2 — The behaviour of immature porcine *femoral* and *humeral* cortical bone samples will under three-point bending at the loading rates

5 mm/min, 50 mm/min, and 100 mm/min show no significant difference across ages.

The observed age-related changes in the mechanical behaviour of immature porcine bone may offer potential insights into the biomechanical properties of paediatric bone. This study contributes to the growing body of knowledge regarding the biomechanical properties of immature porcine bone and provides a foundation for future research. Combining these bone material testing approaches with the assessments of immature bone material at higher loading rates and whole bone in later chapters 6 and 7 respectively will contribute to a more comprehensive understanding of immature bone mechanics. This research can help identify features of bone that are important to consider in future research regarding fracture mechanics.

5.3 Material

5.3.1 Specimen collection and preparation

Fifteen British Large White porcine males from the age range of birth to 40 days were acquired through the assistance of the British Royal Veterinary College. It was decided prior to testing that in consideration of potential variances in size and growth rates between porcine breeds and sex, all the subjects were to be male and Large White. Each femoral and humeral bone was disarticulated and removed immediately upon death and macerated of all soft tissue using a surgical scalpel and pair of tweezers. Whilst manual maceration remains documented as a more time-consuming and physically demanding maceration technique, the method requires no supplemental treatment (e.g. chemicals or enzymes) which may cause unwanted changes to the bone (King and Birch, 2015; Couse and Conno, 2015). Due to the requirement for storage off-site, all specimens were wrapped in saline soaked gauzes, placed in polyethylene bags, and frozen at -20° C. As has been documented by Zhang *et al.* (2018a) and Li *et al.* (2013), the maintaining of specimen hydration is crucial for material testing as fresh bone and fresh-frozen bone has both shown a requirement for saline to maintain cell viability. The added usage of polyethylene bags offers the additional protection and shielding from frost damage and possible bone necrosis. In lieu of observations made by the published literature, the storage of fresh bone at a temperature of -20° C has been documented as having a less destructive impact on the mechanical properties of bone (Torimitsu *et al.* 2014; Topp *et al.* 2012; Pelker *et al.* 1984). See Chapter 4 for further details regarding specimen procurement and preparation.

5.3.2 Sample preparation

Each specimen was thawed in room temperature 12 hours prior to testing. In adherence to Currey and Butler (1975) and the subsequent three-point bending tests, rectangular samples were excised from the specimen diaphysis in the longitudinal direction relative to the bone's in vivo position (Figure 5.1). All samples were excised using a diamond coated cutting blade under constant saline drip and were taken from the diaphysis midpoint measured from each specimen's opposite epiphysis. This included slicing through the whole bone laterally and then longitudinally.

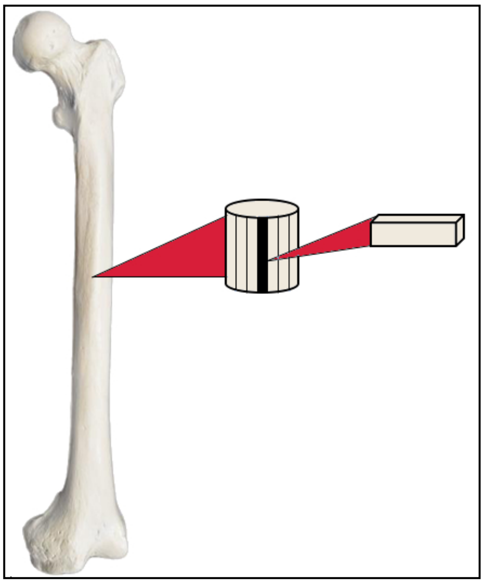

Figure 5.1: Subsequent removal and machining of bone samples to rectangular shapes (Reformatted from Bianucci et al. 2016).

To ensure a uniform sample geometry, each sample was machined to a testable and comparative measurement (Table 5.1) and placed in saline solution prior to testing (Figure 5.1). Regarding standard material testing this study has deviated from the sample sizes utilised in Currey and Butler (1975; Table 5.1). This makes any direct comparison difficult. The reason for this departure in standard was to procure as many test samples as possible. Since the aim of this study was foremost to observe any mechanical changes in the samples used, uniformity in the samples tested herein was prioritised. Although achieving exact sample sizes during the sanding process proved challenging (as indicated by the standard deviations in specimen dimensions in Table 5.1), maintaining a uniform distance between the 3-point bending supports was considered sufficient to ensure that the test results would be comparable across samples.

Core to preparing a suitable specimen was to ensure no cancellous bone was present and only the dense cortex was being tested. Grinding excess material off was therefore performed on the cancellous inner surface first, until all traces of porosity disappeared. Often this was not 100% possible without losing too much wall thickness, and the presence of some porosity in the thickness direction of the specimen cannot be discounted, at least for some specimens. Such an occurrence may well have contributed to the large scatter often seen in tests results and some of the non-linearities seen in the stress-strain responses, and is discussed further in section 5.6

Table 5.1: Means and standard deviations (in parentheses) of the measurements of Currey and Butler (1975) and all the samples tested in this study.

Study	Length (mm) * Depth (mm) * Width (mm)
Currey and Butler (1975)	23-26 * 2 * 3
Colas Aberg (2019)	6.71 (± .85) * 1.92 (± .42) * 2.49 (± .66)

5.4 Method

5.4.1 Three-point bend testing

All the rectangular samples were subjected to three-point bending using a Hounsfield testing machine (HKS fitted with a 2.5kN load cell) at loading rates of 5 mm/min, 50 mm/min, and 100 mm/min (Figure 5.2). These rates were chosen in consideration of the literature and whilst no exact comparison can be made with these studies, choosing these rates offered the opportunity for some speculative exploration of results against them (Currey and Butler, 1975; Cheong *et al.* 2017). Using a custom-developed support rig for sample size purposes, each sample was mounted at a support span of 4 mm to accommodate uniform testing conditions across the various sample sizes (measurements of the support rig by length * width * height was 5 cm * 3 cm * 3 cm. The support span of 4 mm chosen based on the length of the smallest sample tested in this chapter. All samples were loaded at the measured midpoint with the impact head striking perpendicular to the samples and bone's longitudinal direction. Although this position was not mechanically determined (via a fixed arrangement) and so had to be set largely by eye. Errors in setting this centre position could well have led to considerable scatter in some of the mechanical test data, and this aspect is discussed further in section 5.6. Failure was determined as an abrupt and observable change in force and displacement.

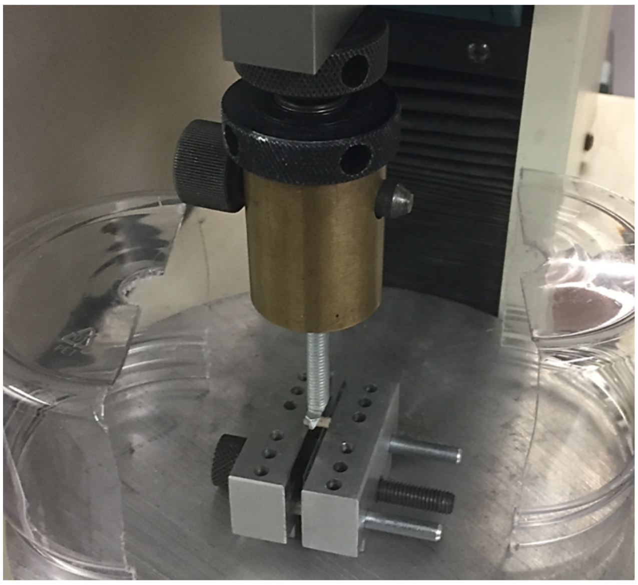

Figure 5.2: Three-point bending set-up.

5.4.2 Data quantification

Data quantification was conducted following Spatz *et* al. (1996), using a modified classic beam theory. Classic beam-theory acts under the assumption that bone is a homogenous material which exhibits linear elastic behaviour and is perfectly rectangular or cylindrical in shape with a uniform cross-section (Cordey *et al.* 2000; Van Lenthe *et al.* 2008). However, since bone exhibits non-linear properties the assumption that bone does not, may lead to an inaccurate identification of bone's material properties. On the other hand, the optimisation approach has, for approximately the last two decades, been used to derive material properties which, due to its consideration of geometry, boundary, and dynamic effects, makes it an option for generating accurate material results (Krone and Schuster, 2006; Hu *et al.* 2009; Guan *et al.* 2011; Zhang *et al.* 2018b).

Classical beam theory is still predominantly used due to its efficiency in solving straightforward mathematical equations to calculate material parameters. It has not been exactly established how erroneous classical beam theory may be when used to obtain material parameters with three-point bending test data (Van Lenthe *et al* 2008). However, if the error is small enough, classical beam theory does provide a reasonable option for the data quantification. In consideration of the published literature, classical beam theory has been found in several well-designed studies which included the estimation of the material properties of cortical bone of long bones (Unger *et al.* 2010; Albert *et al.* 2012; Kourtis *et al.* 2014; Ramezanzadehkoldeh and Skallerud, 2017).

Van Lenthe *et al.* (2008) observed that the tissue modulus was underestimated by 29% when using beam theory in three-point bending tests, with specifically geometry and size having a substantial effect on classical beam theory-derived tissue moduli. Furthermore, Guan *et al.* (2011) found classical beam theory to produce a lower elastic modulus. However, the bone specimens used in both studies were both adult or whole bones (Van Lenthe *et al.* 2008) or cranial bone (Guan *et al.* 2011). Whole bones are both complex in geometry and differing in size and shape whilst cranial bone is generally internally porous and curved. Therefore, it is recommended that even though classical beam theory can and is used for analysing whole bone behaviour the results represent the simplest case possible (Cheong, 2015). However, since material testing allows for the cortical bone of the femur and humerus to be machined to nearly uniform rectangular shapes classical beam theory was deemed a suitable method, certainly for the bone 'material' tests.

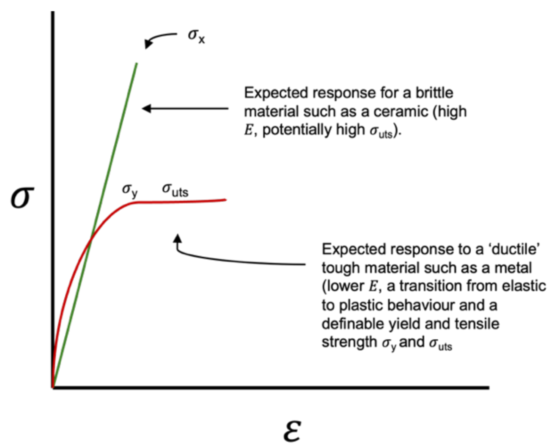

Figure 5.3: hypothetical stress (σ) – strain (ε) responses, for a brittle material (linear to failure) and a ductile material (such as a metal).

Two main properties were targeted using the bend tests – stiffness (E) and strength. As previously discussed in section 3.4.5, the stiffness of a material (also known as the Young's modulus) is traditionally defined as the gradient of the linear elastic portion of a stress-strain response, whereas strength is variously defined depending on the shape of the response, and for brittle materials, such as bone, would normally be the maximum stress applied – hence the stress at which failure by fracture occurred (σ_f). For comparison figure 5.3 below shows two hypothetical stress (σ) – strain (ε) responses, for a brittle material (linear to failure) and a ductile material (such as a metal). The metal's response allows the definition of an additional 'strength', the yield strength (σ_y) which marks the transition between elastic and plastic (permanently deformable) behaviour. The maximum stress applied is now defined as the ultimate tensile strength (σ_{uts}). Such responses are normally measured by conducting a uniaxial tensile test where the specimen is gripped and pulled along its main axis. For brittle materials, such as bone, the act of gripping a specimen can easily affect the σ-ε response and hence the measured values, so a bend test is substituted, where the specimen is loaded between supports (anvils). This was methodology adopted for the bone material tests conducted in this thesis. Classical beam theory can be used to obtain values of σ and ε that are numerically equivalent to those obtained from a uniaxial tensile test and hence plot a similar response to those seen in figure 5.3.

The basic arrangement for a three-point bend test was shown earlier in figure 4.4 and again below for clarity (Figure 5.4), with core dimensions shown. This particular arrangement utilises two supports and a single central load is applied midway been the supports. As discussed in section 4.2.2 this could be replaced with two loads, side by side in a configuration known as four-point bend, which can help improve reproducibility, but requires more space and would have been difficult to implement for the present study, given the diminutive size of the specimens.

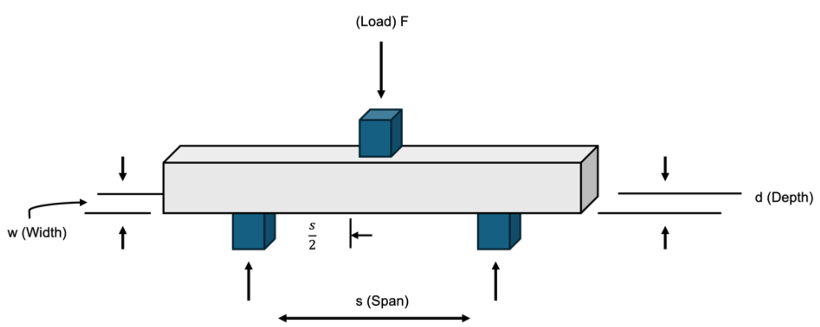

Figure 5.4: Three-point bend configuration utilising bone sample shape

To calculate E, whether in a tensile or bend test you need a continuous measure of stress and strain. For a linear tensile test, the specimen stress (σ) is defined as $\frac{F}{A}$ where, F = tension force and A = cross-sectional area of the sample, and the strain is defined as $\frac{\Delta L}{L}$ or extension over original length (ε). As such E (Young's Modulus, Modulus of elasticity) = $\frac{\sigma}{\varepsilon}$ where σ = stress and ε = strain. E is thus obtained by measuring stress and strain in the elastic region (assuming the material is linearly elastic) and evaluating the ratio of $\frac{\sigma}{\varepsilon}$.

For a bend test, the calculation of E is different but again requires an evaluation of stress and a strain in the linear elastic portion of the σ-ε response. The value of the strain is calculated using:

1)

$$\varepsilon_f = \frac{6Dd}{S^2}$$

Where D = measured deflection at the midpoint, d = depth of the sample, and S = the span. Note that ε_f defines as the flexural strain (not strain at failure) and is the strain along the bottom surface of the specimen. Also note that F (the force applied), does not feature in this equation.

Similarly, we can define a flexural stress, σ_f, which relates to the maximum stress experienced by the bend specimen along its bottom surface. This is given by:

2)

$$\sigma_f = \frac{3FS}{2Wd^2}$$

Both these equations come from standard mechanics treatments of simply supported beams. If one considers a linearly elastic material. Given values of ε_f and σ_f, it is now possible to define a flexural stiffness (called the flexural modulus) as the ratio of $\frac{\sigma_f}{\epsilon_f}$ which is numerically equivalent of the Young's Modulus E.

Crucially, the value of this flexural modulus (which we will now refer to as $E_{apparent}$ or E_{app}) is only the same as E, the Young's Modulus, if the beam is a slender one, and the deflections generated are small. Once the beam becomes substantially fatter with respect to the distance between the supports, and the so called "span to depth ratio" falls below 10, errors occur due to the development of significant shear stresses in the beam, which resist deflection. If we define this "apparent" stiffness (E_{app}) as $\frac{\sigma_f}{\epsilon_f}$ then substituting equation 1 and 2 gives:

3)

$$E_{app} = \frac{Fs^3}{4wd^3D}$$

Where D = deflection at the midpoint. The values of D and F can be taken directly from the load-displacement response of the three-point bending test

The specimen sites chosen for the present study were known from the start to be 'unfavourable' in terms of their aspect ratio, and the span (s) that was feasible. As has been discussed before, there was little choice in this, given the experiment rig was "sized" for bone samples taken from birth onwards. The expectation that the unfavourable span to depth ratio (which was often lower than two) might significantly alter the absolute values of the stiffness (E) measurements was clear, although the magnitude to which this might manifest itself, was not, however the hope (as discussed previously) would be that this would not fundamentally alter the trends seen with age, so three-point bending tests has commenced with the aim of obtaining E using the equations already detailed.

It should be noted that such errors were not expected with the strength at fracture (σ_f) given the literature on span to depth ratio focussed solely on stiffness Subsequently to the first round of testing, preliminary calculations revealed that E measurement were several orders of magnitude to small (Typically MPa rather than

GPa; Currey and Butler, 1975), but mindful that one of the work's core aims was to determine age trends, the testing continued, and the results reported. Following consultation however, it was felt that extra credibility for the conclusions would occur if an attempt was made to correct for the unfavourable span to depth ratio. And these efforts are described in the next section.

5.4.3 Theoretical corrections to bend test data

For 'fatter' beams, a more comprehensive treatment that factors in the contribution to the deflection from shear stresses was required, as highlighted in section 5.4.2. Analysis provided by Timoshenko and Goodier (1970) and later reproduced by Spatz *et al* (1996) was therefore applied to the bend test results.

Thus here, for a beam in three-point bend, the deflection at the centre can be expressed as:

4)

$$D = \frac{FS^3}{4E_\infty wd^3} + \frac{3FS}{10Gwd}$$

Where E_∞ is the flexural modulus for an ideal (infinite span to depth ratio) beam (i.e., the "true" Flexural modulus) and G = the shear modulus – the resistance to deflection under a shear stress.

Rearranging formula 4) and substituting in formula 3) gives (Jackson *et al.* 1986):

5)

$$\frac{1}{E_{app}} = \frac{1}{E_\infty} + (\frac{6}{5}\frac{1}{G}\frac{d^2}{s^2})$$

Spatz *et al.* (1996) successfully used this equation to obtain values for E_∞ and G, by obtaining values of E_{app} (from the three-point bend tests) at different depth (d) to span (s) ratios (i.e., by varying specimen dimensions and evaluating E_{app} via determining $\mathcal{E}_f$ and σ_f). Given that the present data set was calculated to a (nominally) constant depth to span ratio, obtaining G in this way was not possible. However, by assuming a value for G, it was deemed possible to evaluate E_∞ via equation 5) and the values for E_{app} (as obtained in the bend tests). Spatz et al. (1996) provided two possible routes to this. Those were either to assume a book value for G of 0.94 GPa (an average 'calculated' from a review of the literature data) or to assume that the ratio of $\frac{E_\infty}{G}$ is around 20, as similarly determined from an average of several studies of different species and their bone properties.

The fundamental issue with using the fixed value of the shear modulus (G) approach was that the present study was attempting to determine whether E varies with age, but if sensitive to age, then it is unfair to assume G is not also a function of age, hence invalidating the assumption that it is a constant. In addition, the intention with the analysis for the correction of E_{app} to E_∞ for a non-ideal span to depth ratio is to reach a value of E that is more representative of the literature for immature bone using 0.94 GPa for G for immature bone is therefore also likely to generate errors in calculating absolute value of $E_{\infty,}$ given the literature data was used to generate the 0.94 GPa 'average' was applicable to mature not immature bone.

All this suggested that taking $G = \frac{E_\infty}{20}$ was a better approach. Equation 5) then becomes:

$$\frac{1}{E_{app}} = \frac{1}{E_\infty} + [\frac{6}{5}\frac{1}{\frac{E_\infty}{20}}\frac{d^2}{s^2}$$

or

$$\frac{1}{E_{app}} = \frac{1}{E_\infty} + [\frac{24}{E_\infty}\left(\frac{d}{s}\right)^2]$$

$$\frac{1}{E_{app}} = \frac{1}{E_{\infty}} [24 \left(\frac{d}{s}\right)^2]$$

Which rearranges to give:

6)

$$E_{app} = E_{\infty} + [1 + 24 \left(\frac{d}{s}\right)^2]$$

This correction was applied to a small batch of values for E_{app} that were initially obtained during the first round of bend tests to check whether the analysis would return sensible values for E_{∞}. For clarity, the process of applying the correction to representative data is shown below (Table 5.2).

Average' values for Load (F) and deflection (D) were taken from the data (specimens of age category 1 at 5mm/min), together with values for span (s), depth (d) and width (w), to check the sense of the E_{app} values from calculable equation 3), Load was that at fracture, and deflection determined from the raw data at the load chosen:

$$E_{app} = \frac{Fs^3}{4wd^3D}$$

Table 5:1: Average dimensions of specimens tested at 5 mm/min and data for force and deflection.

Parameters	Values
Span (s)	4 mm
Force (F) (max)	96N
Width (w)	2.53 mm
Depth (d)	1.93 mm
Deflection (D) (at given force)	0.68 mm

Which gives:

$$E_{app} = \frac{96 \times (4 \times 10^{-3})^3}{4 \times 2.53x10^{-3})^3 \times (1.93 \times 10^{-3})^3 \times 0.68 \times 10^{-3}}$$

= 124.19 MPa

If we choose to use this and the $\frac{E_\infty}{20}$ method given in equation 6), the corrected result is:

$$E_\infty = 124.19 \left[1 + 24(\frac{1.93 \times 10^{-3}}{4 \times 10^3})^2\right]$$

E_∞ = 818 MPa or 0.82GPa

which is a considerable rise. When the full 5 mm/min data set was analysed, quantification using the corrected beam theory resulted in averages of about 1GPa for the ‘true’ flexural modulus, which is much closer to the expected book values (as witnessed in the literature) and the correction was therefore applied to the remaining strain rates. It should be noted that if, instead of using the $\frac{E_\infty}{20}$ approach, the value of G as 0.94 GPa (Spatz *et al.* 1996) was used, then the corrected values of E (i.e., E_∞) turned out to be almost identical to the initial values (i.e., E_{app}) indicating that this method is certainly not equivalent to the $\frac{E_\infty}{20}$ method, as confirmed by the pre-test using the 5mm/min specimens.

5.4.4 Additional corrections applied to bend test data

Whilst the correction for the unfavourable span to depth ratio appeared to work returning values for E_∞ close to those already published (Currey and Butler, 1975) it nevertheless still generated stiffnesses that differed significantly form accepted values. Additionally significant scatter remained in the data which caused concern and has clouded the ability to extract trends. Applying the initial set of corrections had prompted a re-evaluation of the raw data from the bend tests (i.e., load vs deflection responses as idealised in figure 5.3. In some cases, for example, significant deflection was seen initially, for little load, with the response gradually stiffening up to give a more linear response characteristic of the expected elastic behaviour, suggesting either some movement and setting of the specimen on the anvils and/or local crushing of the edges of the of anvils into the specimen. Such a response is shown below in figure 5.5.

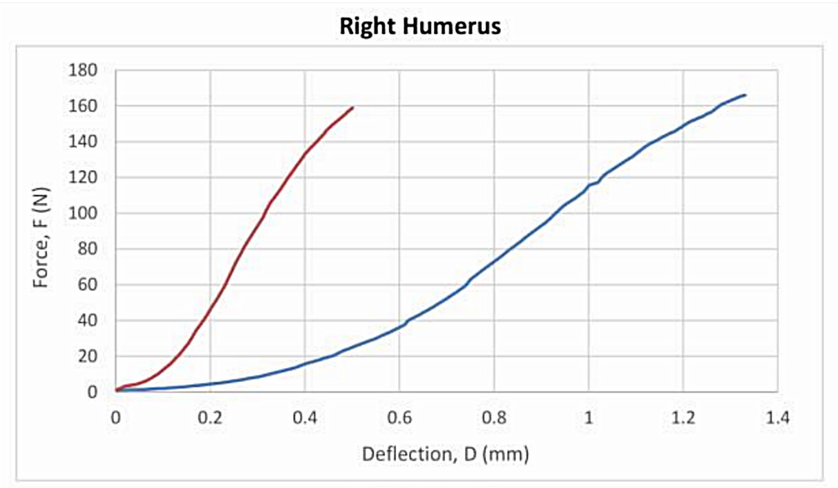

Figure 5.5: stress (σ) – strain (ε) responses from two samples where red shows a more 'unaffected' response and blue where the effect of local crushing or movement on the anvil might have been a stronger factor.

Two further correction methodologies were therefore deemed worthwhile. The first was to alter the fundamental beam bending equations to require the gradient of the load-displacement response to be used to evaluate E_{app} (and thus E_{∞}) rather than the maximum load (and the deflection commensurate this). Additionally, each load-deflection response was. Re-examined to obtain the gradient of the largest magnitude observed, this being the deemed the "true" gradient and hence a better representation of the specimens' stiffness.

The second and final correction methodology was to make an allowance for the stiffness of the testing machine, and the three-point bending rig. this was given to that a separate check of the three-point bending rig indicated that a statistically significant deflection could have been generated in the test apparatus at the typical test loads noted. The stiffness of the experimental set up was measured using a specimen of significant depth (five times that of the bone specimens) of steel such that any deflection observed must have been due to the rig stiffness and not to the specimens. The stiffness was determined to be a value of 0.00045 mm/N (0.045 microns per Newton) and this was then used to calculate a machine deflection at a 100N load, which was then subtracted from the deflections of the bone specimens that would be generated at that load (as given by the previously determined maximum gradient) thus enabling the revised bone stiffness to be calculated.

The results of all the bend tests, after the necessary corrections have been applied, were also subjected to a number of statistical analyses in order to help characterise any trends. These are described below, after which the results are presented, and analysed.

5.4.5 Statistical analysis

Prior to statistical analysis, a further categorisation of the age and sample groups was deemed appropriate in the belief that this would yield a more robust statistical result. All age groups and the commensurate samples were therefore grouped into 3 age categories corresponding to birth to 10 days of age, 11 days to 25 days of age, and 26 to 40 days of age. This age categorisation was deemed appropriate in consideration of the sample ages and the sample size. Having used paediatric data from Hirsch and Evans (1965), Cheong (2015) chose to categorise the Hirsch and Evans' (1965) results and sample groups according to developmental milestones in a child's life. This included categorising the results according to when a child begins to develop the ability to sit up and walk unaided (Adolph and Berger and Adolp, 2007; Adolph *et al.* 2011). A similar categorisation was at first considered for this thesis' studies. However, humans and porcines share no comparable developmental stages this early in life. In regard to the ability to walk, porcines are mobile within a couple of hours of birth. It was therefore decided that human development emulative categories would be unreliable.

In order to statistically test Hypothesis 1, the relationship between the samples' age relative to the resultant force at fracture, flexural strength, and flexural modulus was tested using one-way MANOVA tests (SPSS) with Tukey post-hoc test (A separate one-way MANOVA test was conducted for each loading rate). As previously stated, the sample sizes within the age categories were not equal (Tables 5.3, 5.4, and 5.5). While this disparity in sample sizes can potentially affect the statistical power of the MANOVA tests and introduce bias, the age categorisation was deemed essential to accurately reflect the rapid physiological changes occurring in the porcine samples during this developmental period as well as the limitations of testing samples within the specific age of days. To mitigate potential issues, careful attention was paid to checking the assumptions of MANOVA, and effect sizes are reported alongside p-values to provide a more robust interpretation of the results. The age categorisation was chosen because even with the unequal sample sizes, it was the best method to analyse the developmental trends within the constraints of the available data.

To test Hypothesis 2 and determine the potential significance of age and bone type, the samples mechanical properties were tested using a two-way MANOVA analysis. Statistical significance was set at $p < 0.05$. MANOVA was appropriate as the three mechanical behaviour measures were derived from the same experimental tests and MANOVA is a useful statistical approach is dealing with multiple outcomes of this type (Huberty and Morris, 1989).

5.5 Results

A total of 281 samples were subjected to three-point bending including 111 samples at a 5 mm/min loading rate, 78 samples at 50 mm/min, and 92 at 100 mm/min. For ease, the resultant means by age category for the mechanical properties derived from each experiment are presented in Figures 5.3 through 5.6. Tables 5.3 through 5.6 present the means numerically and allow direct comparison across the mechanical properties for the three different loading rates.

Only the data for flexural modulus (stiffness) was corrected (using the theory given in section 5.4.2) although some “filtering” of data was performed because during the testing and analysis of the resultant data a series of possible test limitations and errors were noted. It was therefore concluded that due to potential human error in the preparation of the test set-up and the actual testing of the samples that a number of the resultant data outputs would need to be removed. Thus, the two following criteria was implemented prior to the descriptive and statistical analysis:

1. Removal of abnormal force-deflection curves. In addition to force-deflection curves which showed initial non-linear elastic responses (as mentioned in section 5.4.2) an initial inspection of the resultant force-deflection result found a series of bell-shaped curves. It was determined in consultation with the experiment notes that the central loading anvil may have not only impacted the sample but the testing rig as well or that some gross form of specimen slippage had occurred. In accordance with this belief, 43 of the 281 sample tests were rejected and removed from the analysed data

2. Removal of samples where the depth was identified as larger than the width. In order to discount the potential of error due to the placement of the samples on the testing rig, all the samples which were loaded but presented a greater depth than the width were rejected and removed from the analysed data. In total, 15 additional samples were removed due to this criterion. It should be noted that the specimens were always placed with the wide edge horizonal, but a few may have rotated in the rig during initial loading.

As a result of implementing these criteria, 58 samples were removed. This left a total of 223 samples for analysis including 90 samples tested at 5 mm/min, 60 samples at 50 mm/min, and 73 samples at 100 mm/min. The numerical results from the experiments for the final samples are presented in Table. 5.3, 5.4, and 5.5. All samples and mechanical properties have been organised according to the specific loading rate, the ages at acquisition, and the age categories used for the statistical analysis.

Overall, the mechanical properties of the immature porcine bone samples, as presented across the three loading rates (5 mm/min, 50 mm/min, and 100 mm/min), demonstrated scattered and variable trends. While a general increase in force at fracture, flexural strength, and flexural modulus, the specific patterns and peak values varied significantly across the different loading rates. Measurements exhibited across different loading rates inconsistent behaviour, with peaks and troughs that did not align across the different loading speeds. This suggested the variation in the results was “experimental” in nature, and not indicative of real trends or phenomena.

As described previously, to maintain uniform testing conditions across the varying sample sizes, a custom-developed support rig was utilised, maintaining a consistent support span of 4 mm. This span was determined based on the length of the smallest sample tested, ensuring all samples could be accommodated. All samples were loaded at their measured midpoint, with the loading anvil set to strike perpendicular to the sample's top surface and the bone's longitudinal direction. However, as mentioned earlier, this anvil was positioned using the naked eye, introducing the possibility that the contact point may not have been loading the samples consistently.

The standard deviations within each age group confirmed the considerable variability observed, suggesting that factors beyond age were influencing mechanical properties.

In truth, biological samples, particularly immature bone were expected to exhibit inherent variability. Factors such as individual growth rates, nutritional differences, and variations in microstructural development likely contributed to the observed scatter. Additionally, some age groups had relatively small sample sizes, which can amplify the impact of individual variations on the overall trends.

Overall, it is important to acknowledge the scattered and variable nature of the results across the different loading rates seen in this work. These variations are attributed in part to the nature of bone tissue and the inherent biological variability of immature bone samples. Furthermore, it is recognised that potential confounding factors, beyond age. These factors may include, but are not limited to, variations in individual growth rates, nutritional differences, and microstructural variations. A significant cause of variation will have been the uniformity of the test specimens, where although great care was taken in specimen preparation, the diminutive size of the specimens generated substantial difficulties in ensuring consistent dimension across the loaded portion, and in maintaining a perfect rectangular cross section throughout. This, and the other experimentally derived error, such as the centrality of the loading, the possibility that the anvils knife edges could locally cut into the specimen, and the non-linearity in the load-line caused by distorted or not properly "seated" specimens, could easily have accumulated and generated large scatter bands in the results. One of the main responses to these variations (in addition to the elimination of some specimens, as described above) was to rely heavily on mean values – and thus upon repeat tests. In truth, many of these repeats were not absolute repeats, and would have entailed calculating means within a particular age group, but across a range of bone types (left and right humerii and femora) which could have introduced their own variations and hence render a calculated mean somewhat presumptive.

Applying a statistical method to the data was therefore deemed appropriate, if meaningful materials' properties and trends were to be extracted from the statistical results, however before such analysis is presented, it is instructive to present the data (or at least the means) in their raw state and consider whether anything meaningful is apparent before the full of the statistical analysis is performed. The following section therefore presents a quick initial "look-see" of the strength and stiffness (corrected) means, with age and draws some initial conclusions, mindful of the large scatter that is present.

5.5.1 Initial consideration of the results

All the data plots presented below are after initial specimen filtering, but prior to any statistical analysis, other than the calculation of means across a given age category. Data is presented for the fracture stress (as calculated from the maximum load read-off the load-displacement plot) and the "true" flexural modulus (E; as calculated using the methodology given earlier in section 5.4.2 and 5.4.3). Additionally, plots are provided for the failure load. All data presented here has been re-evaluated directly from the original load-displacement plots to ensure the appropriate sections of the load-line are considered, rather than just considering the numerical data. This was especially important for the determination of stiffness, where many plots did not follow a 'traditional' response for a stiff brittle material (as discussed in section 5.4.2).

A modified version of equation 3 (section 5.4.2) was employed to calculate E_{app} (which was then corrected to E_{∞}), which did not require deflection data, but only the gradient of the load-displacement plot – hence allowing selection of the most appropriate gradient, if the response showed variations in this, and a commensurate deviation from the 'ideal' response of a linear elastic material.

This took the form of:

$$E_{app} = \frac{ms^3}{4wd^3}$$

Where m is the gradient of the force-deflection response in the linear elastic region, and *s*, *d*, and *w* are the specimen dimensions as illustrated by figure 5.4.

For clarity only the 5 mm/min data is considered here, although the data sets for the other two deflection rates are presented in the next section after statistical analysis. This omission is largely as a result of the repeatable nature of the responses with age, seen across all three loading rates. No significant differences were seen, except perhaps a slight increase in scatter, most probably as a result of the less forgiving nature of testing at higher displacement rates.

Plots (Figures 5,6, 5.7, and 5.8) are presented with no error bars (given the statistical treatment that follows) to help identify any trends, and a simple linear linking of data points is employed without any attempt at cure fitting or supplying a trend line. This is concluded in the follow-on sections.

Response of fracture load with age

The clear trend seen in figure 5.6 is one of a steadily rising force at fracture with age. This could be direct evidence of a rise in strength, with age, as observed from the literature (Currey and Butler, 1975), but in truth was highly influenced by an observed rise in apparent specimen thickness with age. To be clear, the bend test specimens were prepared initially by slicing through the whole bone laterally and then longitudinally (see section 5.3.2). The cancellous bone was then removed primarily by applying light pressure to the cancellous side and rubbing away the friable bone. The resultant specimen was then lightly ground such a flat profile was obtained and some of the cortical bone was removed to ensure complete elimination of any porous cancellous structure. The emphasis on avoiding excessive grinding, due to the very thin cortical portion, meant that no more than a few tenths of mms of this was removed, and therefore the specimen dimensions would have been largely determined by the initial thickness of the cortex, hence upon the whole bone size, and thus upon the age of the source animal. It is not surprising therefore that a good correlation with age is seen. To truly detect whether strength is affected by age, it is clearly necessary to plot failure stress, not failure load, and this is seen below in figure 5.6.

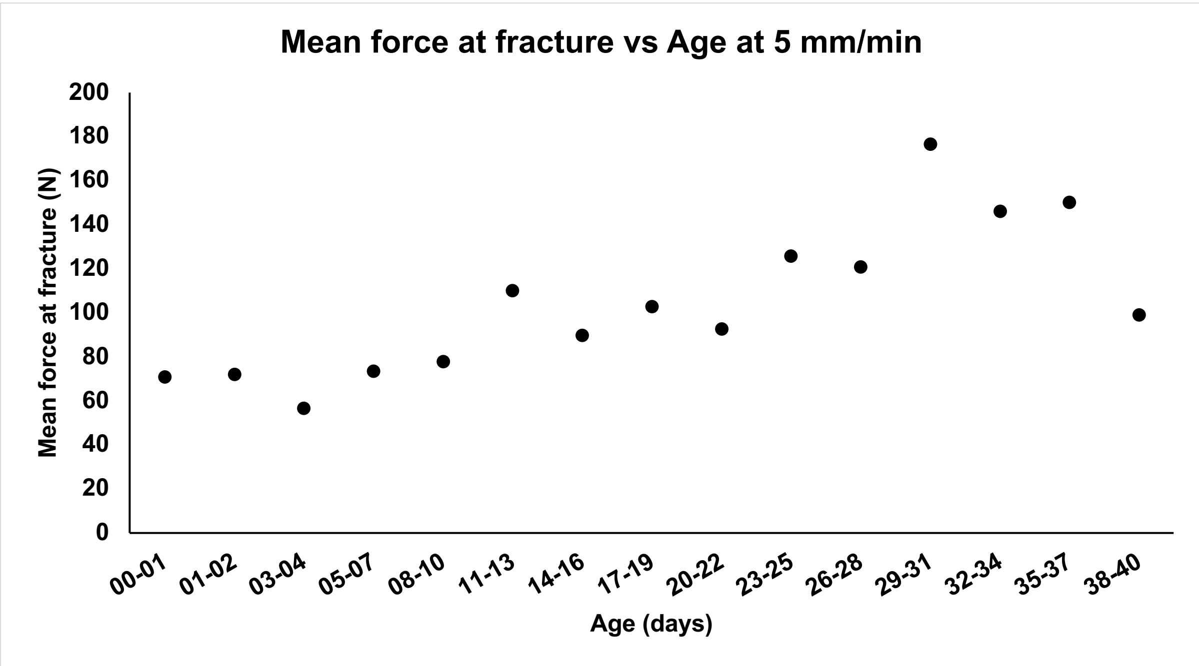

Figure 5.6: Mean force at fracture vs. age at 5 mm/min

Response of strength with age at 5 mm/min

Once the specimen failure loads had been normalised for specimen dimensions, the clear upward trend with age is no longer dramatic, but nevertheless, a plot of average failure stress (i.e., strength) does, as given in figure 5.7 show a subtle rise with age, albeit via substantial scatter in the means for each age category.

The rise in strength, if reproducible, is not entirely unexpected, and follow a trend which has been confirmed by the only previous publications concerned with immature cortical bone (Currey and Butler, 1975).

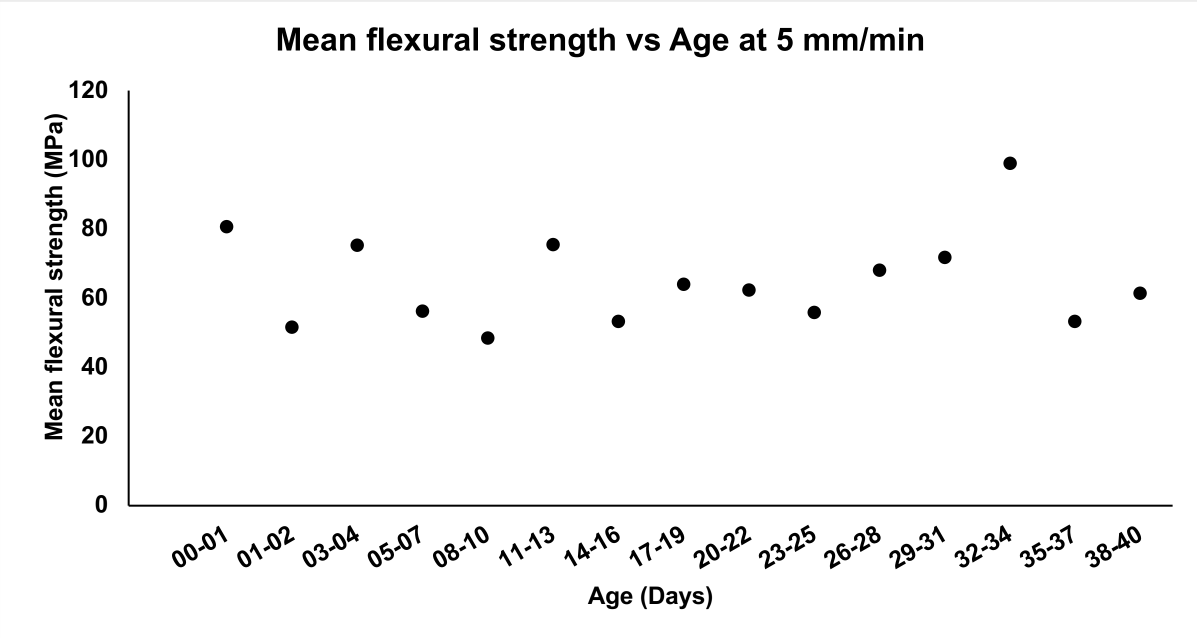

Figure 5.7: Mean flexural strength vs. age at 5 mm/min

Whether this is due to a reduction in porosity and hence an increase in bone density, or to changes in the mineralisation level and/or balance between hard and soft constituents within monolithic bone material, remains a subject for study and debate, however the rise is unlikely to continue and should reach an asymptotic value as age rises. Once again, the data show in figure 5.7 could easily be curve-fitted to predict an asymptotic value of about 65 MPa (See Table 5.3) which is not an unreasonable value, when compared to Currey and Butler (1975).

Response of 'true' stiffness with age at 5 mm/min

Finally, figure 5.8 below reveals the outcome of a substantial body of tests, and a considerable level of post-processing (and applied corrections) to generate a value for the flexural modulus (i.e., stiffness) of the cortical section of immature porcine humeral and femoral bones, with age.

Several observations are worthy of making is a) the stiffness data is now convincingly in GPa (as opposed to MPa in the uncorrected E_{app} values are considered), signifying that the corrections applied have returned averages which are closer to the literature values. In truth, these values are arguably still a factor of 10 away from Currey and Butler (1975) and so could indicate that the corrections applied are insufficient to totally address the unfavourable span to depth ratio, or that other factors are at play, and these have not been adequately considered or identified.

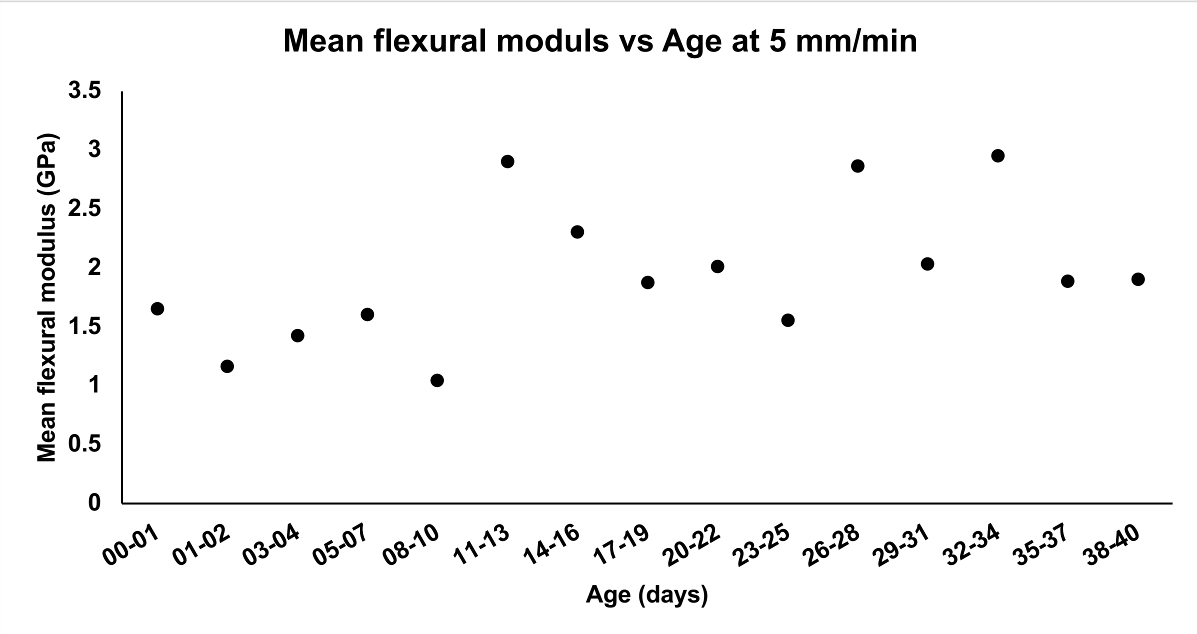

Figure 5.8: Mean flexural modulus vs. age at 5 mm/min

Clearly, a resolution to this apparent discrepancy is required, and would most readily be obtained if it were possible to measure or determine E values in a very different way from that provided through a bend test. Other methods do exist such as utilising ultrasound (Bernard *et al.* 2015; Boughton *et al.* 2019). However, the most obvious route to obtaining corroboratory data would be to create specimens with an ideal depth to span ratio, even if this mean preparing thinner, more delicate ‘whisker’ type specimens. This is clearly a task and recommendation for future work. There is also the possibility that the stiffness data obtained is indeed accurate, and that low stiffnesses are a definite feature of fresh, wet immature bone.

Notwithstanding this debate, a further observation that can be made is b) that the stiffness values appear to rise with age and c) that an asymptotic value of around 2.25 GPa (Loosely based on figure 5.8 and table 5.3) is the likely outcome as the immature porcine bone matures. Again, these trends (ignoring absolute values) are largely as expected – bone mineralisation is known to increase with age, and the stiffness can continue to rise ad infinitum (Boskey and Coleman, 2010).Overall, a rough and ready 'look-see' of the strength and stiffness data does appear to indicate an age-property relationship, with both strength and stiffness rising from birth and reaching a constant level. The rises are seen as subtle but nevertheless consistent with the expectations derived from the Currey and Butler (1975) but should be seen against an experimental backdrop where significant challenges in specimen preparation have resulted in large scatter in the data sets. Attempts have been made to address the scatter and explanations provided for the source(s), and suggestions for improvements to testing protocols and experimental procedure, given, but the work presented here can only be revisited by beginning over again, with a fresh batch of immature porcine specimens across the desired age range. It is tantalising to suggest, however that the present study (Chapter 5) has achieved, in part, what it set to do – namely generate an age-related response for critical bone materials properties that could form the basis of a proxy for determining the age-related properties of human bone, and hence provide computer models, intent on using finite element methods to understand the mechanical response of immature whole bones with the core materials data necessary to do so. The next section applies the previously mentioned statistical treatment to the data presented above, to try and reinforce or refute the conclusion drawn in this section.

5.5.2 Hypothesis 1 – Mechanical properties will demonstrate age-related differences at loading rates of 5 mm/min, 50 mm/min, and 100 mm/min

As a recap, the resultant means by age category for the mechanical properties derived from each experiment are presented in Figures 5.9 through 5.17. The figures showing mean force at fracture, flexural strength, and flexural modulus at 5 mm/min, 50 mm/min, and 100 min with exponential trendlines added Tables 5.3, 5.4, and 5.5 present the means numerically and allow direct comparison across the mechanical properties for the three different loading rates. Results for the loading rates are described in turn and include both a descriptive and statistical analysis.

Table 5:2: Means of samples tested at 5 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	Flexural strength (MPa)	Flexural modulus (GPa)
00-01	1	7	70.8 ± 34.6	80.7 ± 55.4	1.6 ± 0.7
01-02	1	7	72.1 ± 21.2	51.5 ± 21.8	1.1 ± 0.6
03-04	1	7	56.5 ± 18.4	75.3 ± 60.1	1.4 ± 0.7
05-07	1	5	73.4 ± 29.3	56.2 ± 25.8	1.6 ± 1.4
08-10	1	6	77.8 ± 9.1	48.5 ± 13.7	1 ± 0.7
11-13	2	7	110.1 ± 24.5	75.4 ± 24.8	2.9 ± 1.6
14-16	2	7	89.8 ± 50.1	53.3 ± 14.2	2.3 ± 1.7
17-19	2	8	102.7 ± 50.4	64 ± 48.5	1.8 ± 1.7
20-22	2	8	92.7 ± 41.6	62.3 ± 21	2 ± 1.6
23-25	2	7	125.7 ± 24.6	55.8 ± 13.2	1.5 ± 0.7
26-28	3	7	120.8 ± 66.3	68 ±.22.82	2.8 ± 2
29-31	3	4	176.7 ± 87.1	71.8 ± 36.9	2 ± 1.6
32-34	3	2	146 ± 16.9	99 ± 28.4	2.9 ± 0.1
35-37	3	3	150.3 ± 84.8	53.2 ± 17.6	1.8 ± 1.3

Table 5:3: Means of samples tested at 50 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	Flexural strength (MPa)	Flexural modulus (GPa)
00-01	1	4	75.5 ± 47.7	63 ± 21.2	1.6 ± 21.2
01-02	1	3	98 ± 31	70.1 ± 12.7	2.1 ± 1.1
03-04	1	5	65.8 ± 21.8	63.8 ± 23	1.7 ± 0.8
05-07	1	2	56 ± 14.1	34 ± 0.5	0.9 ± 0.1
08-10	1	3	66.3 ± 6.1	1.8 ± 11.6	1.8 ± 0.9
11-13	2	3	59.6 ± 48.4	55.4 ± 29.6	2 ± 2.6
14-16	2	4	92.5 ± 11.7	70.3 ± 31.1	1.7 ± 1
17-19	2	5	135 ± 79.4	73.1 ± 29.3	2.1 ± 1.7
20-22	2	7	101 ± 49.6	105 ± 58.1	3.6 ± .3.6
23-25	2	4	137 ± 41.1	98.9 ± 51.9	2.2 ± 1.5
26-28	3	6	133.8 ± 52.8	89.1 ± 24.3	3.1 ± 1.1
29-31	3	4	120 ± 62.2	55.2 ± 1.6	1.8 ± 0.8
32-34	3	1	137	103.5	1.5
35-37	3	3	130 ± 44.9	77.3 ± 14.5	2.3 ± 0.6
38-40	3	6	105 ± 32.7	69.6 ± 22.6	2 ± 1.2

Table 5:4: Means of samples tested at 100 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	Flexural strength (MPa)	Flexural modulus (GPa)
00-01	1	4	127.6 ± 75.4	100.8 ± 27.9	2.5 ± 2.3
01-02	1	6	51.7 ± 20.9	55.6 ± 28.8	1.6 ± 1
03-04	1	5	62.2 ± 38.9	61.4 ± 39.3	1.6 ± 0.8
05-07	1	3	85.3 ± 35.8	57.9 ± 3	1 ± 0.6
08-10	1	4	42.1 ± 5.9	56.6 ± 26.2	1.1 ± 0.7
11-13	2	4	107.4 ± 18.5	63.5 ± 6.1	1.6 ± 0.3
14-16	2	7	72.9 ± 28.4	75.8 ± 15	1.9 ± 1
17-19	2	3	121.9 ± 101.6	50.8 ± 4.6	1.6 ± 1.1
20-22	2	9	98.2 ± 64.2	74.8 ± 30.6	1.9 ± 1.2
23-25	2	5	115.4 ± 43.3	68.4 ± 23.2	1.4 ± 0.5
26-28	3	6	143 ± 38.5	71.7 ± 22.2	2.1 ± 0.7
29-31	3	6	116.7 ± 33.6	62.6 ± 21	2.3 ± 1.4
32-34	3	1	137.6	122.3	2.6
35-37	3	4	111.4 ± 44.7	53.3 ± 8.4	1.74
38-40	3	6	72.1 ± 17.2	64.9 ± 14.7	1.5 ± 0.5

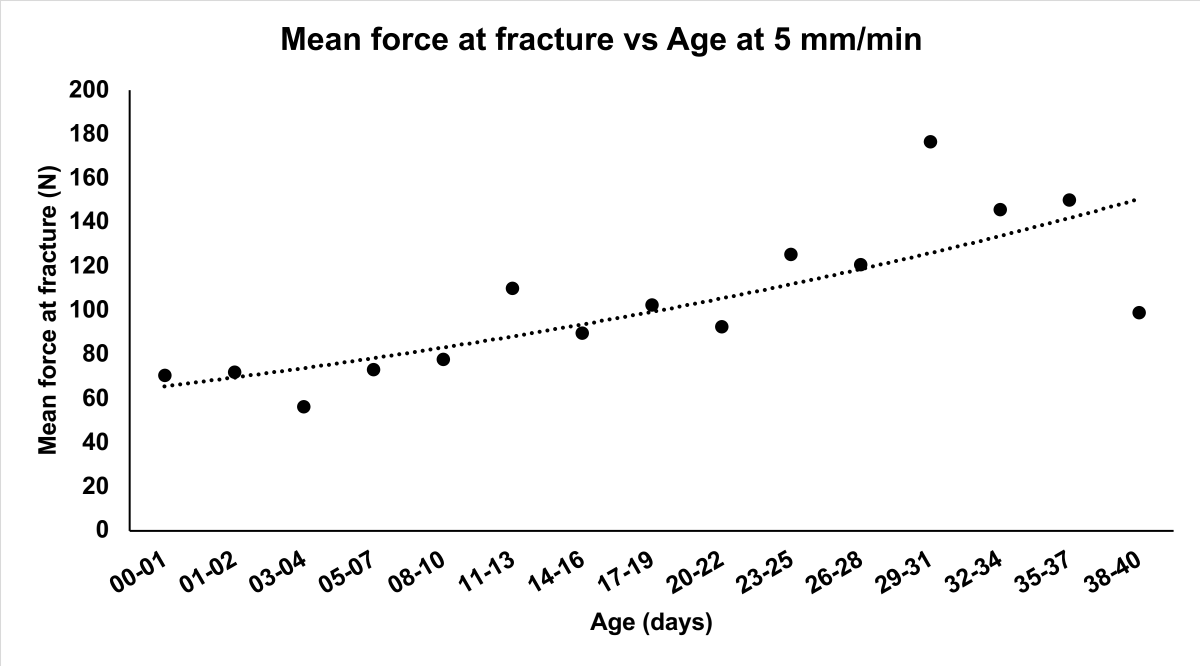

Figure 5.9: Mean force at fracture vs. age at 5 mm/min with exponential trendline.

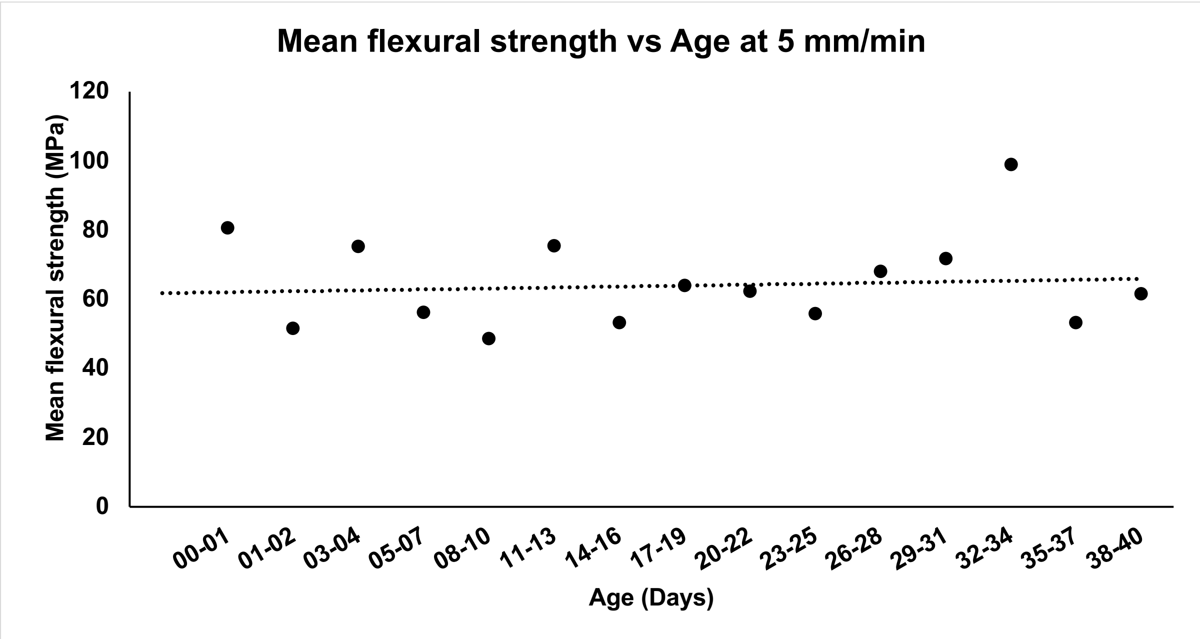

Figure 5.10: Mean flexural strength vs. age at 5 mm/min with exponential trendline.

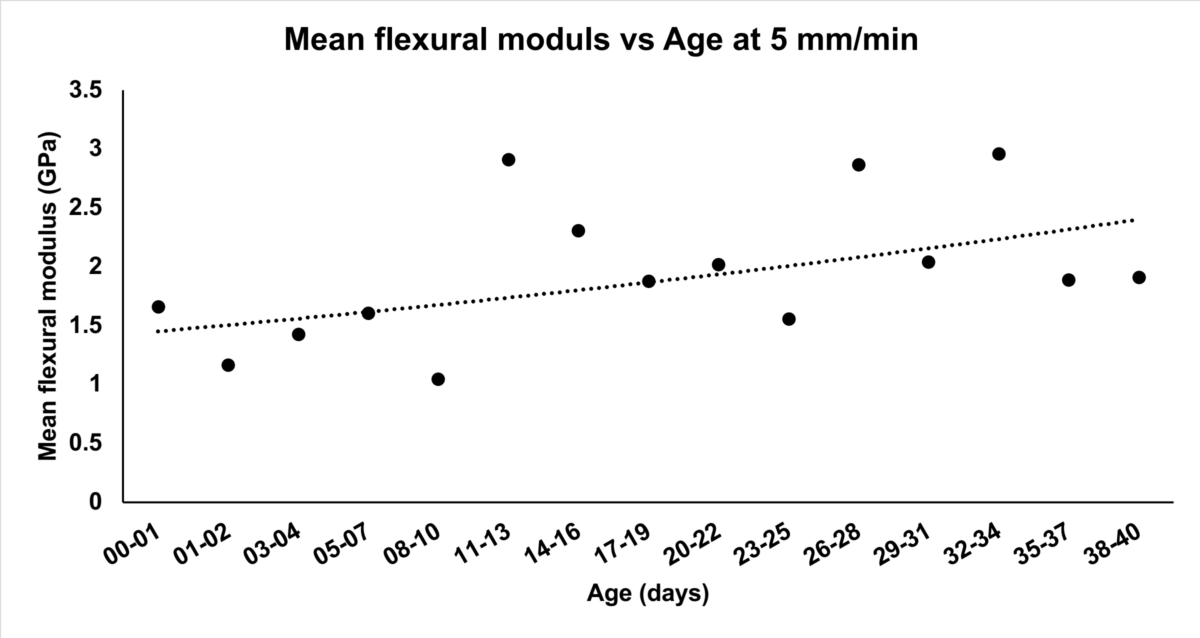

Figure 5.11: Mean flexural modulus vs. age at 5 mm/min with exponential trendline.

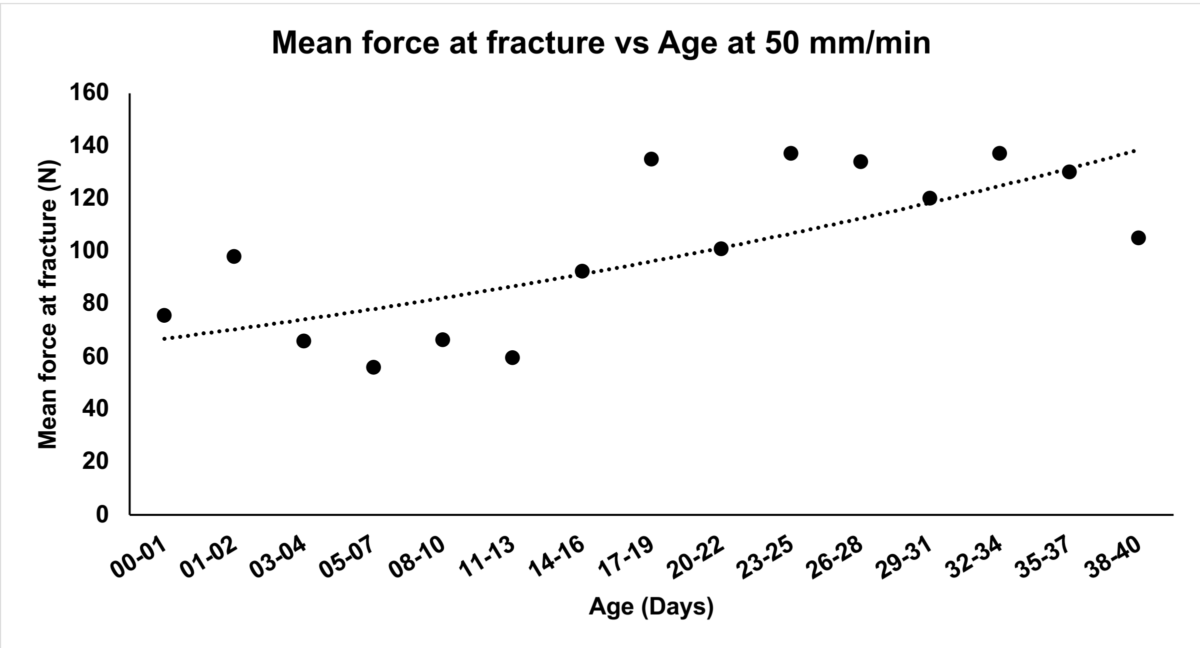

Figure 5.12: Mean force at fracture vs. age at 50 mm/min with exponential trendline.

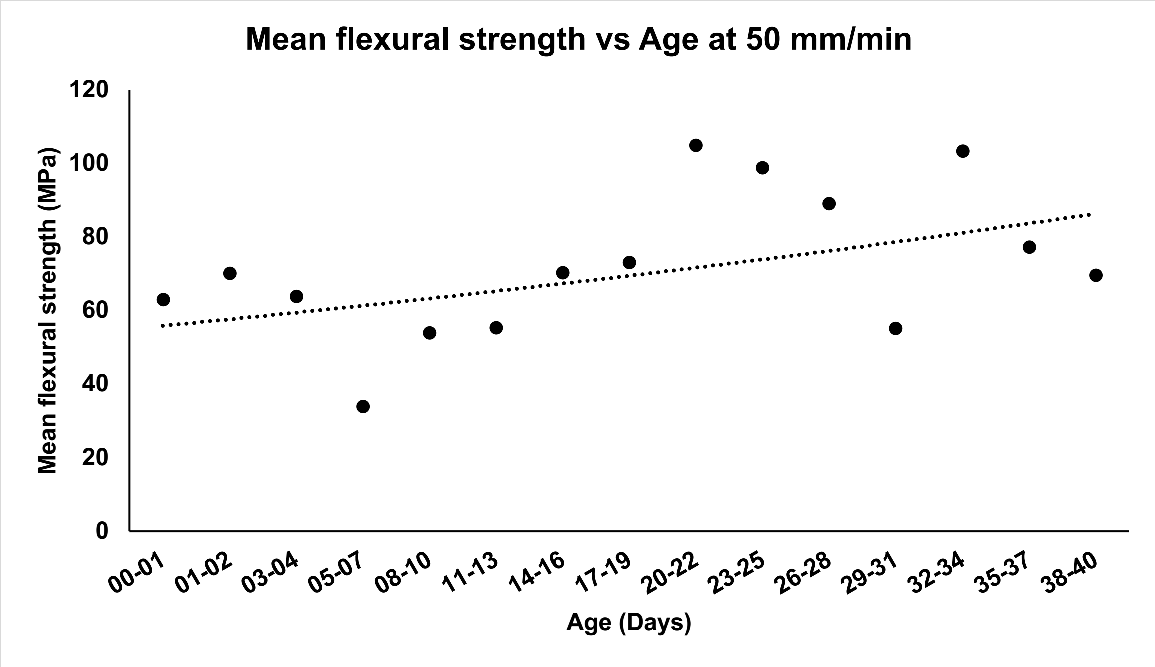

Figure 5.13: Mean flexural strength vs. age at 50 mm/min with exponential trendline.

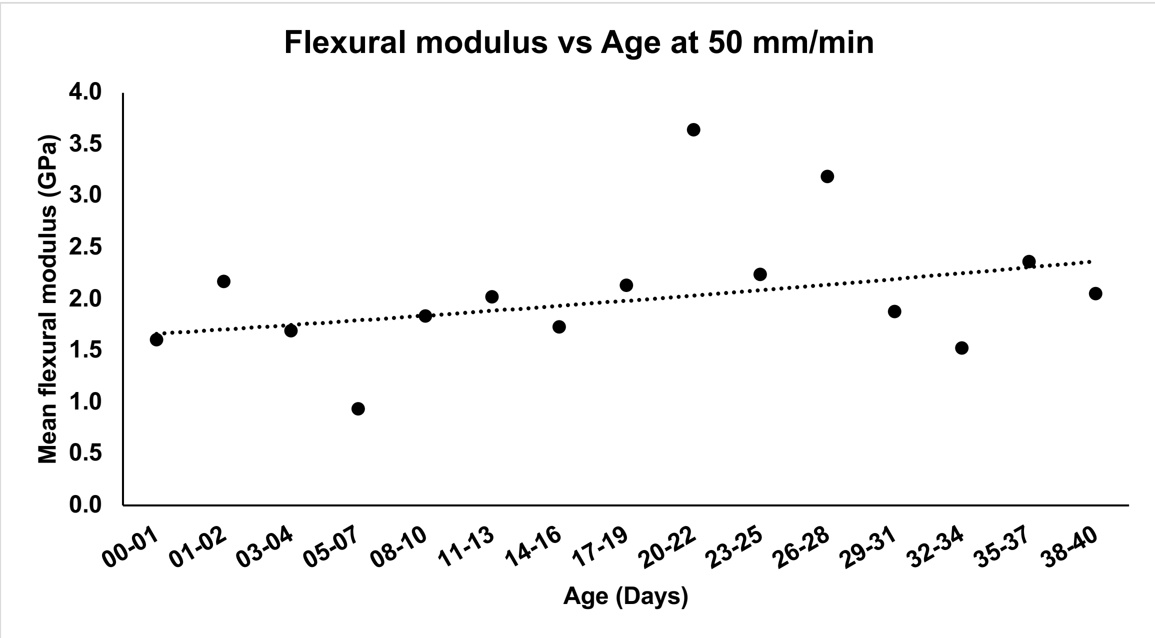

Figure 5.14: Mean flexural modulus vs. age at 50 mm/min with exponential trendline.

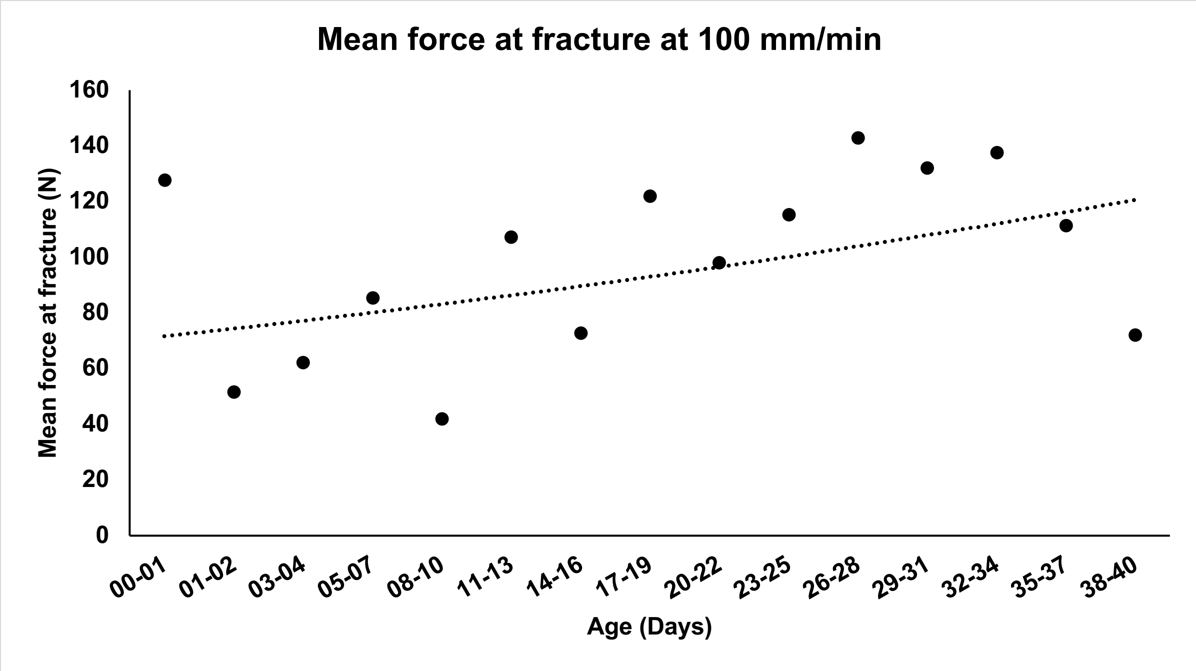

Figure 5.15: Mean force at fracture vs. age at 100 mm/min with exponential trendline.

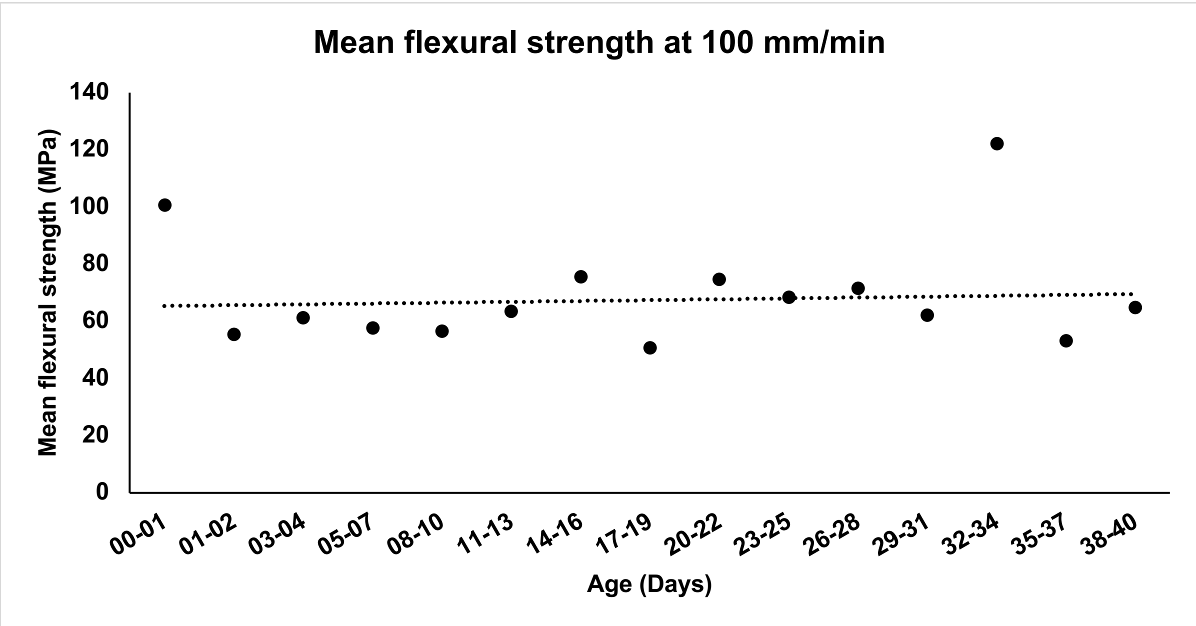

Figure 5.16: Mean flexural strength vs. age at 100 mm/min with exponential trendline.

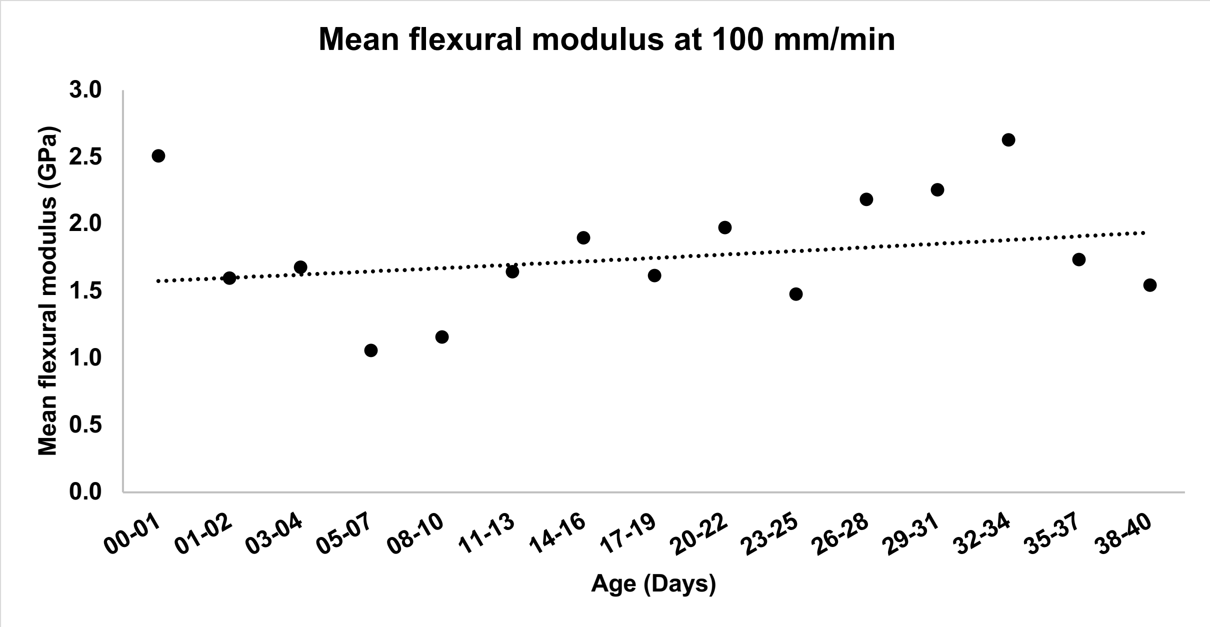

Figure 5.17: Mean flexural modulus vs. age at 100 mm/min with exponential trendline.

Results at 5 mm/min

At a loading rate of 5 mm/min, the mechanical properties of the bone samples, as presented in Table 5.3, demonstrated variable trends across different age groups. The mean force at fracture was found reaching its highest value (176.7 ± 87.1 N) in the 29-31 day age group. The lowest mean force at fracture (56.5 ± 18.4 N) was measured in the 3-4 day age group.

The mean flexural strength showed fluctuations across the age groups. The peak mean flexural strength (99 ± 28.4 MPa) was recorded in the 32-34 day age group. The lowest mean flexural strength (48.5 ± 13.7 MPa was observed in the 8-10 day age group. Similar to flexural strength, the peak mean stiffness (2.9 ± 0.1 GPa) was observed in the 32-34 day age group. The stiffness values, as discussed in section 5.5.1 does indicate an upwards trend.

The one-way MANOVA (Table 5.6) revealed a statistically significant effect at force at fracture ($F(2, 87) = 10.651$, $p = 0.001$) demonstrating age differences. Post-hoc Tukey's test indicated a significant difference in force at fracture between age category 1 and age category 2 ($p = 0.002$), but no significant difference between age category 2 and age category 3 ($p > 0.359$). No statistically significant effects of age were found for flexural strength ($F(2, 87) = 0.101$, $p = 0.904$), and stiffness ($F(2, 87) = 2.590$, $p = 0.081$).

Table 5:5: Statistical results for Hypothesis 1 according to each loading rate

Mechanical parameter	F value	Degrees of freedom	p-value	Post-hoc
5 mm/min				
Force at fracture	10.651	(2,87)	.001	Category 1 – 2 (p =.002) Category 2 – 3 (p = .359)
Flexural strength	.101		.904	Category 1 – 2 (p =.981) Category 2 – 3 (p = .894)
Flexural modulus	2.590		.081	Category 1 – 2 (p =.106) Category 2 - 3 (p = .997)
50 mm/min				
Force at fracture	5.665	(2,57)	.006	Category 1 – 2 (p =.049) Category 2 – 3 (p = .565)
Flexural strength	2.907		.063	Category 1 – 2 (p =.050) Category 2 - 3 (p = .634)
Flexural modulus	1.239		.297	Category 1 – 2 (p =.293) Category 2 – 3 (p = .959)
100 mm/min				
Force at fracture	5.569	(2,70)	.006	Category 1 – 2 (p =.193) Category 2 - 3 (p = .191)
Flexural strength	.159		.854	Category 1 – 2 (p =.841) Category 2 – 3 (p = .953)
Flexural modulus	.626		.538	Category 1 – 2 (p =.858) Category 2 - 3 (p = .794)

Results at 50 mm/min

At a loading rate of 50 mm/min, the mechanical properties of the immature porcine bone samples demonstrated age-related influences, albeit with considerable variability. The mean force at fracture showed a general trend of increase with age, especially notable from the 17-19 day age group onwards. The highest mean values were concentrated in the 17-19 day group (135 ± 79.4 N), 23-25 day group (137 ± 41.1 N), 26-28 day group (133.8 ± 52.8 N), and 32-34 day group (137 N). Notably, the 32-34 day group, with a mean of 137 N, also represented the highest single value recorded. The younger age groups (00-01 to 11-13 days) consistently exhibited lower mean fracture forces, ranging from 56 ± 14.1 N in the 05-07 day group to 98 ± 31 N in the 01-02 day group.

The mean flexural strength also showed a general increase with age, though with significant fluctuations. The highest mean flexural strength was observed in the 20-22 day group (105 ± 58.1 MPa) and the 32-34 day group (103.5 MPa), suggesting potential peaks in bending strength during these periods. The lowest mean flexural strength was observed in the 05-07 day group (34 ± 0.5 MPa). The mean flexural modulus demonstrated a tendency for higher values in older samples, particularly those in the 20-22 day age group (3.6 ± 3.6 GPa) and above. The lowest mean flexural modulus was observed in the 05-07 day group (0.9 ± 0.1 GPa).

In summary, descriptively, at a loading rate of 50 mm/min, the mean force at fracture, flexural strength, and flexural modulus generally increased with age, though with considerable variability and fluctuations.

One-way MANOVA (Table 5.6) revealed a statistically significant effect of age on force at fracture ($F(2, 57) = 5.665$, $p = 0.006$). Post-hoc Tukey's test indicated a significant difference in mean force at fracture between age category 1 and age category 2 ($p = 0.049$), but no significant difference between age category 2 and age category 3 ($p > 0.565$). No statistically significant effects of age were found for flexural strength ($F(2, 57) = 2.907$, $p = 0.063$), and stiffness ($F(2, 57) = 1.239$, $p = 0.297$).

Results at 100 mm/min

The resultant mechanical properties of the immature bone samples tested at a loading rate of 100 mm/min are detailed in Table 5.5. The findings indicate trends in fracture force, flexural strength, and flexural modulus.

The force required to fracture the material appeared to show an initial increase, with the peak value of 143 (± 38.5) N observed at 26-28 days. While a trend in the values for flexural strength was not easily discernible, the values look to have 'stabilised' within a range of approximately 50 to 75 MPa in the samples aged 11-13 days and older. Concerning the flexural modulus, which serves as an indicator of the material's stiffness, an initial decrease was noted from 2.5 (± 2.3 GPa) at 1 day to 1.06 (± 0.6) GPa at 5-7 days. Following this, the flexural modulus appeared to fluctuate, with a possible increase observed around 26-28 days, yielding a value of 2.1 ± 0.3 GPa.

Following the one-way MANOVA (Table 5.6) and compared to the descriptive analysis of the outputs at 100 mm/min, force at fracture (F (2,70 = 5.569, p = 0.006) was found to show a significant relationship with age. A post-hoc Tukey test for force at fracture showed a significant relationship between age category 1 to age category 2 (p = .049) but a non-significant change from age category 2 to age category 3 (p = .565). Flexural strength (F (2,70) = .159, p = .854 and flexural modulus (F (2,70) = 626, p = .538) were both found in turn to be statistically non-significant.

Observations across all the loading rates

A non-statistical comparative analysis of the mechanical properties across the three loading rates (5 mm/min, 50 mm/min, and 100 mm/min) revealed the following trends. At the slowest loading rate of 5 mm/min, a more gradual age-related increase in force at fracture was observed, with the peak force occurring in the 29-31 day age group (Figure 5.9). This trend, however, was accompanied by fluctuations in flexural strength and stiffness across the various age groups (Figures 5.10 and 5.11). In contrast, the higher loading rates of 50 mm/min and 100 mm/min exhibited greater variability in all measured mechanical properties, including force at fracture, flexural strength, and flexural modulus. Notably, the peak values for force at fracture and flexural strength were generally higher at the 100 mm/min loading rate compared to both 50 mm/min and 5 mm/min. For instance, the maximum force at fracture reached 143 N at 100 mm/min, compared to 137 N at 50 mm/min and 176.75 N at 5 mm/min (Figures 5.9, 5.12, and 5.15). Similarly, the highest flexural strength was 122.3 MPa at 100 mm/min, compared to 103.5 MPa at 50 mm/min and 99 MPa at 5 mm/min (Figure 5.10).

5.5.3 Hypothesis 2 - The effect of bone type with age

Hypothesis 2 looked to understand and test the relationship between the mechanical properties of immature porcine bone, age, and different types of bones (humerus and femur). The mechanical properties of the immature porcine femoral and humeral bones were examined across three distinct loading rates: 5 mm/min, 50 mm/min, and 100 mm/min. The results revealed notable differences in the mechanical behaviour of these bone types as a function of both age and loading rate. The values showing the mechanical properties by bone type can be observed in tables 5.7-5.12 as well as the figures 5.18 to 5.35.

Of the femoral samples tested at 5 mm/min, a general trend of increasing force at fracture with age was observed, with higher values typically seen in the older samples. A notable peak in fracture force was evident at 29-31 days. Conversely, the humeral bones tested at 50 mm/min exhibited a more fluctuating pattern, although higher fracture force values were generally observed in older samples, particularly after 17-19 days, with peaks at 23-25 and 32-34 days. At the fastest loading rate, 100 mm/min, femoral samples displayed a unique pattern, with the highest fracture force values concentrated within the middle age range, around 26-28 days. These observations suggest that femoral bones tested at the slower 5 mm/min loading rate showed a more consistent increase in fracture force with age, while humeral bones at 50 mm/min displayed a more variable pattern, albeit with higher strength in older samples. At 100 mm/min, femoral bones presented a distinct pattern, with peak strength occurring in the middle age range. However, caution must be taken in interpreting these peaks as it likely an indication of the scatter error in the experiments.

Of the femoral samples tested at 5 mm/min, flexural strength varied, with higher values observed in the oldest samples. The humeral samples tested at 50 mm/min showed fluctuations but generally exhibited higher flexural strength in older samples. At 100 mm/min, the femoral samples displayed fluctuating flexural strength with no clear trend. These observations suggest that both bone types at slower loading rates tended to exhibit higher flexural strength in older samples. At 100 mm/min, flexural strength in femoral bones appeared more variable.

The femoral samples tested at 5 mm/min showed varying flexural modulus values, with higher values observed in the oldest samples. Humeral bones tested at 50 mm/min exhibited fluctuations but generally showed higher stiffness values in older samples. At 100 mm/min, femoral bones displayed widely varying stiffness values, with high values observed in both middle and older age ranges. Consistent with the flexural strength observations, the stiffness tended to be higher in older samples for both bone types at slower loading rates. At 100 mm/min, femoral bones exhibited high variability in flexural modulus. The patterns of age-related changes differed between humeral and femoral bones, suggesting distinct developmental trajectories for these bone types. It is also important to consider that some age groups had relatively small sample sizes, which could affect the reliability of the observed trends.

Table 5:6:Humeral mean results at loading rate of 5 mm/min

Age (Days)	Age category	Sample size	Force at fracture (N)	Flexural strength (MPa)	Flexural modulus (GPa)
00-01	1	4	83.5 ± 36.5	97.5 ± 56.6	1.3 ± 0.8
01-02	1	4	80 ± 20.1	50.7 ± 21.1	1.6 ± 0.5
03-04	1	4	57 ± 25.3	88.6 ± 81	1.2 ± 0.6
05-07	1	2	101.5 ± 20.5	44.9 ± 5	3.1 ± 0.2
08-10	1	2	84 ± 14.1	60.2 ± 12.9	1.7 ± 1.2
11-13	2	4	128.2 ± 7	86.7 ± 18.7	4.1 ± 0.7
14-16	2	4	106.2 ± 52.8	55.3 ± 11.1	3.3 ± 1.5
17-19	2	4	106 ± 76	63 ± 22.7	1.6 ± 2.2
20-22	2	4	91.7 ± 61	60.6 ± 29.6	2.6 ± 2.2
23-25	2	2	115.5 ± 40.3	61.2 ± 27.5	1.3 ± 0.4
26-28	3	3	135.3 ± 95	58.9 ± 16.6	1.5 ± 0.6
29-31	3	2	105 ± 35.3	41.5 ± 18.3	0.7 ± 0.4
32-34	3	1	158	78.9	3
35-37	3	1	239	59.5	3.1
38-40	3	2	107 ± 19.7	75.5 ± 30	2 ± 1.6

Table 5:7:Femoral mean results at loading rate of 5 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	flexural strength a (MPa)	Flexural modulus (GPa)
00-01	1	3	70.8 ± 34.6	80.7 ± 55.4	1.6 ± 0.7
01-02	1	3	72.1 ± 21.2	51.5 ± 21.8	1.1 ± 0.6
03-04	1	3	56.5 ± 18.4	75.3 ± 60.1	1.4 ± 0.7
05-07	1	3	73.4 ± 29.3	56.2 ± 25.8	1.6 ± 1.4
08-10	1	4	77.8 ± 9.1	48.5 ± 13.7	1 ± 0.7
11-13	2	3	110.1 ± 24.5	75.4 ± 24.8	2.9 ± 1.2
14-16	2	3	89.8 ± 50.1	53.3 ± 14.2	2.3 ± 1.7
17-19	2	4	102.7 ± 50.4	64 ± 48.5	1.8 ± 1.7
20-22	2	4	92.7 ± 41.6	62.3 ± 21	2 ± 1.6
23-25	2	5	125.7 ± 24.6	55.8 ± 13.2	1.5 ± 0.7
26-28	3	4	120.8 ± 66.3	68 ±.22.8	2.8 ± 2
29-31	3	2	176.7 ± 87.1	71.8 ± 36.9	2 ± 1.6
32-34	3	1	146 ± 16.9	99 ± 28.4	2.9 ± 0.1
35-37	3	2	150.3 ± 84.8	53.2 ± 17.6	1.8 ± 1.3
38-40	3	3	99.2 ± 34.1	61.5 ± 22.9	1.9 ± 1.1

Table 5:8: Humeral mean results at loading rate of 50 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	flexural strength a (MPa)	Flexural modulus (GPa)
00-01	1	3	88 ± 49.8	61.6 ± 25.7	1.9 ± 1
01-02	1	2	80 ± 2.8	77.5 ± 0.6	1.9 ± 1.4
03-04	1	3	51 ± 11	60.2 ± 14.7	1.4 ± 0.5
05-07	1	1	66	34.4	1
08-10	1	1	61	1.7	1.7
11-13	2	2	67.5 ± 65.7	54.2 ± 41.8	2.7 ± 3.3
14-16	2	1	84	83.7	2.9
17-19	2	2	72.5 ± 14.8	47.2 ± 9.2	0.9 ± 0.6
20-22	2	4	99.2 ± 19.3	123.8 ± 74.3	2.5 ± 1
23-25	2	1	159	59.5	1.2
26-28	3	4	161.2 ± 29.6	97.4 ± 24.8	3.8 ± 0.8
29-31	3	2	148.5 ± 79.9	51.4 ± 16.7	1.1 ± 0.07
35-37	3	1	137	103.5	1.5
38-40	3	2	143	64.4	2.3

Table 5:9: Femoral mean results at loading rate of 50 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	flexural strength (MPa)	Flexural modulus (GPa)
00-01	1	1	38	67.2	0.5
01-02	1	1	134	55.4	2.6
03-04	1	2	88 ± 5.6	69.4 ± 39.8	2 ± 1.3
05-07	1	1	46	33.5	0.8
08-10	1	2	69 ± 5.6	1.8 ± 13.9	1.8 ± 1.3
11-13	2	1	44	57.7	0.5
14-16	2	3	95.3 ± 12.6	65.9 ± 36.5	1.3 ±0.8
17-19	2	3	176.6 ± 77.5	90.3 ± 23.7	2.9 ± 1.9
20-22	2	3	103.3 ± 82.5	79.9 ± 13.4	5 ± 5.7
23-25	2	3	129.6 ± 47	112 ± 54.9	2.5 ± 1.7
26-28	3	2	79 ± 48	72.7 ± 17.1	1.8 ± 0.1
29-31	3	2	91.5 ± 44.5	58.9 ± 0.7	2.6 ± 0.5
32-34	3	1	137	103.5	1.5
35-37	3	2	123.5 ± 62.5	83.8 ± 13.1	2.3 ± 0.8
38-40	3	4	88 ± 24.7	58.6 ± 12.9	1.2 ± 0.5

Table 5:10: Humeral mean results at loading rate of 100 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	Flexural strength (MPa)	Flexural modulus (GPa))
00-01	1	3	150.5 ± 73.5	87.8 ± 12.2	2.8 ± 2.7
01-02	1	4	56.8 ± 20.6	43.8 ± 10.3	1.9 ± 1.1
03-04	1	3	36.1 ± 15.5	60.4 ± 49.4	1.6 ± 1.0
05-07	1	1	124.9	58.4	0.9
08-10	1	2	38.2 ± 2.5	60 ± 40.8	0.7 ± 0.1
11-13	2	4	107.4 ± 18.5	63.5 ± 6.1	1.6 ± 0.3
14-16	2	3	84.3 ± 39.3	82.7 ± 16.9	2.1 ± 1.5
17-19	2	1	65	51.2	0.5
20-22	2	5	64 ± 10.8	67.1 ± 19.8	1.6 ± 0.8
23-25	2	2	85.5 ± 9	49.1 ± 10.5	1.1 ± 0.3
26-28	3	3	139.9 ± 35.2	69.4 ± 21.9	2.3 ± 1
29-31	3	3	168.2 ± 35.1	59.4 ± 21.8	2 ± 1.1
32-34	3	1	137.6	122.3	2.6
35-37	3	2	259.7 ± 137.3	70.2 ± 12.4	1.7 ± 0.4
38-40	3	2	71.2 ± 31.3	68.5 ± 23.9	1.2 ± 0.3

Table 5:11: Femoral mean results at loading rate of 100 mm/min.

Age (Days)	Age category	Sample size	Force at fracture (N)	Flexural strength (MPa)	Flexural modulus (GPa)
00-01	1	1	59.1	140	1.5
01-02	1	2	41.5 ± 24.2	79.4 ± 46.45	0.9
03-04	1	2	101.3 ± 22.1	63 ± 36.1	
05-07	1	2	65.6 ± 15	57.6 ± 48.0	1.1 ± 0.9
08-10	1	2	46 ± 6.1	53.2 ± 18.5	1.5 ± 0.9
14-16	2	4	64.3 ± 18.8	70.5 ± 13.3	1.7 ± 0.6
17-19	2	2	150.4 ± 125.7	50.6 ± 6.6	2.1 ± 1
20-22	2	4	140.9 ± 80.3	84.6 ± 41.9	2.4 ± 1.6
23-25	2	3	136 ± 46.1	81.3 ± 20.1	1.7 ± 0.5
26-28	2	3	146.1 ± 46.5	74.1 ± 27.2	2 ± 0.3
29-31	3	3	95.8 ± 25.1	65.3 ± 19.5	242.1 ± 109.3
35-37	3	2	85.8 ± 8.2	49.3 ± 6.7	119 ± 30.8
38-40	3	4	72.6 ± 12.7	63.1 ± 12.5	176.9 ± 39.9

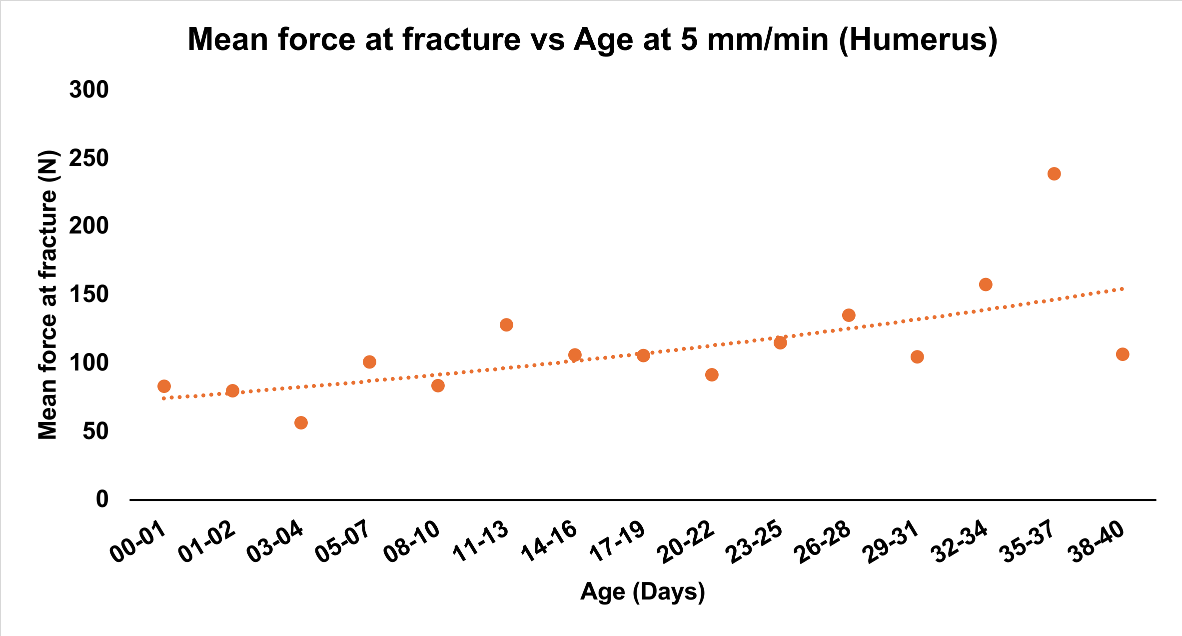

Figure 5.18: Mean humeral force at fracture vs. age at 5 mm/min

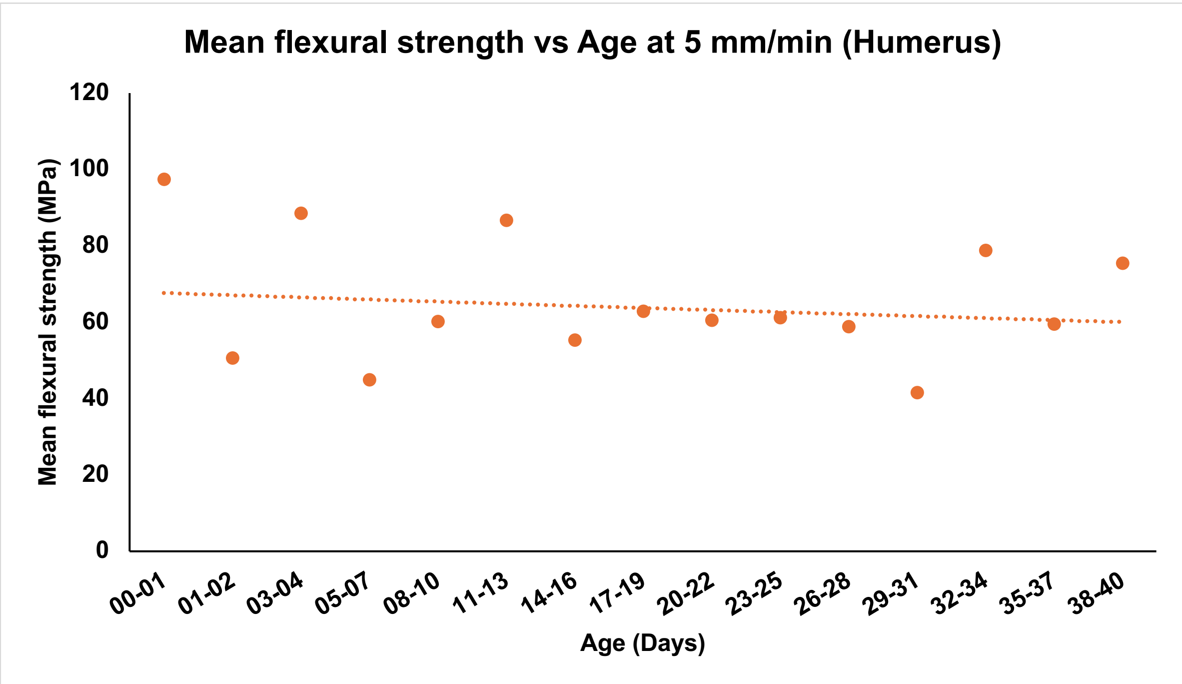

Figure 5.19: Mean humeral flexural strength vs. age at 5 mm/min with exponential trendline.

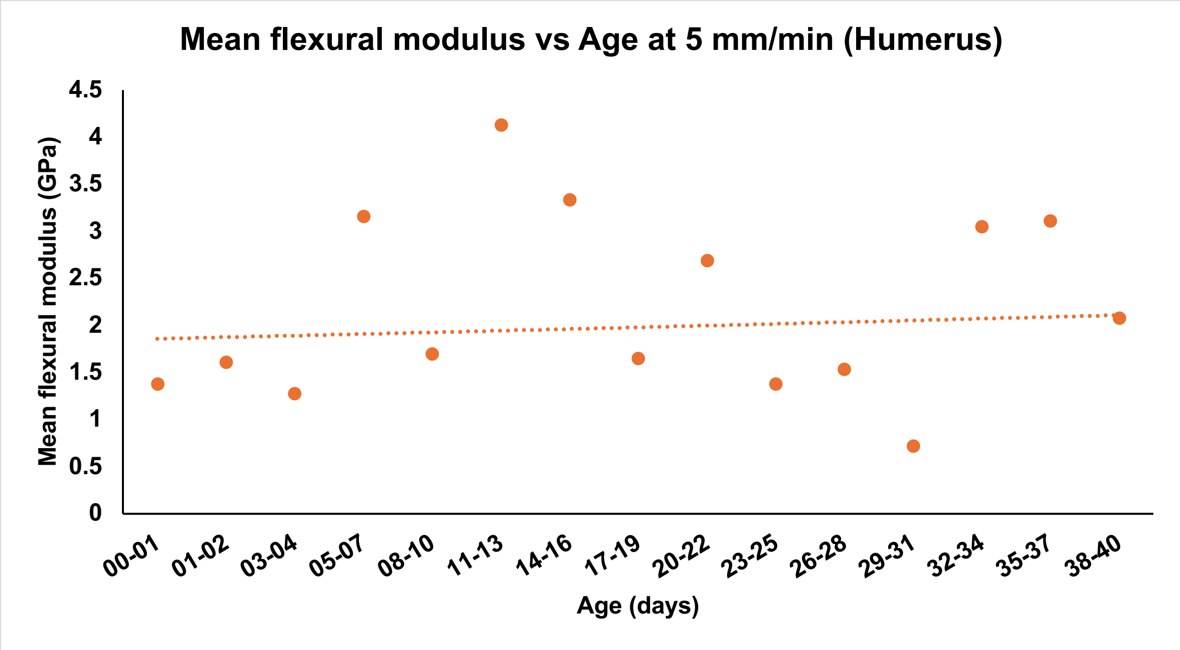

Figure 5.20: Mean humeral flexural modulus vs. age at 5 mm/min with exponential trendline.

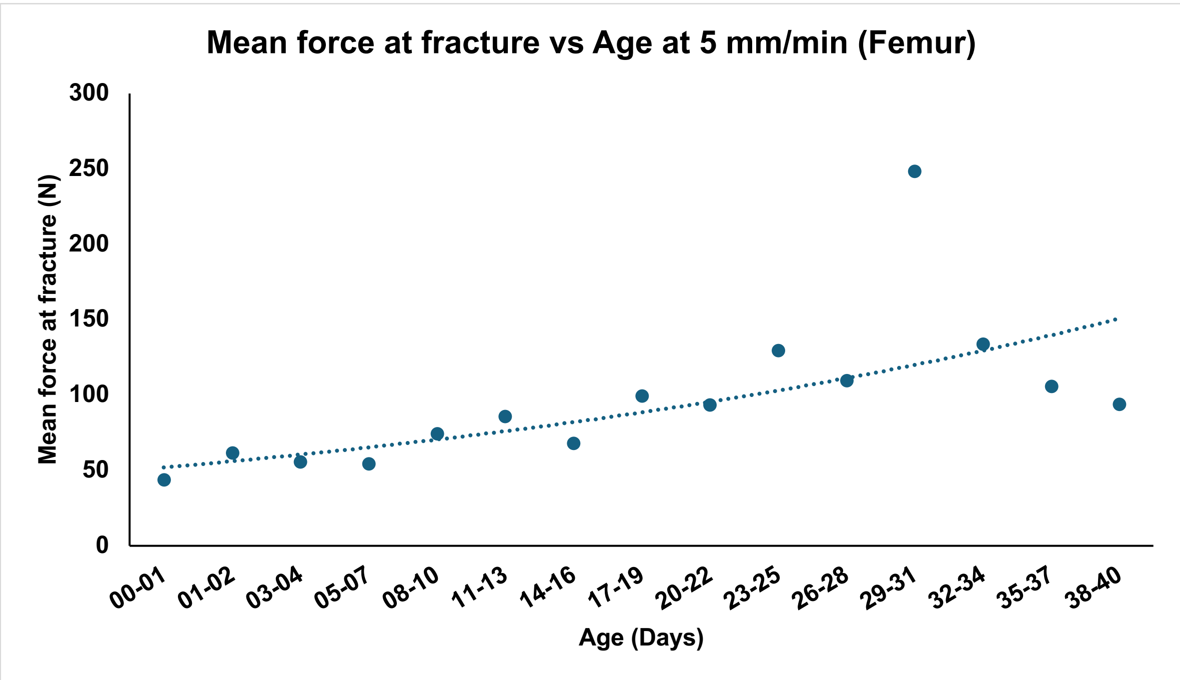

Figure 5.21: Mean femoral force at fracture vs. age at 5 mm/min with exponential trendline.

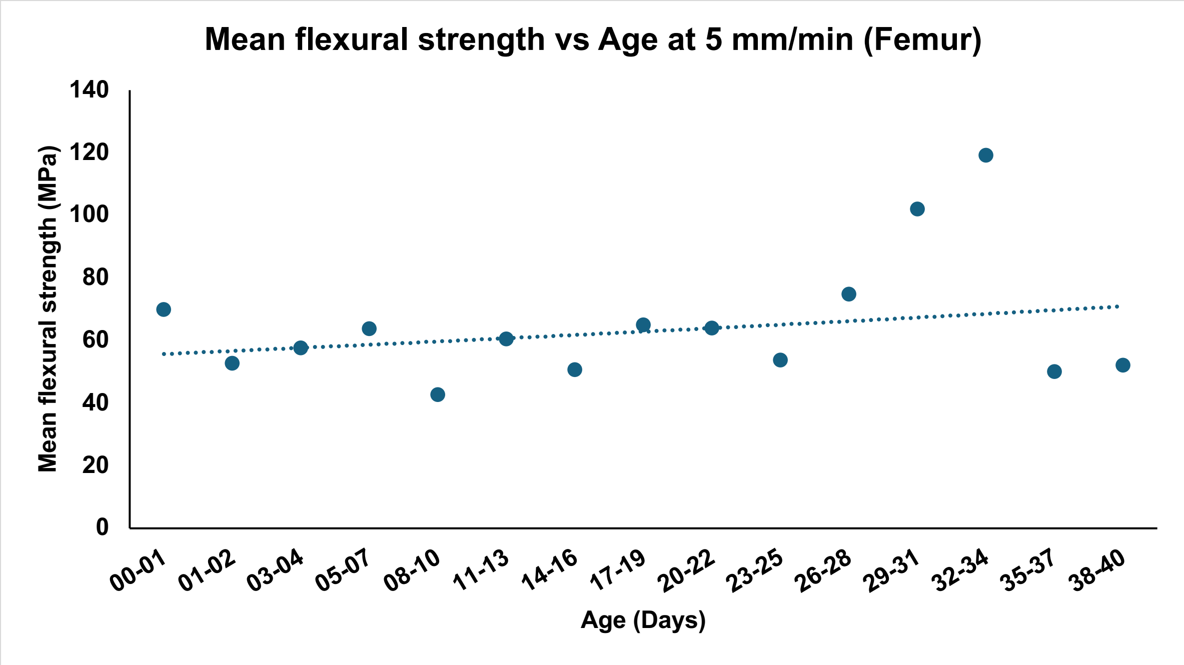

Figure 5.22: Mean femoral flexural strength vs. age at 5 mm/min with exponential trendline.

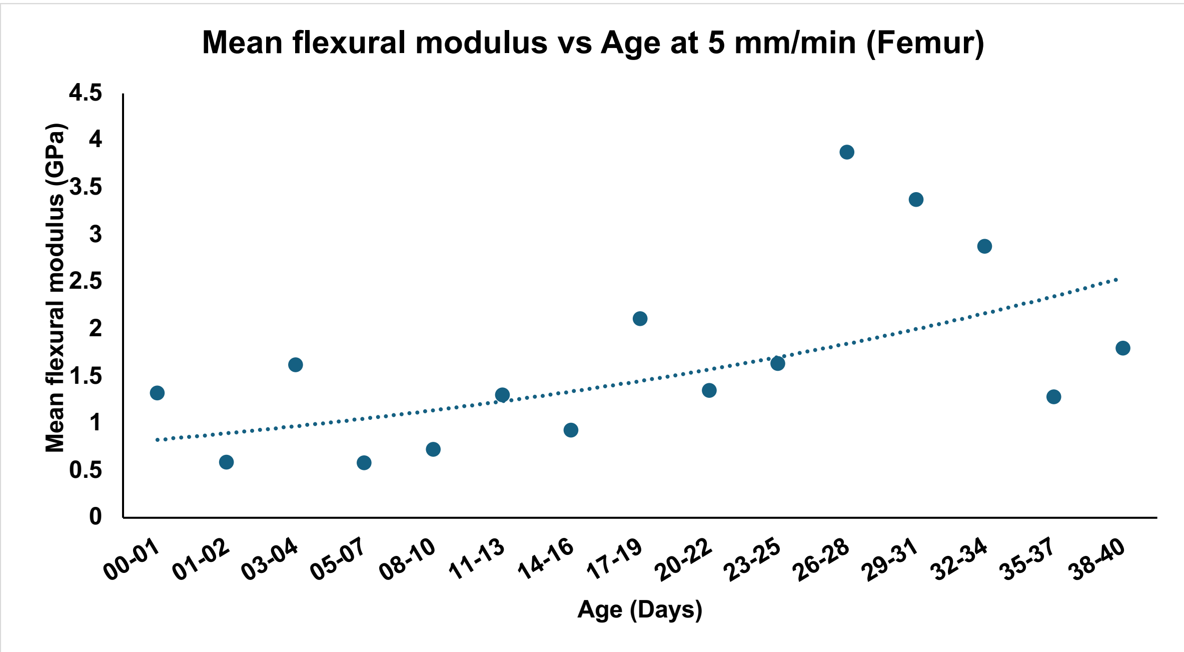

Figure 5.23: Mean femoral flexural modulus vs. age at 5 mm/min with exponential trendline.

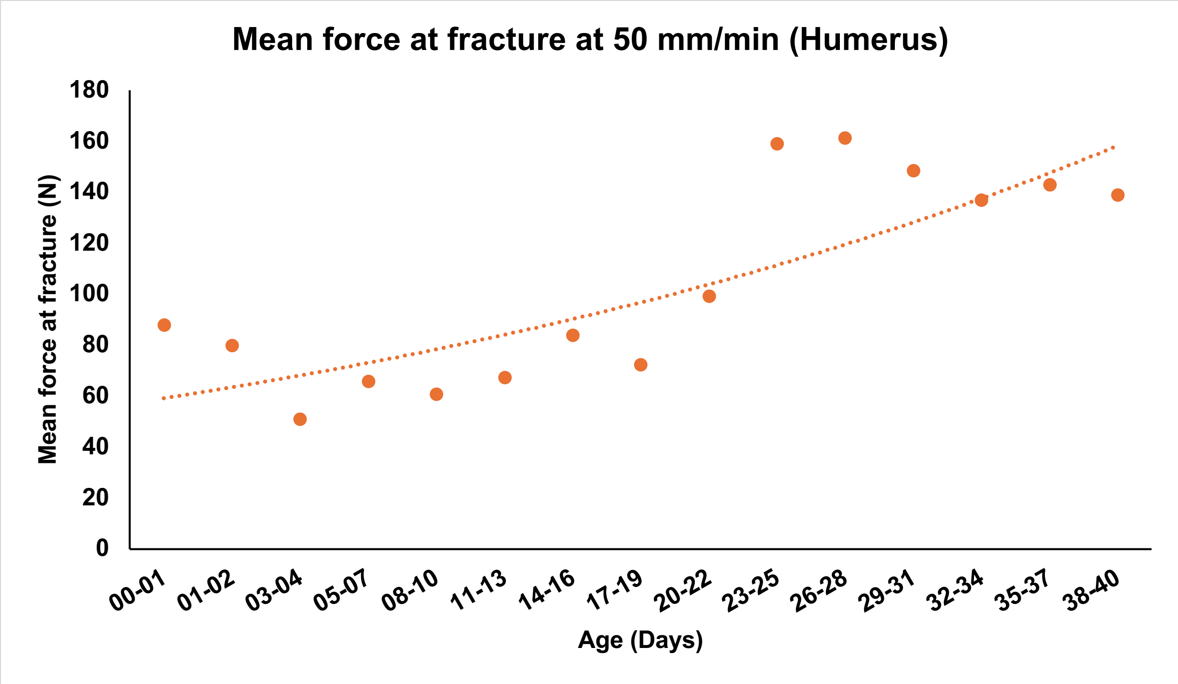

Figure 5.24: Mean humeral force at fracture vs. age at 50 mm/min with exponential trendline.

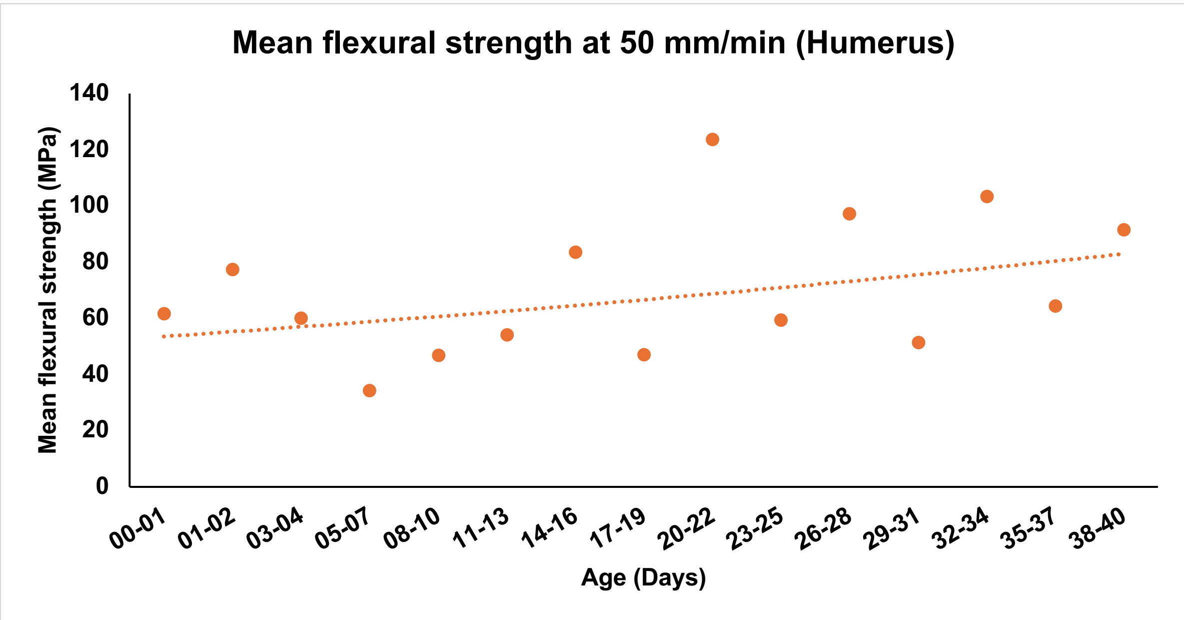

Figure 5.25: Mean humeral flexural strength vs. age at 50 mm/min with exponential trendline.

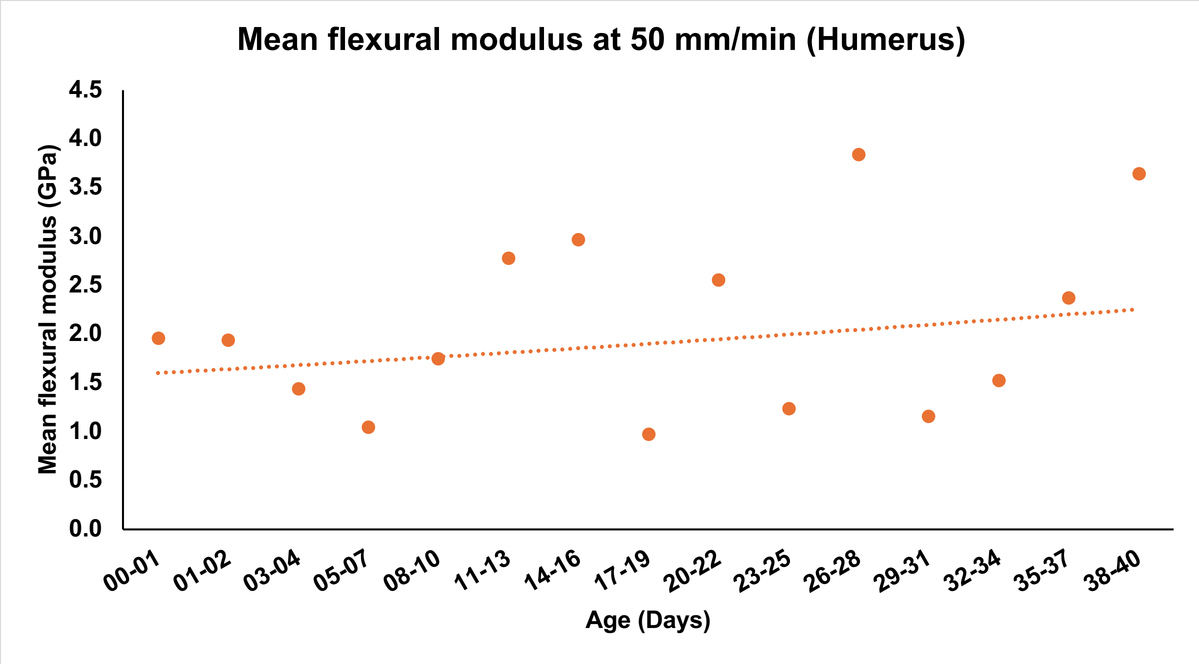

Figure 5.26: Mean humeral flexural modulus vs. age at 50 mm/min with exponential trendline.

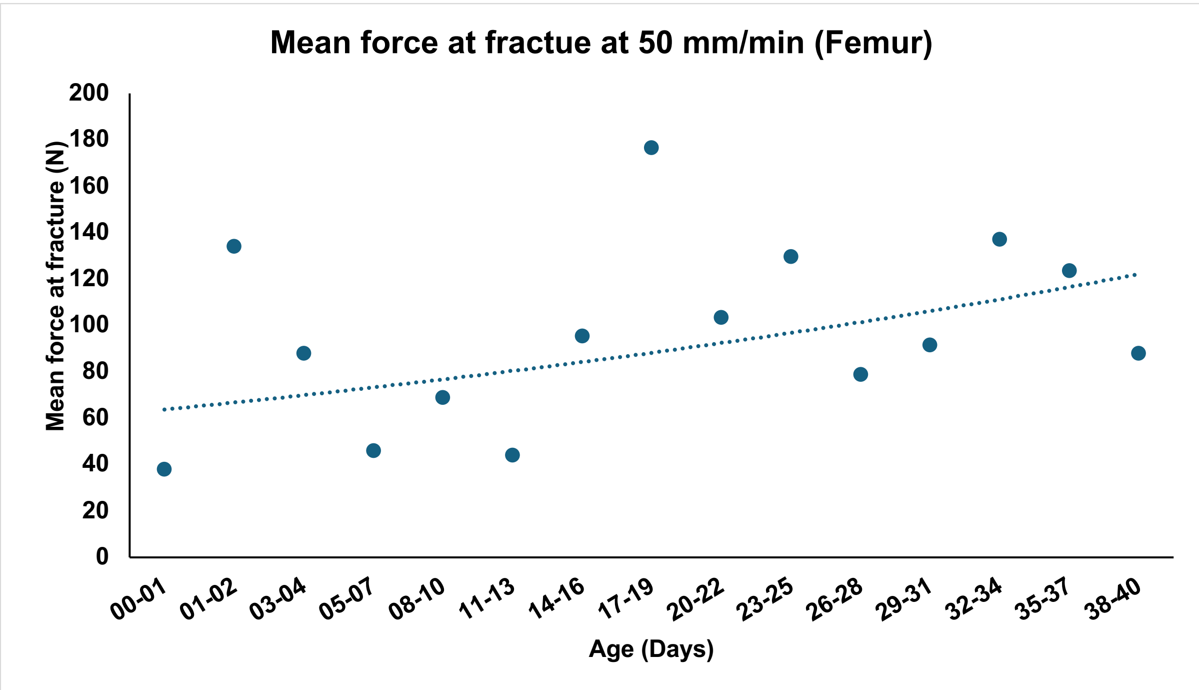

Figure 5.27: Mean femoral force at fracture vs. age at 50 mm/min with exponential trendline.

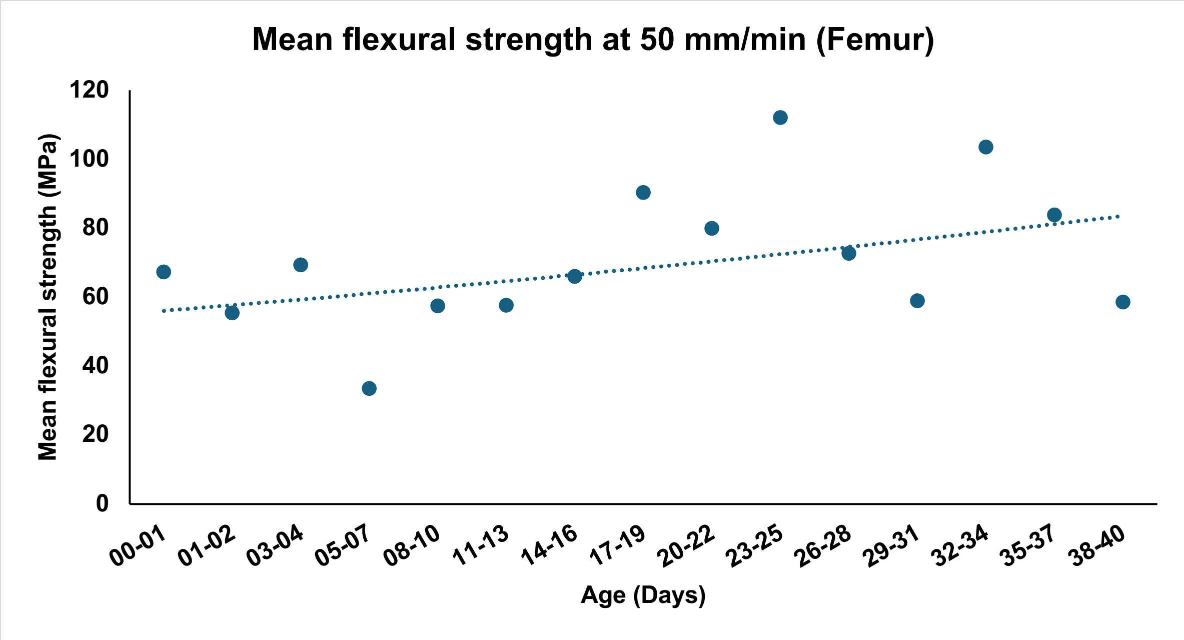

Figure 5.28: Mean femoral flexural strength vs. age at 50 mm/min with exponential trendline.

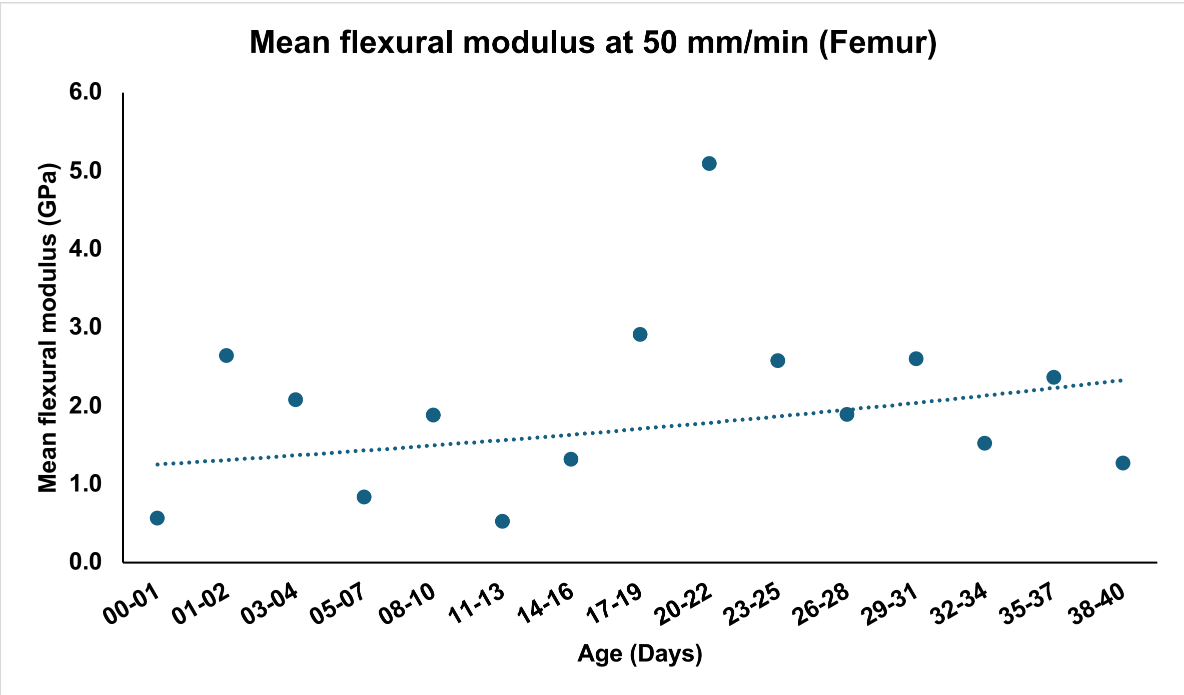

Figure 5.29: Mean femoral flexural modulus vs. age at 50 mm/min with exponential trendline.

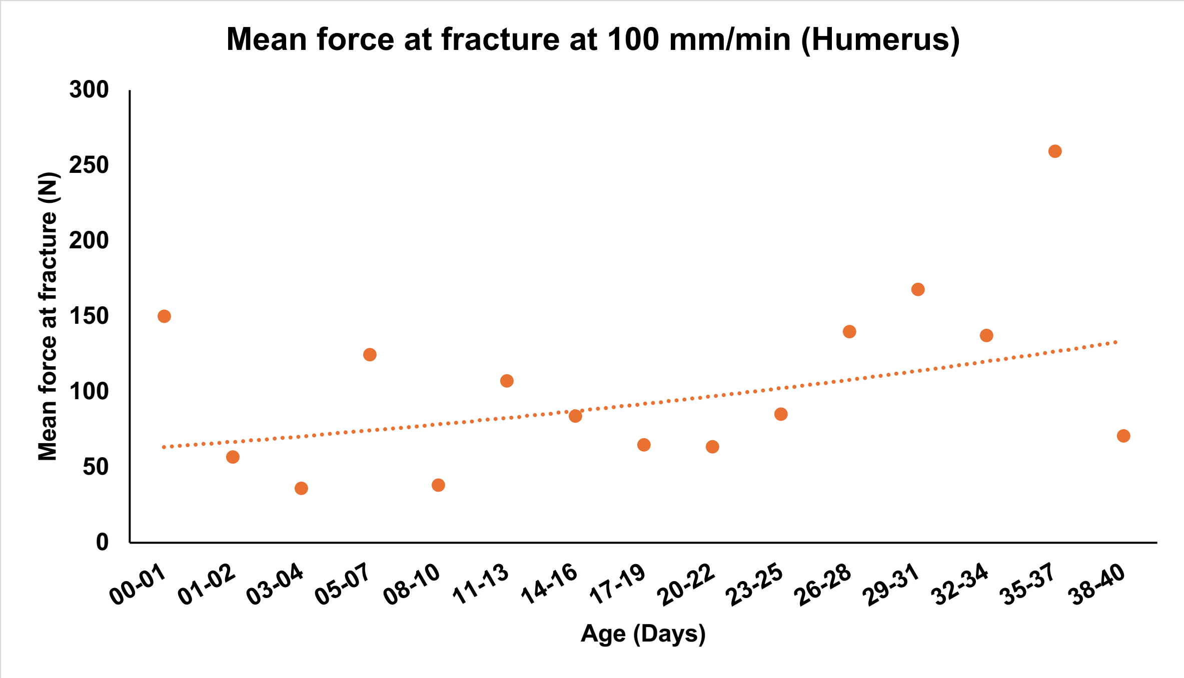

Figure 5.30: Mean humeral force at fracture vs. age at 100 mm/min with exponential trendline.

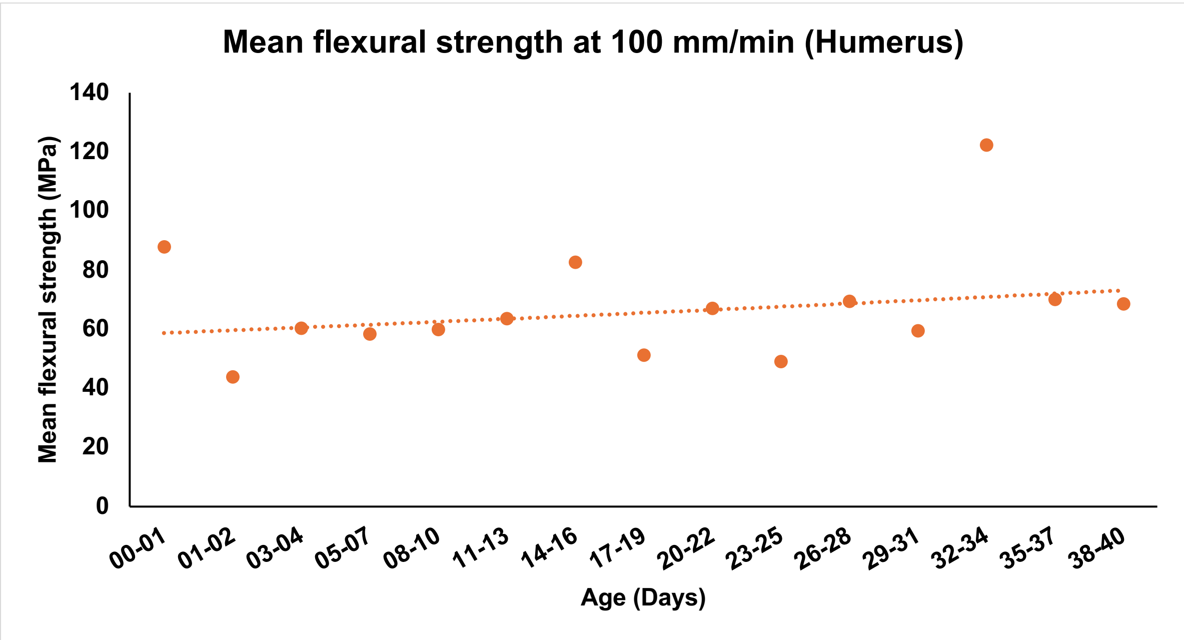

Figure 5.31: Mean humeral flexural strength vs. age at 100 mm/min with exponential trendline.

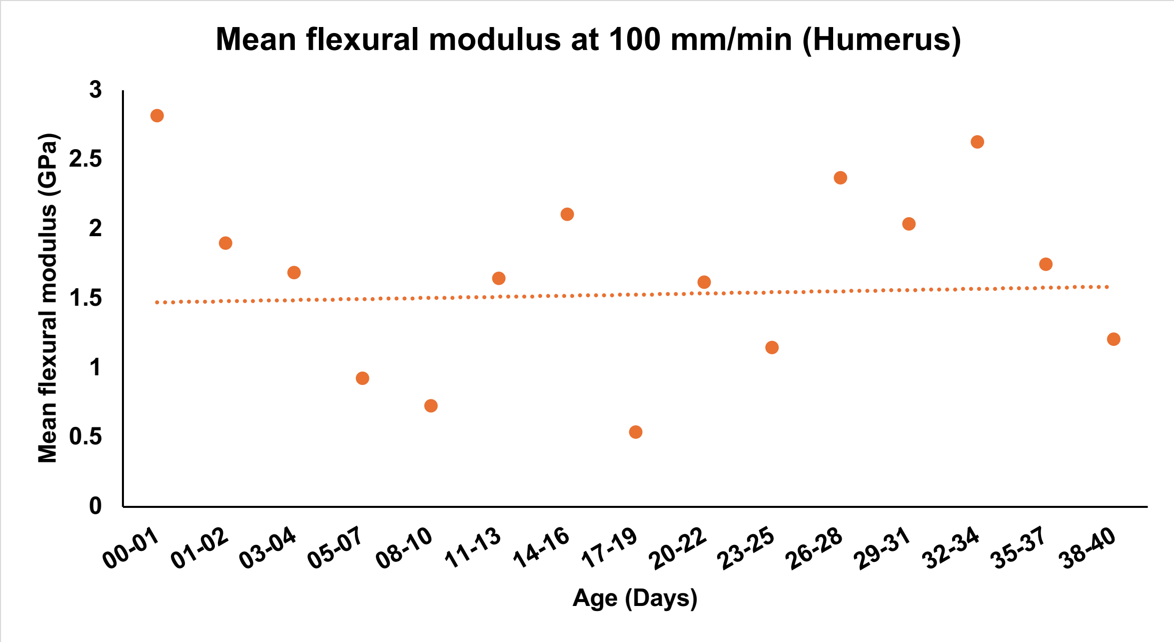

Figure 5.32: Mean humeral flexural modulus vs. age at 100 mm/min with exponential trendline.

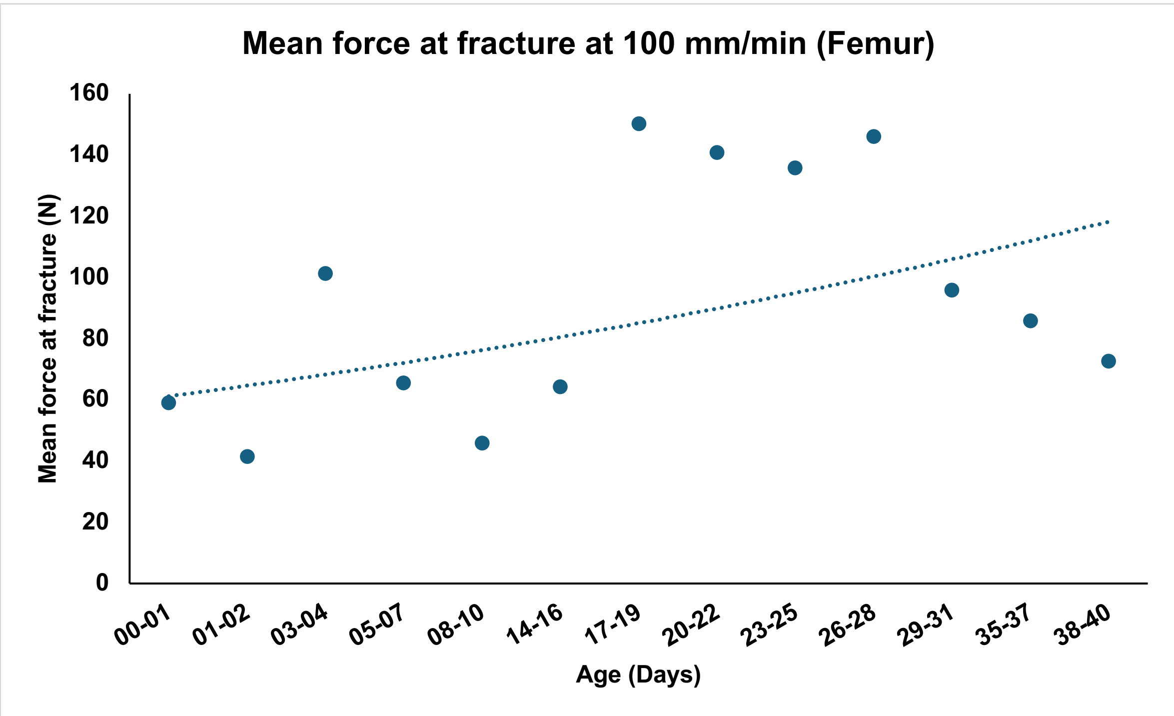

Figure 5.33: Mean femoral force at fracture vs. age at 100 mm/min with exponential trendline.

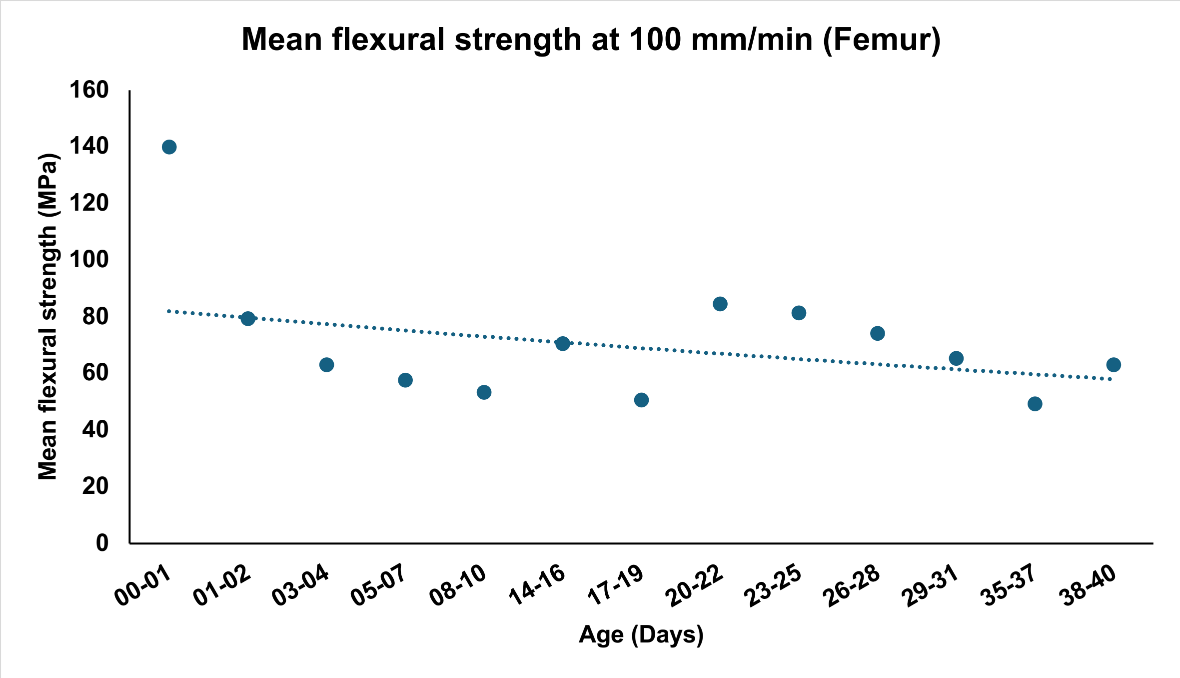

Figure 5.34: Mean femoral flexural strength vs. age at 100 mm/min with exponential trendline.

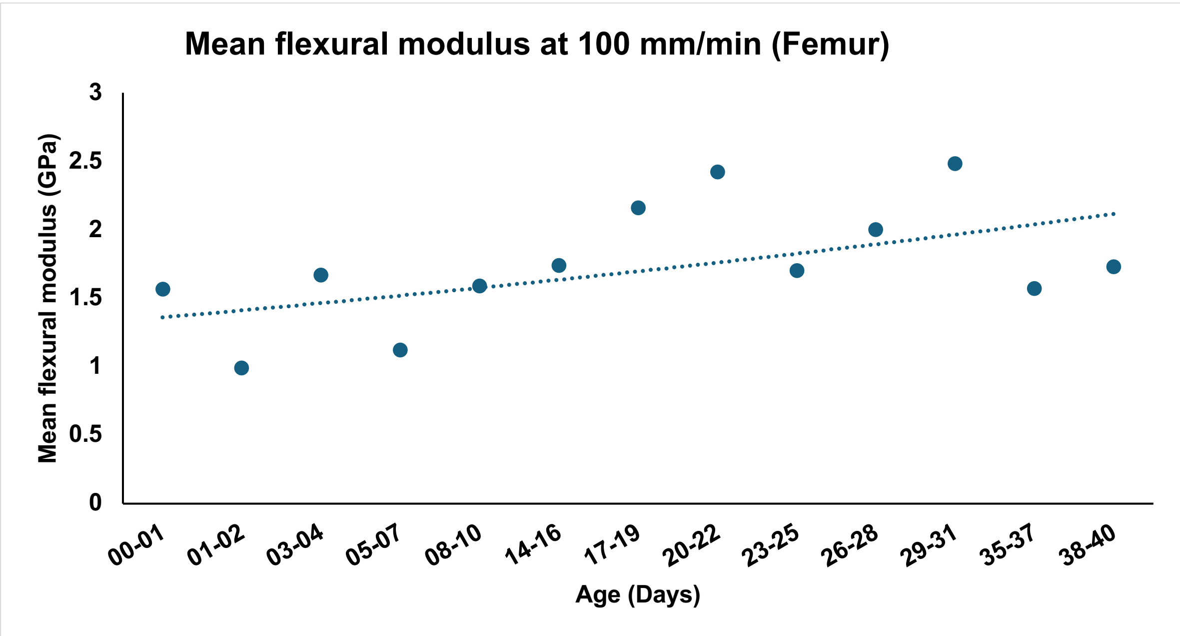

Figure 5.35: Mean femoral flexural modulus vs. age at 100 mm/min with exponential trendline.

A two-way multivariate analysis of variance (MANOVA) was conducted to investigate the effects of age category (1,2 and 3) and bone type (femoral vs. humeral) on the mechanical properties of immature porcine bone samples. The statistical results for bone type for each of the three tested loading rates can be found in Table 5.14

through Table 5.16. The statistical tests for differences in mean test scores by bone type, age, and mechanical properties found bone type to be non-significant across the loading rates 5 mm/min ($F(4, 81) = .530$, $p = .714$), 50 mm/min ($F(4,51) = .316$, $p = .866$), and 100 mm/min ($F(4, 64) = .526$, $p = .717$). This lack of bone type influence suggests that the mechanical properties of these immature porcine bones are not significantly different between the femur and humerus under these testing conditions. The interaction between age category and bone type was not statistically significant at any of the tested loading rates. At 5 mm/min, the interaction was found to be non-significant ($F(8, 164) = 1.084$, $p = 0.337$). Similarly, at 50 mm/min ($F(8, 104) = 1.214$, $p = 0.298$) and 100 mm/min ($F(8, 130) = 1.448$, $p = 0.183$), no significant interaction was observed.

It is important to acknowledge that the variable sample sizes across age categories and bone types at each loading rate may have influenced the observed trends. Despite these variations, the statistical analyses consistently revealed no significant effect for bone type (femoral vs. humeral), except for force at fracture. However, due to the variability of the sample sizes and sample dimensions, it was deemed appropriate to test for potential normalisation issues. This includes determining whether sample size and the sample dimensions were a confounding factor for force at fracture at 5 mm/min, 50 mm/min, and 100 mm/min.

Table 5:12: Number of bone samples across age category and loading rate

Sample size	**5mm/min**	**50mm/min**	**100 mm/min**
Age Category 1	32	17	22
Age Category 2	37	23	28
Age Category 3	21	20	23
Total	90	60	73

To explore the potential impact of sample size Table 5.13 summarises the number of samples across age category and loading rates. A Chi-Square test demonstrates that there is no significant association between age category sample size and loading rates (Chi-Square = 2.3449, $p = 0.673$). This shows that there is no systematic variation in sample size across age group across the three loading rates.
To address the concern that differences in bone size might be confounding the observed age effects on force at fracture, we normalised force at fracture by the

cross-sectional area of the bone samples. One-way ANOVA revealed no significant effect of age category on force at fracture at 5 mm/min ($F(2, 87) = 1.550$, $p = 0.218$), 50 mm/min ($F(2, 57) = 1.726$, $p = 0.187$), and 100 mm/min ($F(2, 70) = 0.201$, $p = 0.818$). To address the potential confounding effect of bone size on the relationship between age and bone mechanical properties, an analysis of covariance (ANCOVA) was conducted. Cross-sectional area was used as the covariate, and force at fracture was the dependent variable. The results of the ANCOVA revealed that, after controlling for cross-sectional area, there was no significant effect of age category on force at fracture. This non-significant finding was consistent across all loading rates: 5 mm/min ($F(2, 86) = 0.560$, $p = 0.573$), 50 mm/min ($F(2, 56) = 0.777$, $p = 0.464$), and 100 mm/min ($F(2, 69) = 0.815$, $p = 0.447$). These results indicate that differences in bone size, as measured by cross-sectional area, accounted for the observed effect of age category on force at fracture, effectively removing the age-related influence when controlled.

In summary, while initial analyses suggested a potential effect of age on force at fracture, subsequent investigations, specifically normalisation and ANCOVA controlling for cross-sectional area, revealed that this effect was confounded by bone size. Once bone size was accounted for, the age effect was no longer significant. Therefore, the observed variations in force at fracture are primarily attributed to differences in bone size, rather than an independent effect of age. This variability in bone size, despite efforts to group samples by age, highlights the inherent challenges in working with biological samples. Future studies could explore methods for non-destructive assessment of bone dimensions prior to mechanical testing to enable more precise matching of samples.

Table 5:13: Statistical results for Hypothesis 2 at 5 mm/min – effects of bone type in regard to age

Mechanical parameters	**F value**	**Degrees of freedom**	**p-value**
Age category	2.701	(8, 164)	.008
Bone type	.530	(4, 81)	.714
Age category * Bone type	1.084	(8, 164)	.337
Between properties			
Age category	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	10.505	(2, 84)	.001
Flexural strength	0.64	(2, 84)	.938
Flexural modulus	2.719	(2, 84)	.072
Bone type	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	1.099	(1, 84)	.297
Flexural strength	.136	(1, 84)	.714
Flexural modulus	1.977	(1, 84)	.163
Age category * Bone type	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	.448	(2, 84)	.641
Flexural strength	1.063	(2, 84)	.350
Flexural modulus	2.759	(2, 84)	.069
Post-hoc			
Force at fracture Category 1 – 2 = .002 Category 2 -3 = .361			
Flexural strength Category 1 – 2 = .981 Category 2 -3 = .895			
Flexural modulus Category 1 – 2 = .089 Category 2 -3 = .997			

Table 5:14: Statistical results for Hypothesis 2 at 50 mm/min – effects of bone type in regard to age

Mechanical parameters	**F value**	**Degrees of freedom**	**p-value**
Age category	2.816	(8,104)	.007
Bone type	.316	(4, 51)	.866
Age category * Bone type	1.214	(8, 104)	.298
Between properties			
Age category	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	6.601	(2,54)	.003
Flexural strength	2.755	(2,54)	.073
Flexural modulus	1.188	(2,54)	.313
Bone type	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	.352	(1,54)	.555
Flexural strength	.261	(1,54)	.611
Flexural modulus	.176	(1,54)	.676
Age category * Bone type	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	4.991	(2,54)	.010
Flexural strength	.223	(2,54)	.801
Flexural modulus	1.458	(2,54)	.242
Post-hoc			
Force at fracture Category 1 – 2 = .035 Category 2 -3 = .526			
Flexural strength Category 1 – 2 = .057 Category 2 -3 = .646			
Flexural modulus Category 1 – 2 = .293 Category 2 -3 = .959			

Table 5:15: Statistical results for Hypothesis 2 at 100 mm/min – effects of bone type in regard to age

Mechanical parameters	**F value**	**Degrees of freedom**	**p-value**
Age category	3.265	(8,130)	.002
Bone type	.526	(4,64)	.717
Age category * Bone type	1.448	(8, 130)	.183
Between properties			
Age category	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	5.866	(2,67)	.004
Flexural strength	.120	(2,67)	.887
Flexural modulus	.768	(2,67)	.468
Bone type	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	.444	(1,67)	.516
Flexural strength	.338	(1,67)	.423
Flexural modulus	.211	(1,67)	.906
Age category * bone type	**F value**	**Degrees of freedom**	**p-value**
Force at fracture	3.947	(2,67)	.029
Flexural strength	2.369	(2,67)	.585
Flexural modulus	2.749	(2,67)	.348
Post-hoc			
Force at fracture Category 1 – 2 = .174 Category 2 -3 = .172			
Flexural strength Category 1 – 2 = .844 Category 2 -3 = .954			
Flexural modulus Category 1 – 2 = .860 Category 2 -3 = .796			

5.6 Discussion

Study 1 developed and utilised a custom-designed experimental set-up to conduct three-point bending tests on immature porcine cortical at the loading rates of 5 mm/min, 50 mm/min, and 100 mm/min. To this author's knowledge no previous study has conducted three-point bending of immature porcine cortical bone to failure or conducted such a study with samples at consecutive ages. This study initially observed a potential age-related increase in force at fracture, suggesting that older immature porcine bone samples exhibited greater strength. However, subsequent statistical analyses, specifically normalisation by cross-sectional area and ANCOVA, revealed that this apparent age effect was significantly confounded by variations in bone size. Once bone size was accounted for, the age-related effect on force at fracture was no longer statistically significant. This outcome underscores the critical influence of bone dimensions, particularly cortical thickness and bone mineral density, on certain mechanical properties, aligning with findings reported by Augat and Schorlemmer (2006) and Gosman *et al.* (2013).

Furthermore, while statistical analyses indicated potential relationships between femoral and humeral bone samples across loading rates, MANOVA results showed no significant interaction between bone type and age. These apparent relationships were likely influenced by residual variations in bone size, highlighting the limitations of current statistical methods in fully eliminating this confounding factor. The observed differences in force at fracture between bone types at higher loading rates may be attributed to developmental variations in bone dimensions, rather than intrinsic material property differences. It is worth adding that in all of the work presented here.

5.6.1 Hypothesis 1 - Mechanical properties with age at a loading rate of 5 mm/min, 50mm/min and 100 mm/min

Hypothesis 1 posited that, similar to the literature, three-point bend tested immature porcine cortical bone samples at the loading rate of 5 mm/min, 50 mm/min, and 100 mm/min would exhibit an increase in the mechanical properties force at fracture, flexural strength, and flexural modulus across ages (age range of birth to 40 days). Descriptive trends suggested age-related changes in mechanical properties statistical significance was limited. While this effect was initially considered to be due to the inherent biological variability within the immature bone samples, coupled with the relatively small sample sizes in some of the age categories additional statistical analysis including normalisation by cross-sectional area and an ANCOVA identified bone size as a confounding factor for force at fracture.

This finding fundamentally alters the interpretation of the initial descriptive trend, indicating that bone size, rather than pure biological variability, significantly influenced the observed force at fracture. The observed (but broadly non-significant) trend of increasing flexural strength and modulus with age may still be attributed to the progressive mineralisation and collagen cross-linking that typically occurs during bone development. It is plausible that heightened osteoblast activity and subsequent bone remodelling processes contribute to the observed enhancement of stiffness and resistance to fracture in the maturing porcine samples, though further microstructural analyses would be necessary to confirm this relationship (Eleniste *et al.* 2014).

Descriptive observations initially mirrored Currey and Butler (1975), showing age-related increases in flexural strength and modulus. However, these trends lacked statistical significance after accounting for bone size, highlighting the limitations of relying solely on descriptive data. Differences in sample dimensions and test setups between this study and Currey and Butler (1975) prevent direct comparisons and emphasise the need for further research to identify other contributing factors

Discrepancies in flexural modulus values compared to Currey and Butler (1975) were significant. Their reported values (79.2 to 98.5 GPa; Table 5.16) were substantially higher than our findings (1.17 to 2.96 GPa), a difference of approximately 190%. This necessitates a detailed examination of methodological variations, including sample preparation, testing setup, and data quantification. McPherson and Kriewall (1980) yielded results more akin to this study's, despite differences in species and bone type. It's important to note the age difference in samples; Currey and Butler's youngest sample was two years, while this study included neonatal samples (Table 5.17). Even considering the Baumer *et al*. (2009) conversion of human months to porcine days, the rapid developmental changes in early life, as noted by Altai *et al.* (2017), make direct comparisons challenging. The strain-rate dependency of immature bone, as highlighted by Margulies and Thibault (2000) and Cheong *et al.* (2017), further complicates comparisons. Addressing the discrepancy in flexural modulus requires careful consideration of potential errors in measurement or calibration. Future research should prioritize standardised protocols and comparative studies to enhance the reliability of immature bone biomechanical data. Baumer *et al.* (2009), who proposed the human month to porcine day conversion, used four-point bending on cranial bone at 25 mm/sec. This suggests the age correlation may be rate-dependent or specific to cranial bone. Comparing studies with varying methodologies, including sample preparation and testing parameters, requires careful analysis of underlying assumptions and potential biases.

Table 5:16: Mechanical values derived from three-point bending tests by Currey and Butler (1975)

TABLE I

MEANS AND STANDARD ERRORS (IN PARENTHESES) OF THE VALUES OF THE BONE IN EACH SUBJECT

Age (*Yrs.*)	Sex	No. of Specimens	Bending Strength (*MN* m^{-2})	Modulus of Elasticity (*GN* m^{-2})	Work Absorbed (*J* $m^{-2} \times 10^{-3}$)	Deflection (*mm*)	Ash (*Per cent*)	N (*Ash*)
2	F	4	157.8 (20.9)	82.2 (14.1)	21.5 (5.0)	1.91 (0.31)	59.98 (0.52)	4
2.5	M	4	168.8 (2.7)	91.5 (7.3)	19.1 (2.0)	1.91 (0.28)	60.57 (1.05)	4
3	M	4	157.0 (12.0)	79.2 (8.9)	19.4 (5.3)	2.12 (0.44)	62.12 (0.17)	4
3.5	M	8	150.0 (9.1)	97.1 (6.7)	16.0 (2.0)	1.69 (0.09)	61.04 (0.33)	7
4	F	10	176.8 (5.5)	98.5 (5.7)	19.7 (1.6)	1.76 (0.10)	61.55 (0.14)	4
6	M	5	207.2 (14.3)	137.8 (10.3)	21.6 (2.7)	1.79 (0.11)	63.08 (0.42)	7
8	F	11	190.4 (4.8)	122.7 (3.2)	16.3 (0.8)	1.50 (0.07)	63.20 (0.29)	4
13	M	7	185.7 (7.6)	117.7 (4.4)	17.1 (1.9)	1.63 (0.14)	63.02 (0.25)	4
14	M	6	183.5 (9.1)	114.5 (5.6)	15.2 (2.2)	1.58 (0.14)	62.61 (0.20)	7
16	M	9	204.7 (4.7)	120.8 (5.0)	18.1 (1.1)	1.56 (0.09)	61.35 (0.41)	4
17	M	12	194.2 (4.6)	122.1 (5.5)	16.2 (1.3)	1.50 (0.09)	65.02 (0.37)	4
26	M	10	206.4 (6.4)	143.8 (6.5)	15.2 (0.9)	1.33 (0.04)	63.70 (0.38)	4
28	M	6	195.0 (6.5)	133.5 (6.0)	11.6 (1.0)	1.19 (0.09)	65.18 (0.43)	4
32	M	8	206.4 (2.2)	148.1 (3.2)	15.6 (0.5)	1.32 (0.04)	64.79 (0.24)	6
39	M	6	188.0 (6.4)	141.7 (3.8)	9.6 (0.7)	1.01 (0.05)	64.52 (0.36)	8
44	M	7	225.4 (3.2)	162.0 (4.8)	13.7 (1.2)	1.11 (0.08)	65.98 (0.16)	4
46	F	6	218.8 (7.9)	153.8 (4.2)	12.0 (1.1)	1.13 (0.08)	64.60 (0.20)	6
48	M	6	220.8 (3.5)	154.3 (3.9)	14.9 (0.6)	1.26 (0.05)	64.58 (0.38)	8

5.6.2 Hypothesis 2 - The effect of bone type with age

Hypothesis 2 aimed to assess the effects of bone type (femur vs. humerus) on the intrinsic mechanical properties of immature porcine bone and their relationship with age. Statistically, bone type was found to have no significant effect, suggesting that, within this sample, cortical bone harvested from the diaphysis of long bones exhibits similar mechanical properties regardless of origin. This aligns with the concept that, at a material level, cortical bone may demonstrate similar mechanical behaviour across different anatomical sites, though comparative studies are limited (Burstein et al. 1976; Smith *et al.* 1976; Wang *et al.* 2010). However, it is crucial to acknowledge that while bone type did not show a statistically significant effect, the underlying influence of bone size variations, as previously identified, could have masked subtle differences between femoral and humeral samples.

Burstein *et al.* (1976) observed differences in mature human tibial and femoral bone tissue, with tibial bone exhibiting a greater elastic modulus and ultimate stress. However, the mature age range of their samples (20-89 years) limits direct comparison with our immature porcine samples. Furthermore, Burstein *et al.* (1976) noted age-related degradation in femoral tissue properties, a trend that may not be directly applicable to the rapid developmental changes observed in immature porcine bone.

5.7 Limitations

A primary limitation of this study concerned the scatter of the results, significantly influenced by variations in bone size. To standardise testing conditions in the face of varying sample dimensions, a custom-designed support rig was fabricated, maintaining a fixed support span of 4 mm, determined by the shortest sample. However, this approach, while necessary, introduced potential inconsistencies. As demonstrated by our statistical analysis, including normalisation and ANCOVA, bone size emerged as a significant confounding factor for specifically force at fracture. However, it can be assumed that strength (σ_f) and stiffness (E) are not “confounded” by bone size as they were calculated from forces and deflections that were normalised for specimen dimensions. Despite so it can be most likely argued that unless the specimen sizes are always identical in dimension force of fracture will be difficult to measure.

While there were efforts to standardise the testing procedure, limitations remained. The decision to base the support span on the shortest sample necessitated the use of a relatively short span for all specimens, potentially influencing the bending moment and stress distribution experienced by longer samples. While this was a necessary compromise to accommodate all samples, it might have introduced variability in the stress profiles across the different sample lengths. Additionally, the process of visually estimating and positioning the impact head at the midpoint of each sample was inherently subjective. This introduced the potential for deviations in the impact point, which could have resulted in variations in the applied bending moment and consequently, affected the measured mechanical properties.

A second limitation of this study is the observed variability in results across the different loading rates, which is possibly attributable, in part, to the inherent viscoelastic properties of bone tissue and the recognised biological variability inherent in immature bone samples. However, the influence of bone size cannot be discounted. The observed inconsistencies in sample dimensions, as evidenced by variations in cross-sectional area, likely contributed to variability across all measured mechanical properties, not just force at fracture.

While statistical analyses, including normalisation and ANCOVA, were conducted to account for these variations, the inherent limitations of working with biological samples, particularly those undergoing rapid developmental changes, should be recognised. Future research should prioritise rigorous control of sample dimensions through standardised preparation techniques or the utilisation of non-destructive imaging methods to quantify bone geometry prior to mechanical testing. This would not only improve the reliability of force at fracture measurements but also ensure greater accuracy in the assessment of other mechanical properties. Additionally, future studies could investigate the specific contributions of growth rate, nutritional factors, and microstructural variations to the mechanical behaviour of immature bone, thereby providing a more comprehensive understanding of these complex biological materials. Specifically, future testing should aim to limit the variation of bone size within sample groups, or to create a more robust method of statistically analysing bone size. This could include, but is not limited to, the use of 3D modelling to calculate bone volume, and moment of inertia.

Lastly, the absence of detailed photographic documentation of the immature porcine bone samples, both pre- and post-mechanical testing, as well as the lack of fractographic analysis of the fractured surfaces, is acknowledged. While the methodology provides quantitative data on the mechanical properties, the inclusion of such visual evidence would have offered valuable qualitative insights into the failure mechanisms. Close-up photographs of the samples before testing could have illustrated initial morphology and any pre-existing defects, while post-testing images would have visually confirmed the mode of fracture and the extent of damage. Furthermore, fractography, through techniques like scanning electron microscopy, could have provided a more in-depth understanding of the fracture initiation and propagation, potentially revealing microstructural features influencing the bone's response to loading. This limitation is noted, and future work would benefit from incorporating comprehensive photographic and fractographic analysis to complement the quantitative findings and provide a more complete characterisation of the mechanical behaviour of immature porcine bone.

5.8 Conclusion

In an effort to build a better understanding of the mechanical behaviour of paediatric bone and the viability of immature porcine bone as a human infant bone surrogate, this thesis has chosen a bottom-up approach. This approach indicates beginning with the mechanical testing of the material properties of immature porcine cortical bone before transitioning to more geometrical specimens and finally whole bones. As such, three-point bending tests were conducted on immature porcine cortical bone samples aged newborn to 40 days of age at three different loading rates (5 mm/min, 50 mm/min, 100 mm/min). The results initially suggested an overall change in force at fracture with age. However, as demonstrated throughout this discussion, these results were significantly influenced by the confounding effect of bone size. Specifically for hypothesis 2, loading rate and bone type were found to be statistically non-significant in affecting the results concerning age. The variations observed in the plots, and the apparent differences in force at fracture between humeral and femoral samples, are strongly attributable to developmental variations in bone dimensions, such as cortical thickness or bone mineral density, rather than solely age or bone type.

The measured mechanical parameters (flexural strength, and flexural modulus) showed varying results across the experiments, with an increasing age-related trend across flexural strength and flexural modulus but with the asterisk of scattered values. These variances in results highlight that further validation of the results and more research into the area is required. As previously mentioned, no study using porcine samples this young has been conducted and therefore change in material properties may only become noticeable when in consideration of several factors such as testing set-up, sample size, and loading rate. The fact that specific changes in certain mechanical properties across age groups were found to fluctuate depending on the loading rate and type of bone, it only further reiterates that more research into the area is required specifically regarding the effect of loading rate on immature bone material. Critically, future research must prioritise rigorous control of bone dimensions.

Ultimately, this study has, despite its limitations and the revision of initial interpretation of force at fracture due to bone size, contributed to the growing body of knowledge regarding the biomechanical properties of immature porcine bone and provided a foundation for future research. This includes a greater understanding of the challenges associated with the biomechanical response of immature porcine material, and the approach undertaken when mechanically testing immature bone samples. As such, these findings, particularly regarding the importance of bone size control, offer valuable insights into the mechanical behaviour of immature bone, which could inform future studies on paediatric fracture mechanisms

Chapter 6 Mechanical Properties of Immature Porcine Bone Under High-Impact Loads

Research Question 2: As a material, how does immature porcine bone act under high impact loads?

6.1 Introduction

The accurate evaluation of a bone fracture is in part based on an understanding of how bone may react differently depending on the situation. This includes understanding how the mechanical behaviour of bone is affected by bone type, age, the manner in which an applied load is acting on the bone, and the rate at which the load is applied. While no specific loading rate has ever been accredited to suspected abuse, studies have observed a difference in bone behaviour depending on the strain rate (Margulies and Thibault, 2000; Hansen *et al.* 2008; Zioupos *et al.* 2008; Abdel-Wahab and Silberschmidt, 2011; Cheong, 2015). McElhaney (1966) tested 24-year old human cortical bone samples in compression rates ranging from 0.001 to 1500 s^{-1} and observed the elastic modulus and ultimate compressive stress to increase with strain rate. The strength and energy absorption of bone have also been found to increase correspondingly at increasing torsional (Panjabi *et al.* 1973) and tensional loading rates (Crowninshield and Pope, 1974; Hansen *et al.* 2008; Zioupos *et al.* 2008). In a trauma-related and forensic context, Hansen *et al.* (2008) and Zioupos *et al.* (2008) reported adult human cortical bone under tension to exhibit a transition from ductile to brittle bone behaviour at moderate to high loading rates. Cohen *et al.* (2016) subjected whole porcine femora (5 to 6 months of age) to various high energy impact tests and observed that the velocity of the impacts affected the type of fracture generated. Margulies and Thibault (2000) tensile tested machined cranial bone from paediatric human infant samples (25 weeks of gestation to 6 months of age) and three-point bend tested immature porcines samples (2 to 3 days of age) at 2.54 mm/min and 2540 mm/min. Margulies and Thibault (2000) observed both sample groups, human and porcine, to exhibit a greater flexural modulus and energy absorbed (per unit volume) when tested at 2540 mm/min. In consideration of age and the mechanical properties of paediatric cortical bone, Currey and Butler (1975) found a decrease in the energy-absorbing properties with age at a loading rate of 5 mm/min. Currey (1979) found this age-related decrease in energy absorbed to also be present at greater loading rates.

Both bone material and whole bones show signs of reacting mechanically different depending on the age and loading rate. When loads are applied to whole bones, a structural behaviour is exhibited, and as previously mentioned, factors such as the material properties and geometry, as well as the magnitude and orientation of the applied loads, will affect this behaviour (Wang, 2010). Fractures occur when bone is exposed to loads which generate stresses exceeding the bone's ultimate strength (Forestier-Zhang and Bishop, 2016). As such, a fracture event initially occurs at the material level before proceeding to affect the whole bone (Forestier-Zhang and Bishop, 2016). Therefore, a good starting point for investigating the strain rate dependency of bone is to begin at the material level (Cullinane and Einhord, 2002). Since bone is a viscoelastic material, both the mechanical properties of bone material and whole bone has to be considered when dealing with spontaneous events such as high energy impacts (Abdel-Wahab and Silberschmidt, 2011). As employed in Hypothesis 1 in Chapter 5, the loading rates of 5 mm/min, 50 mm/min, and a 100 mm/min are considered quasi-static loads (Miltner and Kallieris, 1989; Cristofolini *et al.* 2010; Cheong *et al*. 2017). There is therefore a need for the mechanical testing of immature porcine material at higher loading rates.

Within the context of the experimental research into the strain-rate dependency of immature bone only a few studies have tested at loading rates which may be compared to that of a violent trauma event. Cheong *et al.* (2017) and Vaughan (2017) subjected immature ovine and porcine long bones to low-rate and high-rate 4-point bending tests. While both studies identified a change in bone behaviour depending on strain rate, the studies' primary aim, similar to Cohen *et al.* (2016), was the resultant change in fracture pattern. Margulies and Thibault (2000) focused on cranial bone, which may facilitate possible comparisons to other bone types and sites. Both Currey and Butler (1975) and Currey (1979) measured the energy absorbing properties of paediatric cortical bone at lower and higher loading rates.

However, since Currey (1979) did not include the data from the tests, as well as any differences in testing approach, any direct quantitative comparisons are not possible and can only be explored descriptively. Hence there exists further room for researching the strain-rate dependency of immature cortical bone in regard to both age and bone type. A scan of the existing literature reveals the scarcity of studies that have applied consistent testing conditions to test immature material cortical bone samples under high impact rates. Further, there appears to be a gap of studies examining the variation of high impact effects across age.

6.2 Aim

The primary aim of this chapter is to address the identified gap of knowledge, through an experimental approach which will evaluate immature porcine cortical bone under high energy strains. The study will statistically test two sets of hypotheses looking at changes in energy absorption across age and bone type. Based on the already published literature on immature bone, animal or human, this study will pose and attempt to answer two hypotheses. Hypothesis 1 primarily concerns any age-related differences in energy absorbed (per cross-sectional area). In accordance with the literature, it is hypothesised that a decrease in energy absorbed will be observed with age (Currey, 1979; Margulies and Thibault, 2000). It is interesting to note that Baumer *et al*.'s (2009) results, which were conducted at 1.5 m/sec, reported no consistent age-related decrease in energy absorbed. Hypothesis 1 is as follows:

Hypothesis 1 Immature porcine cortical bone which is subjected to high energy impact tests will exhibit an age-related decrease in energy absorbed.

As there exists a scarcity of studies looking at the variances between humeral and femoral samples but with the knowledge that hypothesis 2 in Chapter 5 found there to be a non-statistical relationship between bone types (or observable trend), the second hypothesis of this experiment is as follows:

Hypothesis 2	There will be no significant difference between femoral and humeral porcine cortical bone samples in amount of energy absorbed during high impact testing.

6.3 Material

Samples for this experiment were initially collected and prepared as detailed in Chapter 5 (section 5.3).

6.3.1 Specimen collection and preparation

Each bone was thawed at room temperature 12 hours prior to testing. In adherence to the manner of sample preparation conducted in Chapter 5, rectangular samples were excised from each specimen's diaphysis in the longitudinal direction relative to the bone's in vivo position. A diamond coated cutting blade under constant saline drip followed by use of a hand-held motor driven saw were used to excise a sample from the measured midpoint of each specimen's diaphysis. To ensure measurable and smooth sample geometry, each sample was machined to a testable and comparative measurements (Table 6.1). A total of 60 samples were prepared for testing.

Table 6:1: Means of impact sample measurements

Length * Depth * Width (mm)
6.71 (± .85) * 1.92 (± .42) * 2.49 (± .66)

Despite attempts at preparing a sample geometry completely uniform in shape and size, it was soon realised that when working with organic tissues no group of samples will be completely identical, and as such the machining and filing of the samples was conducted with the care of not excessively compromising the sample. The nature of bone material is hierarchical, consisting of levels which act in unison to provide bone with its material properties and is as thus dependant on being tested as unaltered as possible. Ge *et al.* (2015) observed in the impact testing of steel samples that energy absorption (measured in J/m^2, Joules per meter squared) does differ if the samples vary in thickness. However, additional studies have also found that the presentation of energy absorbed as a function of the samples' cross-sectional area will exhibit properties nearly equal to standardised samples (Sokolov and Alexander, 1997: Ge *et al.* 2015). In response to potential variances between the samples which may have been missed during preparation it was deemed that the samples tested in this chapter (also see Chapter 4 for similar issue and explanation) would be compared according to energy absorption across the samples' cross-sectional area.

By traditional standard, high energy impact tests are conducted using either Charpy or Izod impact test configurations (Kenedi, 2013; Chaudhary *et al.* 2016). Predominantly, Charpy and Izod impact tests are conducted using either notched or un-notched samples (see section 6.3; Kenedi, 2013; Chaudhary *et al.* 2016). A notched sample will provide the measure of a material's resistance to a fracture in the presence of a flaw (see Chapter 4). As an un-notched sample is the measure of sample without any noticeable flaws, and the aim for this study is to conduct each test in as natural circumstance as possible, all samples in this study were left un-notched. The anterior and posterior side of the sample was marked with a marker pen and submerged in saline solution until testing.

It bears mentioning that due to sample orientation with two supports and an impacting head across the sample a Charpy impact test is essentially a three-point bending test (with some difference in sample position). However, it is important to note that similarly to Chapter 5 and as discussed in sub-chapter 4.4.1, the sample dimensions used in this chapter are not in line with ASTM standards. As such the designated support span provided for the samples for this study was adjusted depending on each samples' length.

6.4 Method

6.4.1 Impact tests

Impacts were delivered by a pendulum impact tester (Zwick 5102; Figure 4.8). Employing the principle of the standard Charpy impact tests, the energy generated through the impact tester is modulated by controlling the release height of a pendulum and the weight of the impacting portion of the pendulum. The current and peak angle of the pendulum was regulated through the impact tester's analogue-angle-indicator which for all tests was conducted with a set-up angle of 160 degrees. Given the initial angle and the maximum angle reached after impact, loss of the total mechanical energy (potential energy + kinetic energy) during the pendulum's initial swing was determined through calculations detailed in section 6.4.2.

A bespoke holder for the samples allowed for holding samples of the dimensions chosen and for the assumption of negligible friction (Figure 6.1; measurements of holders in Length from longest point * Width * Height was 5 cm * 2 cm * 3 cm). This implies that the energy lost by the pendulum is equal to that absorbed by the sample during impact. For this study, a calibrated and custom-made hammer of 0.1087 kg mass (0.2116 kg with rod) and 225 mm long handle was constructed and used (+ 5.9 cm with hammer) which when used, impacted the sample with a velocity of 2.93 meters per second (m/s). Due to samples' varying sizes, a customised holder was developed and manufactured along with the impact hammer. Each sample was placed so that impacted surface was to the anterior of the sample.

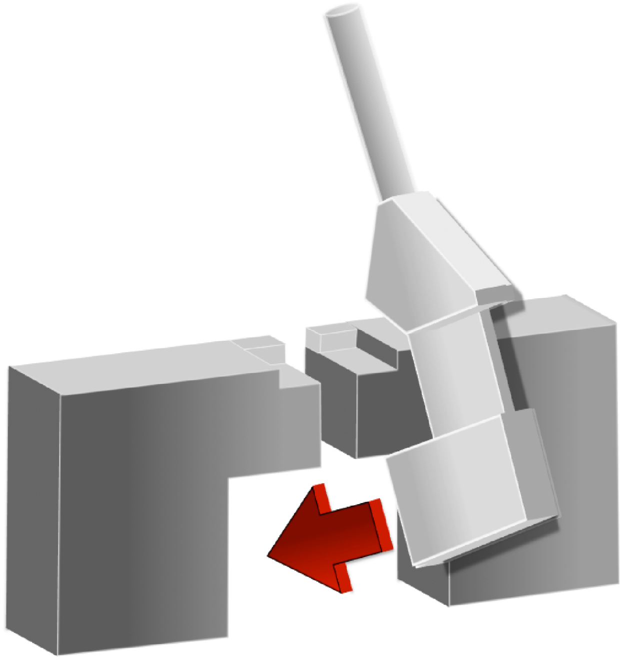

Figure 6.1: Custom sample holder for impact test

6.4.2 Data quantification

The initial data was recorded as energy absorption (J), enabling the relationship between angle and energy to be established. Further calculations, seen below, in turn revealed the presence of a compound pendulum energy exchange system. In this system, the hammer head and attached handle possess the mass capable of creating a moment while swinging about its rotating axis. Therefore, when lifted to the elevated starting position, the compound pendulum was estimated to possess a gravitational potential energy which can be calculated according to the Figure 6.2.

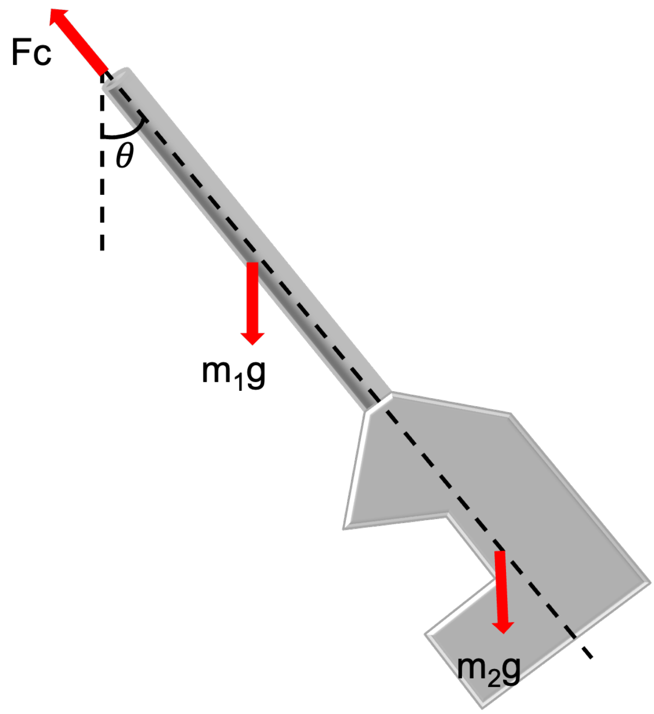

Figure 6.2: The forces acting on the impact rig pendulum during its swing.

The potential energy of the pendulum at any given point in its swing (θ) was calculated in the following manner:

$$PE = g(am_1 + bm_2)(1 - \cos\theta)$$

By conservation of mechanical energy and neglecting frictional forces, the kinetic energy of the pendulum at any given point (θ) in its swing can hence be calculated to:

$$PE_{starting} - PE_{\theta}$$

The energy absorbed during impact can hence be calculated to be the difference between the energy associated with the theoretical peak of the pendulum's swing (identical to its starting point) and that of the actual peak measured after impact, as shown below:

$$\Delta E = PE_{peak\ angle} - PE_{starting}$$

6.4.3 Statistical analysis

Considering the three hypotheses presented in this chapter, three statistical tests were conducted. The statistical relationship between the sample's age and the sample's resultant energy absorbed was analysed through one-way ANOVA with a Tukey post-hoc test. A two-way ANOVA with a Tukey post-hoc test was conducted in order to determine the potential significance of using either femoral or humeral samples across age in regard to energy absorbed. Statistical significance was set at $p \leq 0.05$. As was found during testing in Chapter 5, certain age groups only contained one sample and such for statistical strength, the samples were categorised into three broader age categories: newborn to 10 days, 11 days to 25 days, and 26 to 40 days of age.

6.5 Results

A total of 40 samples (humerii n = 11, femora n = 29) were subjected to un-notched Charpy impact tests at 2.93 meters per second (m/sec). The resultant means and the relationship between age and output of energy absorbed (addressing Hypothesis 1) are presented numerically in Table 6.2 and graphically in Figure 6.3. The resultant means and the relationship between either femoral or humeral samples over age regarding energy absorbed (addressing Hypothesis 2) are presented numerically in Table 6.3 and graphically in Figures 6.4 and 6.5. The relationship between age and resultant energy absorbed at 'high' and 'low' (Currey and Butler, 1975) loading rates is presented numerically in Table 6.5, and Figure 6.6 presents the means graphically.

6.5.1 Hypothesis 1 - mechanical properties with age a loading rate of 2.93 m/sec

The resultant means by age category for energy absorbed from the experiment are presented numerically in Table 6.2 and as a plot in Figure 6.3. The data presented in Table 6.2 showed no consistent chronological pattern across the samples' age groups, the highest measurement was observed in sample group aged 23 to 25 days of age (Table 6.2). The lowest measurement was observed in the age group 14 to 16 days of age.

Table 6:2: Means for energy absorbed (J/m^2) per cross-sectional area by age.

Age (days)	Sample Size	Energy absorbed (J/m^2)	SD (±)
00-01	1	33500	0
01-02	4	13412.6	1999.9
03-04	5	34138.8	8733.6
05-07	2	18840.9	932
08-10	7	21592.5	13117.2
11-13	3	12758.3	3838.1
14-16	1	5000	0
17-19	3	43333.3	18819.3
20-22	2	35750	4596.1
23-25	4	52656.2	10562
29-31	3	21250	8196.7
35-37	2	9097.2	491
38-40	3	21333.3	2309.4

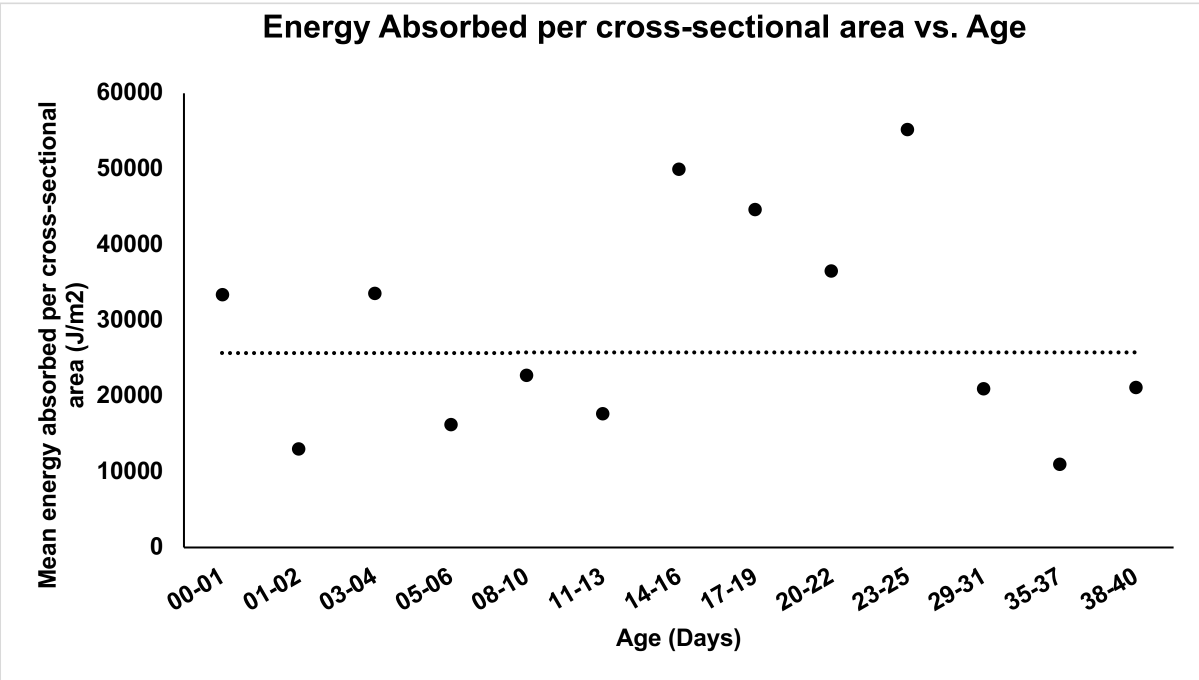

Figure 6.3: Means for energy absorbed per cross-sectional area vs. age with exponential trendline.

The statistical results derived from the One-Way ANOVA found energy absorbed to show a significant difference in means with age (F (2, 39) = 6.851, p = .003). It is important to clarify that this ANOVA result indicates statistically significant differences between the age categories, but it does not necessarily imply a linear trend with age. For ease of reference the three age categories and resultant means used for the statistical analysis of Hypothesis 1 was newborn to 10 days [age group 1] (14384.6 ± 8757 J/m^2), 11 days to 25 days [age group 2] (27761.9 ± 11658.2 J/m^2), and 26 to 40 days of age [age group 3] (15076.9 ± 6572.8 J/m^2). Post-hoc Tukey tests showed significant changes in mean measurements from age category 1 to age category 2 (.009) and age category 2 to age category 3 (.007).

To account for a result where the data was not normally distributed a non-parametric Kruskal-Wallis test was conducted for comparison. The results of the Kruskal-Wallis test also indicated a significant difference in energy absorbed across the age categories ($H(2) = 7.589$, $p = .022$). A subsequent Dunn's post-hoc tests with Benjamini-Hochberg correction indicated a significant difference between age category 1 and 2 (adjusted $p = .0315$), with a significant result between category 2 and 3 (adjusted $p = .045$).

It is acknowledged that the absence of a prior power analysis constitutes a significant limitation of this study. The observed high variability in the data, as evidenced by the data scatter, likely reduced the statistical power of the conducted Kruskal-Wallis test. Consequently, the study might have been underpowered, increasing the risk of failing to detect true differences in energy absorbed across age categories (Type II error). This implies that, while the Kruskal-Wallis test indicated significant differences, the results should be interpreted with caution, as the required sample size to achieve adequate statistical power might have been substantially larger. Future research in this area should prioritise conducting a power analysis prior to data collection to determine the necessary sample size and aim to reduce experimental errors, thereby improving the reliability and interpretability of findings. If possible, using the current data's mean and standard deviations, a power analysis should be conducted to estimate the number of samples that would have been needed to achieve statistical significance.

Furthermore, as observed in Chapter 5, it was found important to determine whether the variable of the bone dimensions influenced the observed trends. To address the concern that differences in bone size might be confounding the observed age effects on force at fracture, we normalised energy absorbed by the cross-sectional area of the bone samples. The one-way ANOVA revealed a significant effect of age category on energy absorbed ($F(2, 57) = 9.268$, $p < .001$). To address the potential effect of bone size on the relationship between age and energy absorbed, an analysis of covariance (ANCOVA) was also conducted. Cross-sectional area was used as the covariate and energy absorbed was the dependent variable. The results of the ANCOVA revealed that, after controlling for cross-sectional area, there was no significant effect on energy absorbed ($F(1, 56) = 1.924$, $p = .171$). This suggests that once the effect of bone size was controlled, it did not explain the variance in energy absorbed.

6.5.2 Hypothesis 2 – the effect of bone type with age at a loading rate of 2.93 m/sec

Hypothesis 2 tested whether there are any differences between energy absorbed and age depending on whether bones were femur or humerus. As observed in Table 6.3 the femoral samples aged 23 to 25 days and respectively humeral samples aged 17 to 19 days showed the greatest output of energy absorbed (Table 6.3).

As observed in Figure 6.3 and 6.4 and if looking at singular age samples the highest femoral specimen was noted in the sample group aged 23 to 25 days whilst the lowest was in age group 1 to 2 days. The highest recording for the humeral samples was observed in age group 17 to 19 days and the lowest in the samples aged between 11 to 13 days. As visible from the trendlines the femoral samples indicate a trending decrease with age in energy absorbed whilst the humeral samples' trendline indicate an increase with age (Figure 6.3; 6.4). However, as can be noted, a wide variety of scatter was observed across the sample ages and bone types, all of which will be discussed further in the next section (6.6).

Table 6:3: Means for energy absorbed (J/m^2) per cross-sectional area by age according to femora and humerii.

Femora				**Humerii**			
Age (days)	**Sample size**	**Energy absorbed (J/m^2)**	**SD (±)**	**Age (days)**	**Sample size**	**Energy absorbed (J/m^2)**	**SD (±)**
00-01	1	33500	0	03-04	2	40833.3	10606.6
01-02	4	13432.5	2308.7	05-07	2	18840.9	932
03-04	3	29675.9	4648.8	11-13	3	12758.3	3838.1
08-10	7	19400.7	13304.6	17-19	2	47916.6	31230.5
14-16	1	50000	0	20-22	2	35750	4596.1
17-19	1	37500	0				
23-25	4	52656.2	10562				
29-31	3	21250	8196.7				
35-37	2	16064.8	491				
38-40	3	21333.3	2309.4				

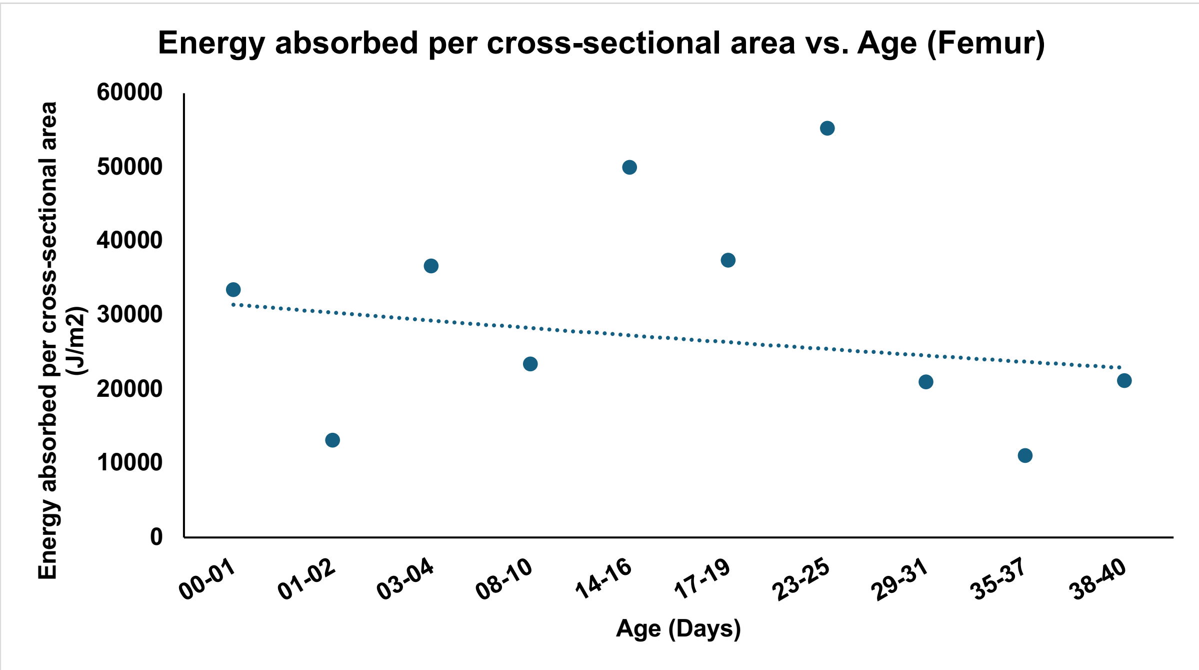

Figure 6.4: Means for energy absorbed (femoral) per cross-sectional area vs. age with exponential trendline.

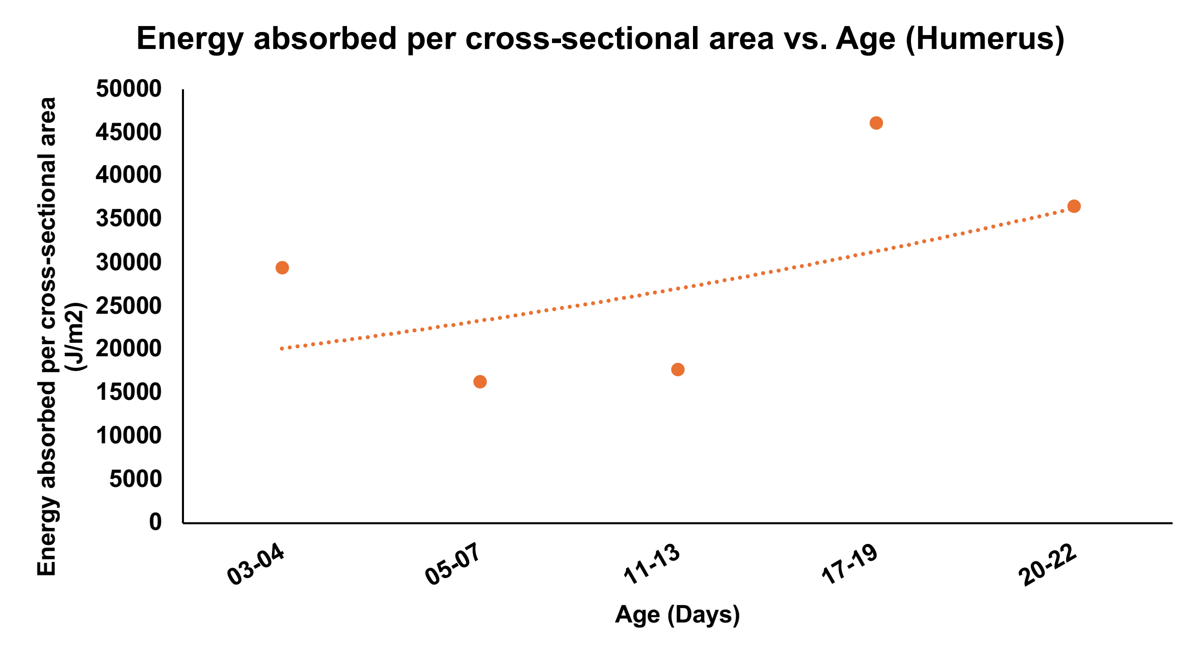

Figure 6.5: means for energy absorbed (humeral) per cross-sectional area vs. age with exponential trendline.

The statistical results for bone type can be found in Table 6.4. The statistical tests for differences in mean test scores by bone type, age, and energy absorbed found bone type to be statistically non-significant (F (1,39) = 1.215, p > 0.05). Post-hoc Tukey tests showed significant changes in mean measurements from age category 1 to age category 2 (p = .004) and age category 2 to age category 3 (p = 003).

Table 6:4: Statistical results Hypothesis 2– effects of bone type in regard to age.

Mechanical parameters	F value	Degrees of freedom	p-value
Age category	7.470	(2,39)	.002
Bone type	1.215	(1,39)	.278
Age category * Bone type	8.219	(1,39)	.007
Post-hoc			
Energy absorbed Category 1 – 2 p < .005 Category 2 – 3 p < .005			

6.6 Discussion

6.6.1 Hypothesis 1 - discussion

As presented in section 6.1, Hypothesis 1 concerned any age-related differences in energy absorbed among the samples. Based on the literature Hypothesis 1 stated that a significant decrease in energy absorbed would be observed with increasing age. It may be noted from Table 6.2 and Figure 6.3 that the trendline is overall suggestive of slight increase in output of energy absorbed across age. However, despite this increase in energy output with age the results show quite a large scatter. In regard to the suggested increase, adult bone, bone mass and bone architecture has been suggested to adapt to external loading conditions (Leblanc *et al.* 1995; Petrtýl *et al.* 1996; Bailey *et al.* 1999). Bone density has been reported to increase if subjected to incremental or continuously applied small loads. As such it could be speculated that bone's capacity for energy absorption would increase with age. However, bone density has also been observed to decrease in situations of immobilisation, bed rest, and under microgravity conditions (Collet *et al*. 1992; Kiratli *et al.* 2000; Rittweger *et al*. 2009). It has been proposed that not only adult bone but also developing bone is affected by these same factors of mechanical stimuli (King *et al.* 2003; Apkon and Kecskemethy, 2008). Growth plates cause longitudinal growth by producing new trabeculae which is subsequently reabsorbed or merged into the cortical shell (Wang *et al.* 2010).

In regard to the observed scatter, a possible explanation is an issue with the test setup. It was noted prior to testing that the depth to width ratio was greater in some samples. While these samples were removed from the data, the difference in the sample dimensions may have influenced the energy absorbed by the samples. Furthermore, while the samples were machined to contain only cortical bone, remains of trabecular bone were noted on some samples. While it was determined on those samples that removing any more trabecular bone would have compromised the samples' physical integrity it worth noted that the trabecular bone might have had a potential effect.

Simultaneously, the sample holder was adjustable to accommodate any difference in the sample length (Table 6.1). While the samples were machined to ensure the amount of available impact area would not impede or limit the impact head, the differing sample lengths may have been a factor. The Charpy impact test has been noted as sensitive to factors such as temperature and specimen size, which can further exacerbate the variability in results if not controlled properly (Wang *et al.* 2022; Ando *et al.* 2019). Lastly regarding the test set-up is that the samples were not fixed but held (See figure 6.1). With the equipment available and smaller dimensions of the samples, there was no possibility of fixing the samples in place other than the holding shelfs and a supporting back at the distal ends of the samples (Figure 6.1). However, there is the possibility that due to the level of impact the samples did move resulting in the scatter of information presented in this chapter. The custom-designed shelf used for securing the samples may not have provided the necessary stability, leading to inconsistent impact responses. This possibility is supported by research findings that emphasise the importance of specimen alignment and fixation in impact testing, as improper mounting can introduce variability in the results due to misalignment during the test (Takashima *et al.* 2016). The observed data scatter is likely due to the combination of the sample dimension irregularities, and the sample holding method. The holding method, that did not fix the samples, probably allowed for sample movement during testing, that would have introduced large variances into the data. Furthermore, aspects of the testing setup, such as the sample holding method, could have contributed to the data scatter

It is acknowledged that the absence of a prior power analysis constitutes a significant limitation of this study. The lack of prior power analysis, which may have led to an underpowered study, adds further uncertainty to the interpretation of these results. Furthermore, the ANCOVA results, that showed that when controlling for cross sectional area, that the effect of age was not significant, further adds to the uncertainty of the results. Because of all these factors it is not possible to conclude with certainty that the observed trend in energy absorption is due to age and not additional factors such as irregularities in the test set-up.

6.6.2 Hypothesis 2 – discussion

The study of bone type in regard to anatomical site remains an unexplored area of research. This study posited that there would be no significant difference between the femoral and humeral porcine cortical bone samples in amount of energy absorbed across age

Whilst a large number of studies have focused on the differences between trabecular and cortical bone, these studies have not extended the research to different anatomical sites (Tanck *et al.* 2006; Carter *et al.* 2016; Hart *et al.* 2017). It may be suggested that striving for consistency in method encourages studies to only use one type of bone. Unfortunately, this hinders any potential comparisons. Validating the suitability of bone tissue across several bones is important for the simple fact that it either provides researchers with a legitimate reason for staying consistent in testing one type of bone (as this study has not) or in providing researchers with a potentially additional source of tissue which can be used for animal model testing in a single test.

While the finding of a significant age-related effect on energy absorbed in the humerus, but not the femur, suggests a distinct developmental or structural difference between these bones influencing the age-related changes, we must acknowledge the considerable scatter observed in the chapter's data across both bone types. This scatter, potentially arising from the test setup, introduces a significant degree of uncertainty. Therefore, while the results warrant a reconsideration of the existing literature, which largely overlooks the influence of anatomical site, and highlight the importance of considering the specific bone element, there must be caution in attributing the observed differences solely to inherent bone type variations.

With no available literature on the potential histological differences between immature porcine cortical bone across different bone elements, it is difficult to definitively determine whether the results in this study are primarily due to developmental differences between the femur and humerus, the actual tested samples, or the test setup. Furthermore, no study to this author's knowledge has investigated possible chronological variances in immature porcine humeral and femoral bone development. In forming a hypothesis, it might be speculated however that due porcines' quadrupedal manner of locomotion that development would occur at a similar time therefore producing no discernible difference between the femur and humerus. However, the impact of the test setup on the data scatter necessitates further investigations to disentangle the true sources of variation.

In consideration of differences between individual samples, Evans (1958) noted that variances in mechanical properties are possible if the samples are from separate regions despite being from the same bone. However, since Evans (1958) also chose to separate the samples into the proximal third, middle, and distal third regions of the tested femora and this study decided upon excising each sample from the midpoint of the femoral and humeral diaphysis, the observations made by Evans (1958) cannot necessarily be applied here. Sedlin and Hirsch (1966) found whilst bending and compression testing femoral samples that those excised from the lateral region of the femoral shaft were found to exhibit greater strength (19.3 MPa) followed by the anterior (18.9 MPa), medial (18.7 MPa), and posterior segment (17.8 MPa) of the bones. Unfortunately, the Sedlin and Hirsch (1996) study contains no reference to the age of the tested samples, only referring to specimens as twenty adult subjects. Since age has been observed to be a factor in the mechanical behaviour of bone, any differentiation between the effect of bone site and age is difficult (McElhaney, 1966; Punjabi *et al.* 1973; Crowninshield and Pope, 1974; Margulies and Thibault, 2000; Hansen *et al.* 2008; Abdel-Wahab and Silberschmidt, 2011; Cheong, 2015). Gauthier *et al*. (2017) who three-point bend tested several machined adult samples from harvested femora, tibiae, and radii at a loading rate of $10^{-4}/s^{-1}$, observed no significant difference in fracture properties between the three anatomical sites. However, this was not considered within the parameters of this research project and as such, the bone samples tested were removed from a cross-sectional site on each bone, but that 'extraction' site was not the same across the samples. This may explain the potential variances observed between the individual samples and the bone types.

Foremost, this study is encumbered by a lack of comparable age groups and sample numbers from each bone. Considering that this meant that it was not possible to test humeral samples in the youngest age group as well as those from 22 days of age and older, it is acknowledged that further testing is required. Whilst similar variances were observed in Chapter 5 despite a larger and more extensive sample size from each bone, it has become apparent that the exactness which is required for results to stand the scrutiny of legal courts demands complete uniformity and consistency in experimentation.

6.6.3 The effects of loading rate

Due to the method chosen for this study, only the measurements for energy absorbed were available (see Chapter 4 for further details). While intrinsic toughness is generally inversely correlated with intrinsic stiffness, the lack of data for flexural strength limits further comparisons (Currey, 2001). If a bone exhibits a greater toughness (energy absorbed) it means that it can plastically deform at a greater level before fracturing and so could elastically deform more as well. It would suggest that as a material, immature bone will not turn brittle at certain high-energy loading rates but stay viscoelastic. However, several studies have noted that an increasing energy absorption on par with increasing strain rates up to a certain level, after which decreases are observed (Piekarski, 1970, Crowninshield and Pope, 1974, Robertson and Smith, 1978, Behiri and Bonfield, 1980, Behiri and Bonfield, 1984, Evans *et al.* 1992; Hansen *et al.* 2008; Zioupos *et al.* 2008). If this strain-rate dependant change occurs at different periods in paediatric and porcine bone it would indicate an additional variable to consider when comparing interspecies results. However, due to the variance in the test results, the only conclusion which can be drawn from this chapter is that in addition to age, multiple other variables such as the test set-up and sample dimensions may have been a contributing factor to the conflicting results.

6.7 Limitations

While multiple limitations have already been discussed in sub-chapter 6.5, it bears repeating for posterity. One of the limitations of this study is the manner of testing. Due to the choice of a pendulum impact tester and not necessarily a universal testing machine (see Glossary) this study sacrificed (due to the unavailability of equipment) the possibility of comparability with the available literature. An additional limitation of this study is that the immature porcine cortical bone tested are significantly tied to the concept of bone density. Bone density plays a crucial role in determining the mechanical properties of bone, including its toughness and ability to absorb energy during impacts.

In the context of this study, the focus on immature porcine cortical bone raises concerns regarding the generalisability of the findings. The mechanical properties of bone are not solely dependent on its mineral content but also on its microstructural organisation and the interplay between cortical and trabecular bone. The absence of trabecular bone testing in this study is a significant limitation, as trabecular bone density and architecture can substantially influence the overall mechanical behaviour of the bone, particularly under dynamic loading conditions. For instance, Fyhrie *et al.* (1993), Keaveny *et al.* (2001) and Barak (2019) highlight that the biomechanics of trabecular bone cannot be fully understood without considering its structure-function relationships. They note that the failure characteristics of trabecular bone often show independence from density. It is important to note that all the samples were prepared manually but the effort across those samples may have been flawed due to the potential remains of trabecular bone. All samples were inspected visually by the naked eye and thus there is the possibility that trabecular bone might have remained or that the removal of the trabecular bone might have generated uneven cortical bone thickness across the sample, thus affecting the testing and subsequent results.

This becomes an even greater question as the samples were all at different developmental levels, all which may have introduced additional variability in bone density (Wolff’s Law being one such factor). The differing amounts of force placed upon the bones throughout development, as described by Wolff's Law, could have impacted the bone's mechanical properties and contributed to the variability in results (Bonney and Goodman, 2021) As noted in previous research, the transition from trabecular to cortical bone during growth implies that the mechanical properties of the bone can change significantly with age and activity levels, potentially confounding the results observed in this study (Bayraktar *et al.* 2004). Additionally, the custom designed sample holder, may have allowed for movement of the samples during testing, adding to the data scatter. The limited data available on the toughness of immature porcine cortical bone further complicates the interpretation of the results, as it suggests that the observed energy absorption may be influenced by both intrinsic factors (such as bone density) and extrinsic factors (such as physical activity prior to testing). Unfortunately, due to the availability of the samples for bone mineral density testing these factors remain as of currently inconclusive. While the study contributes valuable empirical data on the mechanical properties of immature porcine cortical bone, the limitations related to bone density and the lack of consideration for Wolff's law highlight the need for further research. Future studies should aim to include a more specified approach to each sample thus providing a more comprehensive understanding of the mechanical behaviour of immature porcine bone under high-energy strains. Furthermore, the variability in sample dimensions, as discussed above, may explain why the ANCOVA revealed no significant effect of age on energy absorbed when controlling for cross-sectional area. Due to the number of limitations discussed, it is important to interpret the results of this study with caution.

Lastly, as with Chapter 5 (Section 5.7), the lack of detailed photographs of the immature porcine bone samples, both before and after high-impact testing, and the absence of fractographic analysis of the fracture surfaces is noted. While the methodology yielded quantitative mechanical data, including such visual evidence would have enhanced the understanding of failure mechanisms under these specific loading conditions. Pre-test images could have shown initial sample morphology, and post-test photos would have illustrated the fracture mode and damage extent. Fractography could have further elucidated fracture initiation and propagation at a microstructural level. This limitation is acknowledged, and future research into the high-impact behaviour of immature porcine bone would benefit from incorporating photographic and fractographic analysis alongside quantitative measurements for a more comprehensive characterisation.

6.8 Conclusion

The primary aim of this study was to further the current understanding for the mechanical behaviour of immature porcine cortical bone under high-energy strain rates. To answer the natural emerging questions of possible age-related, bone-related, and strain-related differences between the bone samples the following two hypotheses were posed:

Hypothesis 1	Immature bone will exhibit an age-related decrease in energy absorbed per cross-sectional area when subjected to high-energy impacts, and
Hypothesis 2	bone type is not a factor when primarily testing immature cortical bone under high-energy loading rates.

The results derived from the testing of Hypothesis 1 found that there is a statistically significant change in energy absorbed across age, which falls in line with the descriptive analysis showing that energy absorbed increases with age. These findings are in contrast to the only other study (Margulies and Thibault, 2000) that have tested immature porcine bone under both quasi-static and high energy strain rates. Whilst Margulies and Thibault (2000) observed a decreased output of energy absorbed across age, the study tested cranial samples, and not femoral or humeral samples. However, large variances in the results per individual sample and the fact that, when controlling for cross-sectional area, the effect of age was not significant, further shows the impact of the experimental design on the results.

Simultaneously, the testing of Hypothesis 2 indicated a statistically significant difference in energy absorbed between the humeral and femoral samples. However, these results will have been affected by the same test set-up relatable factors mentioned for Hypothesis 1. Furthermore, due to the lack of available data and literature on the differences between immature bone depending on anatomical site the results in this study may be due to reasons not fully understood yet. One suggestion for the difference is Wolff's law which states that bone grows and remodels in response to the forces that are placed upon it. It indicates that varying amounts of loads are placed upon the immature porcine humerus and femur throughout development (Woo *et al.* 1981; Frost, 2004. It would suggest the presence of both internal and external factors which affects the mechanical properties of the various bones (Frost, 2004). However, there is also an additional need to replicate the experiment where the sample size is larger for statistical purposes. Furthermore, there is a need to run a comparative experiment using samples following dimensional standards and conducting the testing under controlled conditions such as fixing the samples during testing. It is thus in consideration of these results that Hypothesis 2 was confirmed to be technically false but with the large added caveat that a need for future research is required. Due to the combined limitations of the test setup, sample variability, and lack of bone mineral density testing, the findings of this study should be interpreted with caution, and further research is essential to validate these results.

In conclusion, immature porcine bone subjected to high-energy impacts does technically illustrate a statistical variation across age but what that effect is due to requires further research involving more controlled testing conditions. Differences between the femoral and humeral samples were also observed, suggesting impacting factors which are unaccounted for. As the experiments conducted in this study were primarily on a material level it begs the question as to whether this information can be extrapolated to better understand whole bones, specifically fracture patterns – this will be explored in the next chapter.

Chapter 7 Fracture Patterns of Whole Immature Porcine Bone Under Different Loads

Research Question 3: What type of fracture patterns can be generated through the mechanical testing of whole immature porcine long bones?

7.1 Introduction

The mechanical testing of the *intrinsic* material properties of bone (See Chapters 5 and 6) contributes to a portion of our current understanding of immature bone behaviour. However, the supplemental testing of immature bone's *extrinsic* properties may help provide additional understanding for the fracture behaviour of immature bone, especially if evaluating bones of different ages. There are several changes that occur throughout the development and aging of bone, which in turn may affect skeletal injury tolerance and the potential manner in which certain fracture characteristics manifest themselves (Kress *et al.* 1995; Ouyang *et al.* 2003). Documented factors which may influence fracture pattern include both the bone's physical size and shape (Carter *et al.* 1996). In a bone's growth and development stages these factors may specifically include increases in length, cross-sectional area, and cortical bone thickness (Carter *et al.* 1996). The greater challenge is developing understanding across two factors: both the mechanical factors at play during fracture and how that relates to age and the extrinsic properties of bone. However, the benefits of first observing the trauma patterns which may be exhibited in developing whole bone of different ages would allow for increased understanding of bone fractures through potentially better calibration of equipment due to age and potential age-dependent forensic characterisation of a bone trauma (Kress *et al.* 1995; Ouyang *et al.* 2003; Ebacher *et al.* 2007; Cheong, 2015).

The existing research on bone injury tolerance have shown multiple age-related variances in the mechanical characteristics of cortical bone (Lindahl and Lindgren, 1967; Yamada, 1970; Cristofolini *et al.* 2007; Juszczyk *et al.* 2011; Zani *et al.* 2015).

At present, only Ouyang *et al.* (2003) and Forman *et al.* (2012) have mechanically tested paediatric whole bone. Both studies conducted three-point bending tests on a sample group of femora between the ages of 1 year and 4 months to 57 years and observed the force at fracture to increase through infancy and adolescence before peaking and plateauing between 25 and 50 years of age. This plateau was followed by a decline with increasing age (Forman *et al.* 2012). However, Oyang *et al.* (2003) and Forman *et al.* (2012) focused on the specific analysis of the *fracture moment* and therefore no analyses of the resultant fracture patterns were conducted.

Studies using immature animal models have particularly observed an interaction between certain fracture patterns, failure mechanisms, and age (Pierce *et al.* 2000; Koo *et al.* 2001; Thompson *et al.* 2015; Bertocci *et al.* 2017; Cheong *et al.* 2017; Vaughan, 2017). This includes bucket-handle, transverse and oblique fractures under bending, and spiral fractures under torsion (Pierce *et al.* 2000; Thompson *et al.* 2015; Bertocci *et al.* 2017; Cheong *et al.* 2017; Vaughan, 2017). No experimental data on axial compression-induced fractures in children or immature animal bone is currently available, but as noted in Chapter 2, oblique, transverse, greenstick, torus, and buckle fractures have all been reported in clinical cases where the mechanism of injury has been indicated to have been compression loads (Pierce *et al.* 2004; Bilo *et al.* 2010). Furthermore, multiple studies have observed condylar fractures, metaphyseal fractures, and growth plate failures during compression and torsion testing (Pierce *et al.* 2000; Aguel, 2004). The growth plates and surrounding tissues are a critical component of the immature skeleton (Nguyen *et al.* 2017) and the fact that immature bone is continuously fusing until adulthood creates for susceptible fracture concentration sites not found in adult bone (Aguel, 2004). Thompson et al.'s (2015) research focused on inducing metaphyseal lesions in whole porcine limbs using a varus and valgus bending method, where bending is applied at the distal ends. In this study, however, the distal ends of the specimens are still in the process of fusing. As a result, applying a varus and valgus method would primarily test the weakness of the growth plates rather than any effects occurring in the diaphysis or metaphysis. Simultaneously, Varus and valgus testing may introduce lateral bending forces, which may cause atypical or unrealistic fracture patterns compared to forces the bone would experience in vivo. As long bones in weight-bearing animals (like porcines or humans) may also fail under axial compression, torsion, or bending due to falls or impacts this current work has chosen to conduct three-point bending, compression, and torsion tests.

As previously mentioned, compression and torsion have been reported to generate metaphyseal fractures or growth plate failures and were expected to do so in the present study (Pierce *et al.* 2000; Aguel, 2004; Ritchie *et al.* 2008; Kleinman, 2008). However, differences in test set-up and goal does not make this study a suitable comparison with Thompson *et al,* (2015). Despite so, Thompson *et al.* (2015) is currently the only study to have employed whole porcine bones under the age of three months (3 and 7-day old specimens). While the literature has provided several references to the mechanisms required to generate certain fracture patterns, little comparative data still relates to the earliest age groups: birth to 3 years of age. In humans, this age group is particularly challenging and of interest due to an accelerated growth rate and change in anatomy (Altai *et al.* 2018). This is of particular importance in the use of the immature porcine as a paediatric model.

To understand the types of fracture patterns generated by common loading types such as bending, compression, and torsion in line with potential developmental changes across age, the objective of this chapter is more fully understanding the fracture characteristics of the whole immature porcine femur and humerus. A specific focus will be given to the analysis of potential differences in fracture characteristics (depending on the type of physical load across the following variables: age and bone type). While Chapters 5 and 6 focused on the mechanical behaviour of immature porcine bone at a material level, this chapter shifts the focus to the structural behaviour of whole immature porcine long bones. The femur and humerus, while both long bones, exhibit distinct differences in length, cross-sectional geometry, curvature, and the distribution of cortical and trabecular bone (Currey, 2002). Furthermore, their functional roles differ significantly, with the femur primarily adapted for weight-bearing in quadrupeds and the humerus for upper limb movement (Frazer, 1946; Currey, 2002; Drake, 2010; Bilo *et al.* 2010). These structural and functional differences are hypothesised to result in variations in how the bones respond to applied loads, leading to different fracture patterns under bending, compression, and torsion. As such this experimental study proposes the following three hypotheses:

Hypothesis 1	Fracture patterns in immature porcine long bones will change with age (newborn to 40 days) when subjected to three-point bending, compression, and torsion loads at a 5 mm/min loading rate.
Hypothesis 2	The effect of age on fracture patterns will differ between immature porcine femora and humerii under three-point bending, compression, and torsion tests at a 5 mm/min rate.
Hypothesis 3	Fracture patterns in immature porcine femora and humerii will vary based on the type of loading applied (three-point bending, compression, and torsion)

7.2 Method

7.2.1 Consideration for method

In the pursuit of establishing a methodology for testing immature whole bones of irregular sizes and shapes, this study employed bespoke designs tailored to the unique characteristics of these specimens. This approach deviates from standard procedures, necessitating a critical examination of the limitations inherent in existing standards, particularly those set forth by ASTM for the testing methods utilised. While ASTM provides guidelines for various mechanical testing methods, including compression and bending tests, these standards have not yet established a universally accepted testing protocol that accommodates the distinct anatomical and mechanical properties of biological specimens, such as immature porcine femurs and humerii. A systematic review by Zhao *et al.* (2018) on compression methods highlighted the significant variability in compression testing methodologies for bone across the literature, emphasizing that while a lack of standardization complicates the comparability and interpretation of results, the unique internal properties and geometries of bones complicates adhering to a true standard, The bespoke nature of the testing equipment developed for this study was necessitated by the anatomical variations and non-homogeneous structure of immature bone. Standard protocols, such as those outlined by ASTM, can lead to misleading results if applied without consideration of these unique characteristics. For example, while ASTM standards may provide a framework for testing compressive strength, they do not account for the unique microstructural properties of immature bone, which can significantly influence mechanical performance.

Several studies have addressed the challenges associated with testing and gripping whole bones in three-point bending, compression, and torsion, particularly highlighting the necessity for bespoke equipment and testing conditions. For instance, Kolbeck *et al.* (2000) noted that while whole long bones are often tested using bending or torsion methods, the application of three-point bending requires careful consideration of the specimen's dimensions to ensure accurate measurements, indicating a gap in standardised protocols for biological specimens. This observation underscores the need for customised testing setups that can accommodate the unique geometries and mechanical properties of whole bones.

Similarly, Lotinun *et al.* (2004) discussed the biomechanical evaluation of whole bones through various testing modalities, including three- and four-point bending and torsional testing. They pointed out that the curvature of rat long bones can affect the precision of bending tests, suggesting that bespoke testing conditions are often necessary to achieve reliable results (Lotinun *et al.* 2004). This highlights the importance of adapting testing methods to the specific characteristics of the biological specimens being studied, rather than relying solely on established standards.

Meganck *et al.* (2013) provided insights into the use of custom torsion testing setups for whole bone specimens, emphasizing that their approach involved potted specimens mounted in a specially designed apparatus to ensure accurate torsional loading (Meganck *et al.* 2013). Furthermore, Vashishth *et al.* (2001) explored the fatigue behaviour of cortical bone under combined loading conditions, noting that traditional testing methods may not adequately capture the complexities of bone mechanics (Vashishth *et al.* 2001). Their findings reinforce the argument for developing bespoke testing methodologies that can better reflect the mechanical behaviour of whole bones under realistic loading scenarios.

As the focus of this specific study chapter is solely on observing fracture patterns resulting from three-point bending, compression, and torsion testing, the argument for using bespoke equipment and non-adherence to ASTM standards and the literature becomes more compelling. As previously mentioned, several studies have highlighted the limitations of existing standards in accurately capturing the complex fracture behaviours of biological specimens, particularly when the aim is to analyse fracture patterns rather than quantify mechanical properties such as stiffness or Young's modulus.

Prodinger *et al.* (2018) underscored the critical issue of non-standardised biomechanical testing protocols, which can hinder accurate comparisons across fracture models. They argue that bespoke setups are essential for enhancing the efficacy of biomechanical testing, as these tailored configurations can accommodate the unique characteristics of the specimens being studied. This customisation is particularly important for bone where anatomical and physiological variations can significantly influence outcomes. While the bespoke approach is strongly advocated for its potential to yield more unique data, it is important to recognize the inherent challenges it poses, particularly when concerning the generalisability of the findings. Nevertheless, this study has adopted a bespoke test set-ups and equipment, prioritising the specificity and relevance of biomechanical assessments over the limitations in comparability, thereby contributing to a more nuanced understanding of bone fractures. By addressing these unique characteristics through bespoke testing, it is the aim that this study can obtain insights that are more reflective of the biological realities, despite the trade-offs in broader applicability.

7.2.2 Potting of the samples

For the testing set-ups of the specimens in this chapter, the proximal and distal ends of each bone to the metaphyseal line were potted in smoothed square-shaped metal holders filled with bone cement (Kemdent® Simplex Rapid Powder). This was conducted so as to ensure the stability and accuracy of the testing. This method is supported by various studies that highlight the importance of securely embedding bone specimens to facilitate reliable mechanical assessments. Recknagel *et al.* (2011) demonstrated the use of polymethylmethacrylate (PMMA) for potting femoral specimens in their biomechanical testing, emphasizing that this technique allows for a stable fixation that minimizes movement during testing, which is crucial for obtaining consistent results in three-point bending tests Recknagel *et al.* (2011). They noted that the distal end of the femur was potted in a cylinder using PMMA, which effectively immobilized the bone and allowed for accurate measurement of mechanical properties without introducing artifacts from specimen movement.

Similarly, Wehrle *et al.* (2014) described a setup where the proximal end of the femur was rigidly fixed to an aluminium cylinder, ensuring that the bone was securely held during the three-point bending test (Wehrle *et al.* 2014). This approach highlights the necessity of using robust potting techniques to maintain the integrity of the specimen throughout the testing process, thereby enhancing the reliability of the observed fracture patterns. In a study by Bell (2024), the authors also utilised PMMA to embed the proximal and distal ends of femurs, ensuring that the longitudinal axis of each bone was aligned with the axis of torsion during testing (Bell, 2024). This alignment is critical for accurately assessing fracture patterns, as it prevents misalignment that could skew results and lead to incorrect interpretations of mechanical behaviour. Moreover, Zhou *et al.* (2019) emphasised the significance of potting techniques in their biomechanical assessments, noting that the use of appropriate materials and methods for embedding bone specimens is essential for minimizing variability in test outcomes (Zhou *et al.* 2019). This is particularly relevant when the goal is to observe fracture patterns, as any instability in the specimen could lead to inconsistent fracture behaviour.

7.2.3 Specimen collection and preparation

A total of 41 femora and humerii were harvested from 15 immature porcines in the age range of newborn to 40 days of age. All specimens were acquired in collaboration with the British Royal Veterinary College (RVC) and manually macerated of all soft tissue at the RVC campus at Potters Bar, England (see Chapter 4 for further details). Following maceration, all specimens were CT-imaged for potential signs of potential pre-experiment trauma. After CT scanning, the whole bones were divided up as follows: 8 femora and 6 humerii for three-point bending; 6 femora and 7 humerii for compression testing; and 6 femora and 8 humerii for torsion testing. In agreement with the published literature and method of sample preparation employed in Chapter 5 and 6, all specimens were wrapped in saline soaked gauzes and placed in a polyethylene bag upon transport to the designated testing facility located off-site. Upon arrival, the samples were stored in -20° C to avoid extensive tissue degradation and frost damage.

7.2.4 Experimental set-up

Three-point bending set-up

Each specimen was removed from the polyethylene bag and gauze and allowed to thaw in room temperature 12 hours prior to testing. For the three-point bending tests, each sample was oriented in the latitudinal direction such that the actuator impact head contacted the anterior aspect of the long bone (face front impact) (i.e. bending in the sagittal plane). For support during testing, each sample's proximal and distal end were potted approximately to the metaphyseal line in smoothed square shaped metal holders filled with bone cement (Kemdent® Simplex Rapid Powder; Figure 7.1). In an effort to minimise stress concentration whilst providing both support and natural movement during testing, each bone was placed within the holder to avoid no contact with the surrounding metal. In consideration of each specimen's varying length, all testing distances were individually measured as the space between the supports (2.93 cm and 10.10 cm).

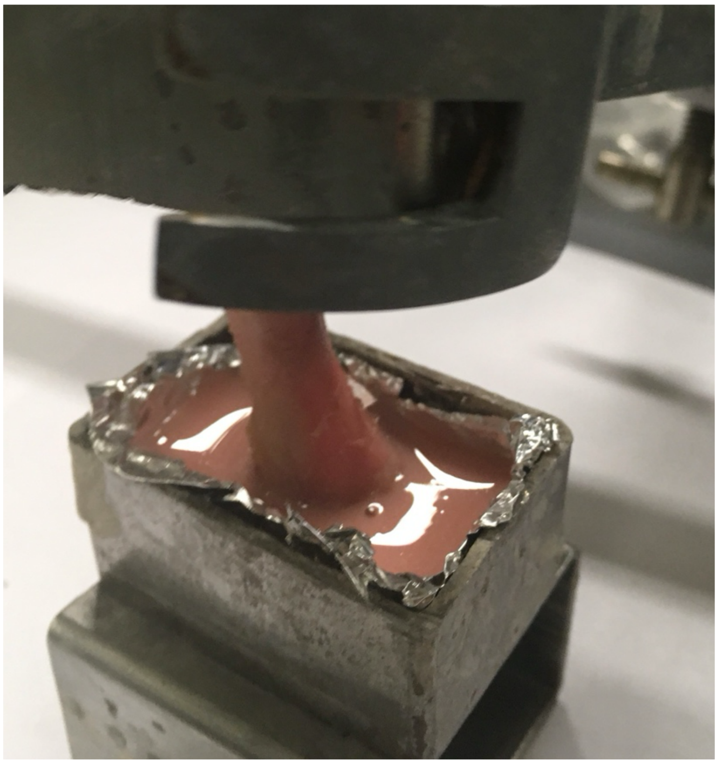

Figure 7.1: Image of a preliminary potting of a specimen post alignment.

Compression set-up

Each specimen was allowed to thaw in room temperature 12 hours prior to testing. Using square potting holders, each specimen's proximal and distal ends were potted using bone cement (Kemdent® Simplex Rapid Powder; Figure 7.1). All samples were aligned by visual inspection (each femur and humerus were aligned using a straight edge ruler to ensure that the midline of each specimen's diaphysis was properly aligned) to minimize potential bending loads acting on the specimens. However, it is important to acknowledge that visual alignment introduces a potential source of error due to the inherent subjectivity of the process. Even with the use of a straight edge, subtle variations in bone geometry and manual placement could result in minor deviations from perfect alignment, potentially leading to unintended bending or shear loads. Future studies could benefit from incorporating more precise alignment techniques, such as laser alignment or specialized jigs, to minimise this source of error. Each specimen was oriented in the longitudinal direction with the proximal holder in contact with the actuator and therefore loaded through the bones' condyles. Each specimen was potted up until the metaphyseal line in order to allow for stability. To ensure as little lateral motion as possible during testing, the distal holder was fixed with a vice around the holders.

Torsion set-up

As with the other conditions, the torsion specimens were allowed to thaw at room temperature 12 hours prior to testing. To eliminate unnatural force concentrations and condylar slipping whilst retaining as much fixation as possible, each end was submerged in bone cement (Kemdent® Simplex Rapid Powder) up until the metaphyseal line and allowed to harden. The context of these experiments was the evaluation of fracture characteristics as they may appear in children admitted for medical care. As such, testing conditions were attempted with the specimens' natural vivo condition in mind. A portion of the literature published on the torsion testing of whole bone have included drilling or the insertion of pins into each specimen's ends for better fixation (Aguel, 2004; Theobald *et al.* 2012; Aziz *et al.* 2014; Cheong, 2015; Bertocci *et al.* 2017; Vaughan, 2017; Malik *et al.* 2019). While Aguel (2004) conducted post-experiment radiographs to control for extraneous trauma due to the effect of insertion pins, it was decided prior to experimentation that since the effects of insertion pins in torsion test has as of yet not been explored no insertion pins would be used for this study.

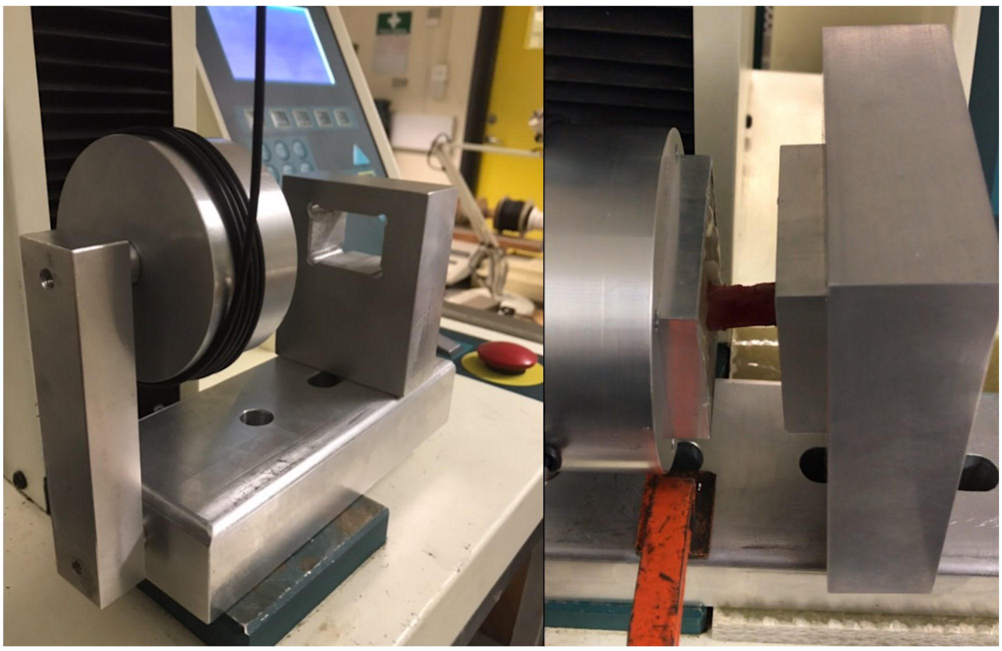

Figure 7.2: The designed and developed custom-based torsion rig.

Given the unavailability of a universal testing machine capable of producing controlled torsional loads, it was determined that a custom-based torsion rig would need to be developed and implemented (Figure 4.10; and Figure 7.2 above; The size of the rig by length *width * height was 25 cm * 12cm * 15cm). While a universal testing machine would have provided a more standardised and stable environment for conducting torsion tests, the bespoke rig was necessary to accommodate the specific requirements of this study. Utilizing the available mechanical testing machine (Hounsfield HKK55 2.5kN load cell), which only allowed for vertical movements, a rig was customized to enable one end of the specimen to remain stationary while subjecting the opposite end to torque loads. The rotational end was attached to the testing unit's tension-driven arm, allowing for controlled rotation of the designated end of the bone. Consequently, the bones were tested in the medial-lateral plane. For consistency of results, only the proximal ends of the specimens were oriented to act under rotation. Translational motion during testing was minimized by securing the supporting arm (containing the rotational element) to prevent movement in any axis.

While the bespoke rig was essential for conducting the torsion tests under the given constraints, it is important to acknowledge the limitations inherent in this approach. Despite the best efforts of the author to avoid, the potential for failure at the distal ends of the specimens was a concern that arose during testing. This eventuality underscores the challenges associated with custom setups, particularly when compared to the stability and control typically offered by standardised universal testing machines. Despite these challenges, the bespoke rig provided a necessary solution to facilitate the investigation of torsional properties in the absence of suitable commercial equipment, thereby contributing valuable insights into the biomechanical behaviour of the specimens.

All specimens (across all conditions) were tested until failure using a Hounsfield testing machine (Hounsfield HKK55 2.5kN load cell). While comparisons between the material tests in Chapter 5 and the whole bone tests in Chapter 7 were not attempted because of dissimilarity in specimens, a loading rate of 5 mm/min was adopted for the whole bone three-point bending and compression tests. For the torsional tests, it was determined that due to the speed of the entire rotational unit, a torque force of 5 mm/min acting on the specimens at the rotational unit's centre would not be equivalent to the same speed. Since the outer radius of the rotational unit was calculated to be 10x that of the torsional rate, a loading rate of 50 mm/min (with an equivalent torsional rate of 5 mm/min on the specimen) was adopted for the torsional tests. To ensure bone hydration each specimen was constantly sprayed with saline solution during testing.

7.2.5 Data quantification

In terms of quantifying the data variables being collected, decisions were made regarding data quantification. Failure was determined as an abrupt and observable change in the force-displacement curve, marking the point at which a fracture occurred. The load-displacement curve derived from each mechanical test (Figure 2.7, Chapter 2) was utilised to analyse the fracture patterns exhibited by each specimen. While the original focus on mechanical properties such as failure load, Young's modulus, and energy absorbed has been set aside, the load-displacement curve remains a critical tool for understanding the nature of the fractures. Observations were made regarding the characteristics of the fracture patterns, including their location, morphology, and any associated features that may indicate the mode of failure. The Instron 3340 provided incremental data in the form of applied force and translational deflection, which were instrumental in identifying the conditions under which fractures occurred. This data was analysed to categorize the fracture patterns observed, allowing for a comprehensive understanding of the behaviour of the specimens under torsional loads.

7.2.6 Statistical analysis

For this chapter, a Fisher's Exact Test was chosen as the primary statistical method for analysing the three hypotheses. This is due to its suitability for small sample sizes and its ability to provide accurate p-values for categorical data, in this case, fracture type, age category, and loading type (Kim, 2017). While Fisher's Exact Test does not allow for post-hoc tests, contingency tables were employed in this study to informally analyse the relationships between categorical variables. The statistical test results were all determined at the significance level of 0.05.

Furthermore, as used in Chapter 5 and 6 (Sub-chapters 5.4.3. and 6.3.3.) all age groups and containing samples were broken down into three age categories corresponding to birth to 10 days of age, 11 days to 25 days of age, and 26 to 40 days of age. While this categorisation resulted in an uneven specimen distribution throughout, it was deemed best to stick to the consistency of the age categories followed throughout this thesis. The specific reasons for selecting Fisher's Exact Test for each hypothesis are as follows:

For the first hypothesis of this chapter, the relationship between the specimens' age (broken down into three age categories) and resultant fracture pattern, analysed according to each separate mechanical test (three-point bending, compression, and torsion), was assessed using Fisher's Exact Test. Given the relatively small sample sizes for each mechanical test, 14 specimens for the three-point bending, 13 specimens for compression, and 5 specimens for torsion the Fisher's Exact Test was found to be the most appropriate. More specifically, the Fisher's Exact Test does not rely on large-sample approximations, therefore allowing for a more precise evaluation of the association between age categories and fracture patterns, even when the expected frequencies in some of the cells are low, which is often the case in studies with limited sample sizes.

Hypothesis two looked to test the association between age (broken down into three age categories), bone type, and fracture pattern. Again, a Fisher's Exact Test was employed in consideration of the small sample sizes (8 femora and 6 humerii for the three-point bending, 6 femora and 7 humerii for compression, and 1 femur and 4 humerii for torsion). Despite such, the method allows for the examination of the interaction between age and bone type on fracture patterns while accommodating the small sample sizes and the categorical nature of the data.

Hypothesis three sought to test the relationship between loading type (three-point bending, compression, and torsion) and fracture pattern, irrespective of age. This relationship was analysed using Fisher's Exact Test which again was found to be the best choice due to the small sample sizes of certain fracture types (e.g., 3 oblique fractures, 11 transverse fractures) which may lead to low expected frequencies in some cells of the contingency table. Fisher's Exact Test provided a robust method for assessing the association between loading type and fracture patterns without the constraints of sample size limitations inherent in other tests, such as the Chi-Square test or One-Way ANOVA.

Lastly, to reiterate that while a limitation of Fisher's Exact Test is that it does not provide post-hoc comparisons to examine differences between the specific age groups, the use of contingency tables allowed for an informal understanding of changes from one category to another. By examining the distribution of fracture patterns across age categories and loading types, it was deemed that insights into the relationships present in the data could be gleamed despite the absence of formal post-hoc tests.

7.3 Results

A total of 41 femora and humerii were subjected to mechanical testing including 14 specimens for three-point bend testing, 13 specimens for compression testing, 14 specimens for torsion testing. For ease, the resultant means by for the mechanical properties and types of fracture patterns are presented numerically in Tables 7.1 through 7.3.

For further ease of reading, the descriptive and statistical results in this study are presented in a joint manner (no separate descriptive and statistical sections). It was deemed easier to format this specific chapter’s results by hypothesis and loading type so avoid multiple sub-sections. The hope is that by presenting the results in this manner will make the results a bit easier to read and digest.

7.3.1 Hypothesis 1 – fracture patterns across age

Hypothesis 1 – Three-point bending

The bone fractures derived from the three-point bending tests are presented numerically in Table 7.1. Specifically, 14 bones [femora (n = 8) and humerii (n = 6)] were tested in three-point bending at a loading rate of 5 mm/min. A post-experiment evaluation of the tested specimens found 11 of the specimens to exhibit a transverse fracture pattern in the middle of the diaphysis and 3 samples to exhibit an oblique fracture pattern (Figures 7.3;7.4; 7.5; Table 7.1). All 3 oblique fractures were present in the specimens older than 17 days (Figure 7.5; Table 7.1). The statistical results derived from the Fisher's Exact Test indicated that there was no significant association between age and fracture type at $p<0.05$ ($p = 0.07$). While the Fisher Exact Test does not include a post-hoc test, a contingency table was created for an informal observation (Table 7.2). As can be observed no relationship or gradual change appears to be occurring from the youngest age category to the middle, and the later (Age Category 3) across any of the fracture patterns.

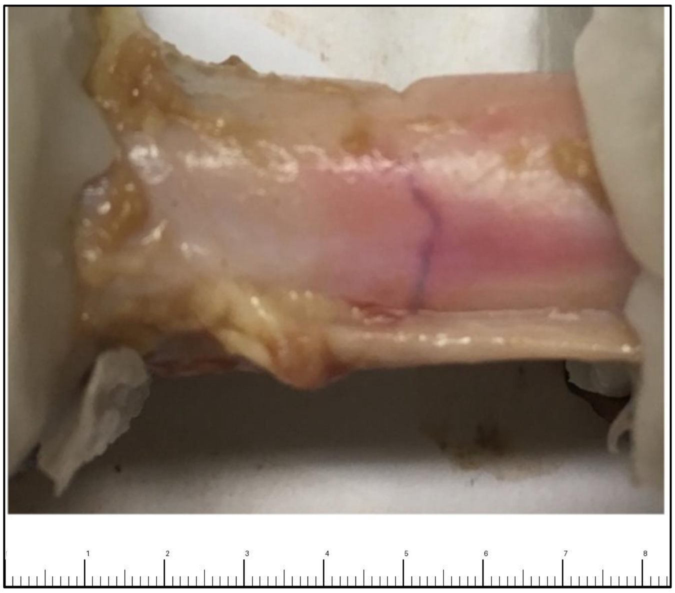

Figure 7.3: Femoral transverse fracture (between 23-25 days of age).

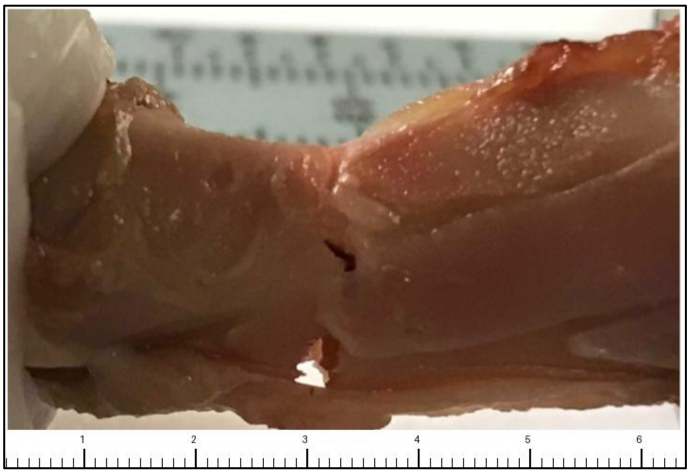

Figure 7.4: Humeral transverse fracture (between 11 and 13 days of age).

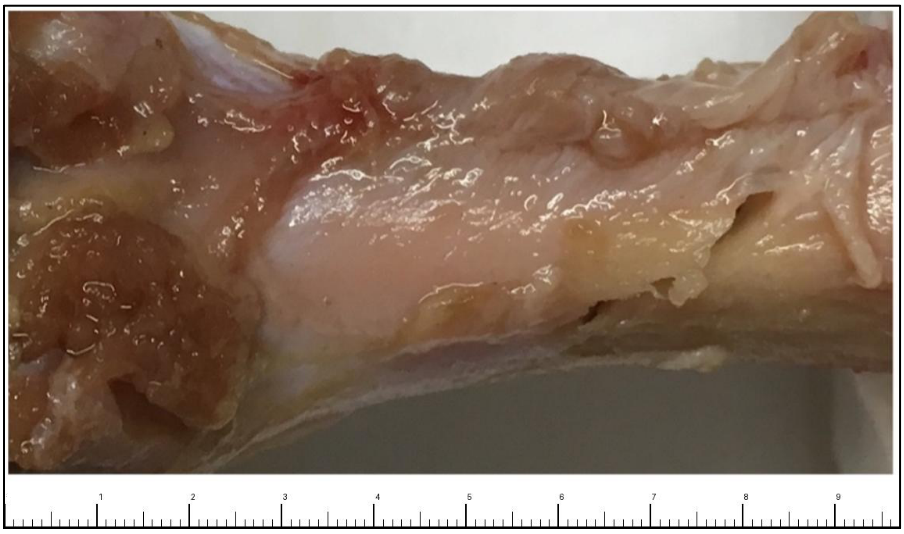

Figure 7.5: Femoral oblique fracture (between 38 to 40 days of age).

Table 7:1: Whole bone three-point bending results at 5 mm/min.

Specimen	Age	Age Category	Bone	Fracture Pattern	Site
1	00-01 days	1	Humerus	Transverse	Diaphysis
2	01-02 days	1	Femur	Transverse	Diaphysis
3	03-04 days	1	Femur	Transverse	Diaphysis
4	03-04 days	1	Humerus	Transverse	Diaphysis
5	05-07 days	1	Humerus	Transverse	Diaphysis
6	08-10 days	1	Femur	Transverse	Diaphysis
7	11-13 days	2	Humerus	Transverse	Diaphysis
8	14-16 days	2	Femur	Transverse	Diaphysis
9	17-19 days	2	Femur	Oblique	Diaphysis
10	20-22 days	2	Humerus	Transverse	Diaphysis
11	23-25 days	2	Femur	Transverse	Diaphysis
12	32-34 days	3	Humerus	Transverse	Diaphysis
13	35-37 days	3	Femur	Oblique	Diaphysis
14	38-40 days	3	Femur	Oblique	Diaphysis

Table 7:2: Contingency table derived from whole bone three-point bending statistical test

		Fracture pattern		
		Oblique	**Transverse**	**Total**
Age Category 1	Count	0	6	6
	Expected Count	1.3	4.7	6.0
	% within Age Category 1	0.0%	100.0%	100.0%
	% within Fracture pattern	0.0%	54.5%	42.9%
Age Category 2	Count	1	4	5
	Expected Count	1.1	3.9	5.0
	% within Age Category 2	20.0%	80.0%	100.0%
	% within Fracture pattern	33.3%	36.4%	35.7%
Age Category 3	Count	2	1	3
	Expected Count	.6	2.4	3.0
	% within Age Category 3	66.7%	33.3%	100.0%
	% within Fracture pattern	66.7%	9.1%	21.4%
Total	Count	3	11	14
	Expected Count	3.0	11.0	14.0
	% within all age categories	21.4%	78.6%	100.0%
	% within Fracture pattern	100.0%	100.0%	100.0%

Hypothesis 1 – Compression

The resultant means by age category for fracture pattern data derived from the compression tests are presented in Table 7.3.

Table 7:3: Whole bone compression results at 5 mm/min.

Specimen	Age (Days)	Age Category	Bone	Fracture Pattern	Site
1	00-01	1	Femur	Condylar	Distal
2	01-02	1	Femur	Buckle	Proximal
3	03-04	1	Humerus	Buckle	Distal
4	05-07	1	Femur	Buckle	Distal
5	08-10	1	Humerus	Buckle	Proximal
6	11-13	2	Femur	Condylar	Distal
7	14-16	2	Humerus	Condylar	Proximal
8	17-19	2	Humerus	Condylar	Distal
9	20-22	2	Femur	Condylar	Distal
10	23-25	2	Humerus	Condylar	Distal
11	26-28	3	Femur	Condylar	Proximal
12	29-31	3	Humerus	Buckle	proximal
13	32-34	3	Humerus	Condylar	Proximal

Thirteen bones femora (n = 6) and humerii (n = 7) were tested in axial compression at a loading rate of 5 mm/min. A post-test evaluation of the resultant fracture patterns identified 5 specimens with a buckle fracture and 8 specimens presented with condylar fractures. As condylar fractures are associated with shearing forces and several of the specimens were observed during testing to move laterally in response to the applied load, the involvement of shearing forces is a possibility. It was also noted during compression testing that the load-displacement curves for the specimens illustrated a somewhat erratic behaviour but with a discernible point of fracture (Figure 7.6). It was therefore decided that due to the specific behaviour of some of the specimens (observed through the stress-strain curve in real-time) all tests were aborted once the physical deformation of the sample could be observed to have reached 50 percent of each sample's diaphysis length.

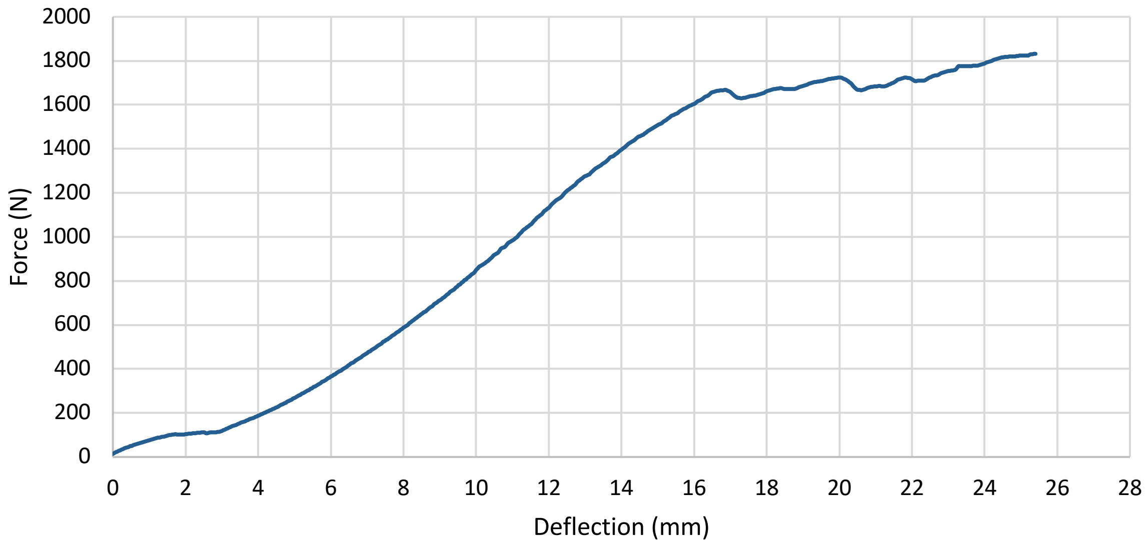

Figure 7.6: Force against deflection (mm) for whole bone specimen under compression.

The statistical results derived from the Fisher's Exact Test indicated that there was not a significant association between age and fracture type at $p<0.05$ ($p = 0.07$). However, it is crucial to interpret these results with caution due to the possibility of unintended shear loads during the compression tests. The observed fracture patterns may have been influenced by these lateral forces, potentially affecting the accuracy of the data in reflecting pure axial compression. As previously mentioned for the three-point bending results, the Fisher Exact Test does not include a post-hoc test. However, a contingency table was created for an informal observation (Table 7.4) and as can be observed no relationship or gradual change appears to be occurring from the youngest age category to the middle, and the later (age category 3) across any of the fracture patterns. It may be noted that four out of the five generated buckle fractures were found in age category 1 (less or equal to 10 days of age) whereas five of the seven condylar fractures were identified in age category 2 (11 to 26 days of age).

Table 7:4: Contingency table derived from the whole bone compression statistical test

		Fracture Pattern		
		Buckle	Condylar	Total
Age Category 1	Count	4	1	5
	Expected Count	1.9	3.1	5.0
	% within Age Category	80.0%	20.0%	100.0%
	% within Fracture Pattern	80.0%	12.5%	38.5%
Age Category 2	Count	0	5	5
	Expected Count	1.9	3.1	5.0
	% within Age Category	0.0%	100.0%	100.0%
	% within Fracture type	0.0%	62.5%	38.5%
Age Category 3	Count	1	2	3
	Expected Count	1.2	1.8	3.0
	% within Age Category	33.3%	66.7%	100.0%
	% within Fracture Pattern	20.0%	25.0%	23.1%
Total	Count	5	8	13
	Expected Count	5.0	8.0	13.0
	% within Age Category	38.5%	61.5%	100.0%
	% within Fracture Pattern	100.0%	100.0%	100.0%

Hypothesis 1 -Torsion

During the torsion testing of the 14 specimens, several failures were observed at the growth plates, which significantly impacted the ability to accurately assess how the loads were affecting the bone specimens complicating the interpretation of the resulting data. For ease of reference, the remaining results from the torsion tests are summarized in Table 7.5.

Table 7:5: Whole bone torsion results at 5 mm/min (50 mm/min).

Specimen	Age	Bone	Fracture Pattern	Site
1	00-01 days	Humerus	Spiral	Diaphysis
2	01-02 days	Humerus	Spiral	Diaphysis
3	14-16 days	Humerus	Spiral	Diaphysis
4	23-25 days	Humerus	Spiral	Diaphysis
5	26-28 days	Femur	Spiral	Diaphysis

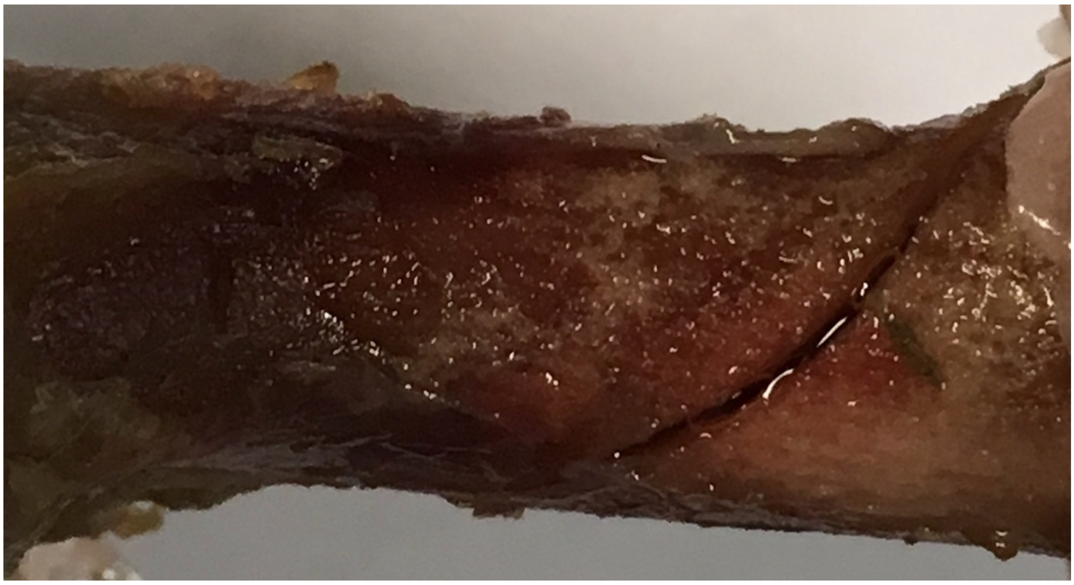

Figure 7.7: Humeral spiral fracture (between 1 and 2 days of age).

The torsion testing yielded a total of 5 spiral fractures (femur, n = 1; humerus, n = 4; figure 7.7) and 9 growth plate failures (femur, n = 5; humerus, n = 4). An overview of the load-displacement curves for the 9 growth plate failures revealed some angular deflection, which hindered reliable interpretation of the data. This is exemplified in Figure 7.8, which illustrates continuous increases in torque and angular deflection, followed by steep drop-offs in torque. Notably, post-experiment evaluations of the bones did not reveal any visible fractures. Instead, a removal of the bone cement showed what appeared to be dislocations at the specimens' growth plates. Figure 7.8 can be referenced against the load-displacement curve of one of the successfully generated spiral fractures (Figure 7.9), where a continuous increase in torque and angular deflection was followed by a singular drop-off in torque.

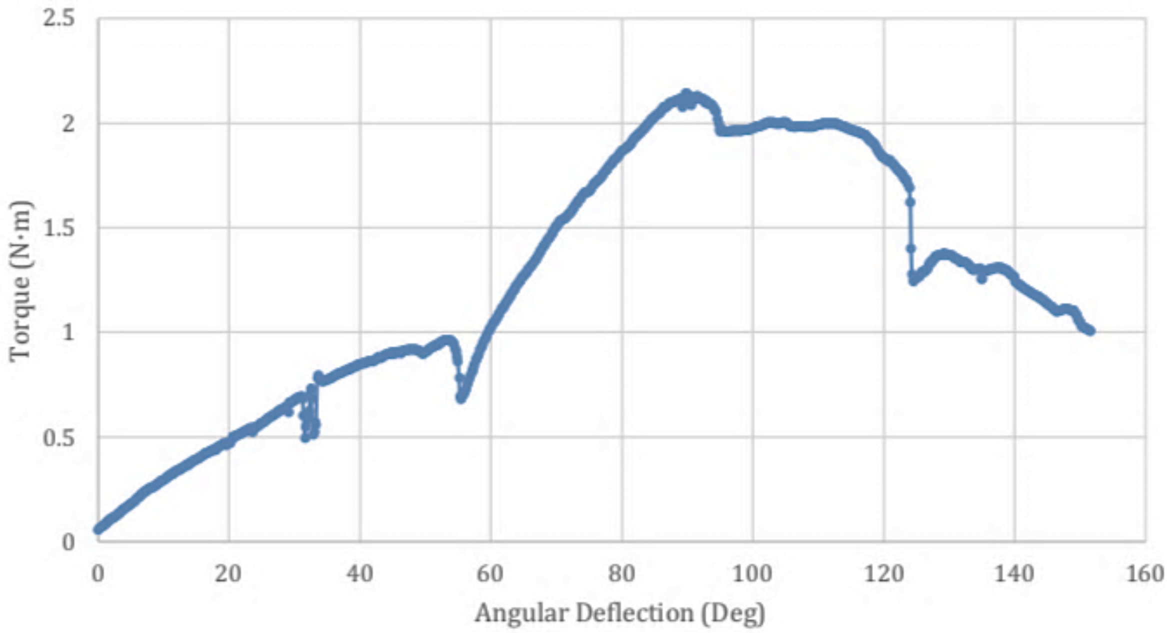

Figure 7.8: Torque against angular deflection of bone (growth plate failure).

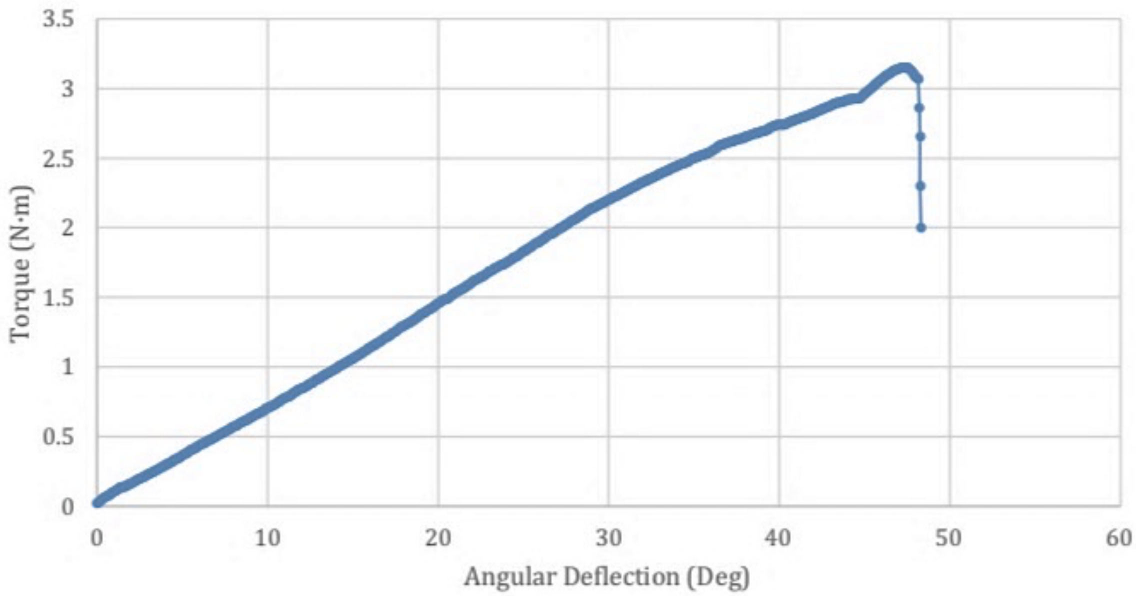

Figure 7.9: Torque against angular deflection of bone (spiral fracture).

Despite the post-experiment indicating a failing at the growth plates, the observed load-displacement curves may be attributed to multiple factors, including potential debonding between the bone and cement, cement cracking, or actual fractures within the bone itself. Whilst video footage of the tests was reviewed, any indication as to whether the initial failure was due to one specific factor or multiple was not possible. Due to the limited sample size and the amount of corresponding age groups, a statistical analysis was not conducted for the torsion tested specimens. However, a speculative discussion based on the literature will be presented in sub-section 7.4.

7.3.2 Hypothesis 2 – age-related mechanical properties and fracture patterns across bone type

Hypothesis 2 tested whether any of the generated fracture patterns vary with age across the two bone types. Tables 7.6, 7.7, 7.8, and 7.9 presents separate tables of the femoral and humeral results in accordance with the three-point bending and compression tests.

Hypothesis 2 -Three-point bending

The numerical results for the femoral and humeral specimens used for the three-point bending tests are presented in Tables 7.6 and 7.7. As observed, transverse fractures were observed across both the femoral and humeral specimens. However, only the femoral specimens were found to exhibit oblique fracture. As can be observed in Table 7.7, all humerii exhibited a transverse fracture which based on the limited sample size reveals little about any potential change with age regarding the humerii or any potential association with the femora. However, for the femoral data a contingency table was created (Table 7.8). The statistical results derived from the Fisher's Exact Test indicated that there was not a significant association between age and fracture pattern at $p<0.05$ ($p = 0.20$) for the femoral data. Based on an informal observation a change can be observed across the different fracture patterns with the oblique fractures an increase in frequency can be observed from Age

Category 1 (n= 0) to Age Category 2 (n= 1) and Age Category 3 (n= 2). Comparatively, the resultant transverse fractures in the femora showed a slight decrease from Age Category 1 (n= 3) to Age Category 2 (n= 2). As has already been stressed, the small sample size does not indicate a significant association and there should be future testing utilising larger samples.

Table 7:6: Three-point bending results at 5 mm/min according to whole bone femoral specimens.

Specimen	Age (Days)	Bone	Fracture Pattern	Site
1	01-02	Femur	Transverse	Diaphysis
2	03-04	Femur	Transverse	Diaphysis
3	08-10	Femur	Transverse	Diaphysis
4	14-16	Femur	Transverse	Diaphysis
5	17-19	Femur	Oblique	Diaphysis
6	23-25	Femur	Transverse	Diaphysis
7	35-37	Femur	Oblique	Diaphysis
8	38-40	Femur	Oblique	Diaphysis

Table 7:7: Three-point bending results at 5 mm/min according to whole bone humeral specimens.

Specimen	Age	Bone	Fracture Pattern	Site
1	00-01	Humerus	Transverse	Diaphysis
2	03-04	Humerus	Transverse	Diaphysis
3	05-07	Humerus	Transverse	Diaphysis
4	11-13	Humerus	Transverse	Diaphysis
5	20-22	Humerus	Transverse	Diaphysis
6	32-34	Humerus	Transverse	Diaphysis

Table 7:8: Whole bone three-point bending contingency table showcasing any change in fracture pattern depending on bone type.

		Fracture Pattern		
		Oblique	**Transverse**	**Total**
Age Category 1	Count	0	3	3
	Expected Count	1.1	1.9	3.0
	% within Age Category 1	0.0%	100.0%	100.0%
	% within Fracture Pattern	0.0%	60.0%	37.5%
Age Category 2	Count	1	2	3
	Expected Count	1.1	1.9	3.0
	% within Age Category 2	33.3%	66.7%	100.0%
	% within Fracture Pattern	33.3%	40.0%	37.5%
Age Category 3	Count	2	0	2
	Expected Count	.8	1.3	2.0
	% within Age Category 3	100.0%	0.0%	100.0%
	% within Fracture pattern	66.7%	0.0%	25.0%
Total	Count	3	5	8
	Expected Count	3.0	5.0	8.0
	% within Age Categories	37.5%	62.5%	100.0%
	% within Fracture Pattern	100.0%	100.0%	100.0%

Hypothesis 2 - Compression

Tables 7.9 and 7.10 show the results for the fracture patterns under compression testing by bone type. A number of observations can be made from these results. Both the femoral and humeral specimens were found to exhibit condylar and buckle fractures during testing. Reviewing the data descriptively there appears to be no strong associations with age. However, as noted in the results derived from the compression tests in Hypothesis 1 (Section 7.3.1), several of the specimens were subjected to what may have been shear loads. As such the identified fracture patterns may not have been generated under axial compression, so the results need to be interpreted with caution and statistical testing is not deemed appropriate. Fisher’s exact test was used to determine if there was a significant association between age category, fracture pattern and bone type (Contingency table 7.11). Regarding the added variable of age category the result indicated that there was no statistically significant association between age category, fracture type and bone type in age category 1 at $p<0.05$ ($p= 1.00$), age category 2 no relationship as all fracture types were condylar, and age category 3 at $p<0.05$ ($p= 1.00$).

Table 7:9: Whole bone compression test results at 5 mm/min in accordance with the humeral specimens.

Specimen	Age (Days)	Bone	Fracture Pattern	Site
1	03-04	Humerus	Buckle	Distal
2	08-10	Humerus	Buckle	Proximal
3	14-16	Humerus	Condylar	Proximal
4	17-19	Humerus	Condylar	Distal
5	23-25	Humerus	Condylar	Distal
6	29-31	Humerus	Buckle	proximal
7	32-34	Humerus	Condylar	Proximal

Table 7:10: Whole bone compression test results at 5 mm/min in accordance with the femoral specimens.

Specimen	Age (Days)	Bone	Fracture Pattern	Site
1	00-01	Femur	Condylar	Distal
2	01-02	Femur	Buckle	Proximal
3	05-07	Femur	Buckle	Distal
4	11-13	Femur	Condylar	Distal
5	20-22	Femur	Condylar	Distal

Table 7:11: Compression contingency table showcasing any change in fracture pattern depending on bone type.

		Fracture Pattern		
		Buckle	**Condylar**	**Total**
Femur	Count	2	4	6
	Expected Count	2.3	3.7	6.0
	% within Bone Type	33.3%	66.7%	100.0%
	% within Fracture Pattern	40.0%	50.0%	46.2%
Humerus	Count	3	4	7
	Expected Count	2.7	4.3	7.0
	% within Bone Type	42.9%	57.1%	100.0%
	% within Fracture Pattern	60.0%	50.0%	53.8%
Total	Count	5	8	13
	Expected Count	5.0	8.0	13.0
	% within Bone Type	38.5%	61.5%	100.0%
	% within Fracture Pattern	100.0%	100.0%	100.0%

Hypothesis 2 - Torsion

The numerical results for the torsion tests are presented in Table 7.5. Due to having determined only 5 femoral (n =1) and humeral (n = 4) specimens to have exhibited viable data any comparisons between the two bone types is difficult.

7.3.3 Hypothesis 3 – fracture pattern depending on loading mechanism

Hypothesis 3 concerned the mechanical relationship between fracture pattern and loading type. Specifically, it proposes that there will be a difference in fracture pattern depending on loading type. Table 7.12 summarises the data and shows that no specific fracture pattern was found to be generated across multiple loading types. Instead, oblique and transverse fractures were generated exclusively during three-point bending testing, buckle and condylar fractures were exclusively exhibited during the compression tests, and spiral fractures were in turn exclusive to the torsion tests. A noted secondary effect of both the compression and torsion tests was the development of 7 condylar fractures and 9 growth plate failures (the latter are not included in Table 7.12). This may suggest the involvement of shearing forces in both tests (See also section 7.3.1; Hypothesis 1). An additional observation was the constant fracturing and failing at the distal ends, areas where the bones are still fusing, which may suggest those distal ends as areas of stress concentration.

Table 7:12: fracture pattern across loading type.

Fracture pattern	Specimen size	Loading type
Oblique	3	Three-point bending
Transverse	11	Three-point bending
Buckle	6	Compression
Condylar	7	Compression
Spiral	5	Torsion

Fisher’s exact test was used to determine if there was a significant association between fracture pattern and bone type (Contingency table 7.13) and is as follows:

Table 7:13: Contingency table showcasing variations in fracture types depending on loading type.

		Loading type			
		Three-point bending	Compression	Torsion	Total
Transverse	Count	11	0	0	11
	Expected Count	4.8	4.5	1.7	11.0
	% within Fracture pattern	100.0%	0.0%	0.0%	100.0%
	% within Loading type	78.6%	0.0%	0.0%	34.4%
Oblique	Count	3	0	0	3
	Expected Count	1.3	1.2	.5	3.0
	% within Fracture pattern	100.0%	0.0%	0.0%	100.0%
	% within Loading type	21.4%	0.0%	0.0%	9.4%
Buckle	Count	0	5	0	5
	Expected Count	2.2	2.0	.8	5.0
	% within Fracture pattern	0.0%	100.0%	0.0%	100.0%
	% within Loading type	0.0%	38.5%	0.0%	15.6%
Condylar	Count	0	8	0	8
	Expected Count	3.5	3.3	1.3	8.0
	% within Fracture pattern	0.0%	100.0%	0.0%	100.0%
	% within Loading type	0.0%	61.5%	0.0%	25.0%
Spiral	Count	0	0	5	5
	Expected Count	2.2	2.0	.8	5.0
	% within Fracture pattern	0.0%	0.0%	100.0%	100.0%
	% within Loading type	0.0%	0.0%	100.0%	15.6%
Total	Count	14	13	5	32
	Expected Count	14.0	13.0	5.0	32.0
	% within Fracture pattern	43.8%	40.6%	15.6%	100.0%
	% within Loading type	100.0%	100.0%	100.0%	100.0%

The statistical results derived from the Fisher’s Exact Test indicated that there was a significant association between fracture pattern and loading type at $p<0.05$ ($p = 0.001$).

However, in consideration of the exclusivity of all the fracture patterns to a specific loading type, the statistical results are hardly surprising. Despite this, the statistical the results suggest that loading type is a variable when observing a resultant fracture pattern in immature porcine femora and humerii. However, these results need to be interpreted with caution due to the sample size and mentioned affecting factors. These factors are further discussed in the discussion (7.4) and limitations section (7.5).

7.4 Discussion

7.4.1 Hypothesis 1 - fracture patterns across age

Hypothesis 1 posited that there will be a change in fracture pattern with age. To test this hypothesis whole bone immature femora and humerii in the age range of birth to 40 days was subjected to three-point bending, axial compression, and torsion tests. A more detailed discussion about each separate mechanical test can be found in the following sections. Throughout the three mechanical experiments no consistent chronological pattern was observed across any of the resultant fracture patterns. However, that is not say that small and informal trends were not observed between certain fracture patterns or specific age groups, only that those patterns were 1) not consistent through all three age categories, or 2) of such a small frequency that further research is required. In order to explore Hypothesis 1 more fully, separate analyses were conducted for each mechanical test where the focus was the relationship between the specimen's age, and resultant fracture patterns.

Three-point bending

The resultant fracture patterns of the three-point bending tests produced transverse and oblique fractures and is in line with the literature (Pierce *et al.* 2000; Bertocci *et al.* 2017). Specifically, the direct trauma to a bone has been reported to create transverse fractures (Pathria, 2002). In accidental situations, a transverse fracture may result from of a parent falling onto steps while carrying a child (Pierce *et al.* 2004; Bilo *et al.* 2010). If the child's leg is impacted between the caretaker and the edge of the step, the potential mechanism of injury could be a bending load and the resultant injury a transverse fracture (Pierce *et al.* 2004). The three-point bending tests conducted in this chapter were able to consistently generate transverse fractures in all the specimens within the age range of birth to 16 days. No other study as of yet has evaluated fracture pattern by age and across both the immature porcine femur and humerus. However, Vaughan (2017) subjected adult (1 to 2 years of age) and immature (1 to 7 days of age) ovine femora to four-point bending and observed the older specimens to exhibit reverse tensile wedge and oblique fractures. The immature specimens exhibited transverse and partial tensile wedge fractures. While the testing configurations did differ from the current study there is an indication that certain fracture patterns may be more prominent in immature specimens.

As observed by the contingency table an informal increase in oblique fractures could be observed from age category 1 (n= 0) to age category 2 (n= 1) to age category 3 (n= 2) (Table 7.8). The observed informal age-related increase in oblique fractures among some of the specimens may be attributed to several biomechanical and developmental factors. As immature bones undergo growth and development, their structural integrity and mechanical properties evolve, which in turn can influence the types of fractures that occur under stress. Oblique fractures may result from a combination of shear and rotational forces applied to the bone, which may be more prevalent in younger specimens due to a relatively weaker and more compliant bone structure (Jiang *et al.* 2021). In a three-point bending configuration, the application of force is not purely axial; rather, it induces both bending moments and shear forces along the length of the bone. This combination of forces can lead to complex loading scenarios that are conducive to oblique fracture patterns, particularly in immature bone, which may be more susceptible to such stresses due to its unique biomechanical properties. However, it is crucial to emphasise that these findings are based on a very small sample size of only three specimens, which is therefore not entirely indicative of broader patterns and necessitates further research to validate these observations. A study encompassing a larger sample size would be essential to draw more definitive conclusions regarding the relationship between age and the incidence of oblique fractures in immature bones

Compression

The mechanical data derived from the compression tests of immature porcine bones may be contentious due to the varying results and documented failure modes (See section 7.3.1). Nonetheless, certain factors warrant consideration regarding the compression testing of these specimens. The unfused and developmental state of immature bone makes the growth plates, located at the distal and proximal ends, particularly susceptible to stress concentrations. This is evidenced by the resultant condylar fractures observed during the compression experiments.

Growth plates are primarily composed of cartilage, which is more compliant than bone (Aguel, 2004). Consequently, the cartilage is likely to undergo significant strains, leading to fractures. In contrast to the torque mechanism, which can dislodge cartilage from the bone, axial compression tends to crush the cartilage into the bone, resulting in condylar fractures rather than growth plate failures. Condylar fractures are not uncommon in paediatric injuries, often resulting from accidental falls (Pierce *et al.* 2001). Pierce *et al.* (2001) noted that discrepancies between real-world paediatric fractures and laboratory-induced condylar fractures may stem from the absence of stabilizing structures, such as ligaments, during testing.

Interestingly, condylar fractures were observed in both the proximal and distal regions of the femoral and humeral specimens, suggesting that the growth plates serve as sites of stress concentration under compression. As children mature, the fusion and hardening of growth plates may reduce the likelihood of fractures occurring at these sites. To mitigate condylar fractures in experimental studies, it is advisable to ensure pure axial compression through the bone, achievable through the use of attached platens and precise alignment prior to testing (Badiei *et al.* 2007; Bosisio *et al.* 2007; Bourgnon *et al.* 2014).

The condylar fractures produced during the compression tests were unexpected and may have resulted from inadvertent secondary loads. The natural curvature of long bones can expose them to shear loads (Zhao *et al.* 2018). Lundin *et al.* (2000) reported differing fracture patterns when subjecting mature and immature porcine spines to compression loads, suggesting that the age of the porcine bone directly influences fracture type. This observation reinforces Pierce *et al.'s* (2001) findings that unexpected shearing and fractures at growth plates are not uncommon.

Additionally, buckle fractures were the remaining types sustained during the compression tests. These fractures are frequently reported in children during both accidental and non-accidental situations (Berthold *et al.* 2018). The narrow age range of the samples, all within the immature phase, may have contributed to these results. Future research should include mature samples to better evaluate bone behaviour. The mechanisms leading to buckle fractures involve a combination of compression and shearing loads, which create tension on the convex side and compression on the concave side (Bilo et al. 2010). If the bone fails on the compression side, as reported in immature bone, the resultant fracture is a buckle fracture (Bilo *et al.* 2010). Thus, incorporating adult samples would facilitate distinguishing fracture patterns on either the convex or concave side.

Regarding the observed age-related specificity of buckle and condylar fractures, the prevalence of buckle fractures in specimens aged 10 days or younger may be attributed to the inherent structural characteristics of immature bone. Bone tissue is generally weaker in tension than in compression, leading to buckling under compressive loads, particularly in thinner-walled structures found in younger specimens (Kocher *et al.* 2004). The higher proportion of cartilage in immature bone may also contribute to its susceptibility to buckle fractures, as the cartilage can deform under stress, resulting in characteristic fracture patterns (Mckissick *et al.* 2008). Conversely, the increased occurrence of condylar fractures in specimens older than 10 days could be linked to the maturation process of the bone. As growth plates begin to fuse and harden, the mechanical properties of the bone change, potentially making the condylar regions more susceptible to axial compression forces (Theobald *et al.* 2012).

In summary, while age may influence the types of fractures observed, complications during mechanical testing complicate straightforward interpretations of these patterns. Further investigation with a larger sample size and more controlled loading conditions is essential to gain deeper insights into these relationships, but it has been possible to draw some useful conclusions.

Torsion

The torsion tests were, in this study, foremost illustrated by bone failures that can be attributed to multiple factors such as failure at the growth plates, potential debonding between the bone and cement, cement cracking, or fractures within the bone itself.

Whilst 5 out the 14 torsion tests produced spiral fractures, the potential causes for failure at specifically the growth plates could be due to a failure to properly stabilise and secure the bone during testing. Malik *et al.* (2019) reported that a common issue in the torsion testing of immature bone is failure at the growth plate before the bone is fractured. As observed during the compression tests this is due to the connective tissue (e.g. cartilage) being weaker than the cortical bone (Malik *et al.* 2019). However, whereas the compression tests actually fractured the bone through the direction of the mode of the applied, no such application of forces were observed during the torsion tests.

It is important to note that, as of now, there has been no established method for successfully setting up torsion tests on immature bone that adequately replicates in vivo conditions. While the insertion of metal pins has been shown to enhance stability in torsion testing (Cheong *et al.* 2017; Vaughan, 2017; Malik *et al.* 2019), this approach was deliberately avoided in this study to better simulate the natural conditions of immature bone, recognizing that such modifications may not accurately reflect physiological loading and injury mechanisms. Future studies could explore alternative methods to mitigate growth plate failures while maintaining a degree of physiological relevance. For instance, modifying the potting technique to provide more robust support at the metaphyseal regions, or utilizing a custom-designed jig that mimics the support provided by surrounding soft tissues, could be investigated. Building on Thompson *et al.* (2015) by maintaining the knee joint and ligaments could provide more in-vivo like results. Additionally, exploring the application of non-invasive imaging techniques, such as micro-CT, to assess bone-cement interfaces and identify potential failure points prior to testing may also be beneficial.

However, as evident by nine failures at the growth plates immature porcine femora and humerii can fail in such a manner if not secured. Although, it needs to be considered that the insertion of metal pins is not necessarily representative of the natural in-vivo events. Any mechanism of injury can be generated should the testing conditions be manipulated enough. It may also be noted that the knee ligaments, capsules and meniscus may combine to naturally in-vivo prevent the shearing of the growth plates. Thus, there may be value in researching manners of bone constraints during torsion testing which best emulates natural conditions. As to why no statistical test was conducted for the torsion test due, the lack of an appropriate sample size (n=5) as well as only one type of fracture pattern observed (in the successful tests). It is important to acknowledge that the high prevalence of distal end (growth plate) failures observed in the torsion tests of this study is not typically reported as a primary fracture pattern in forensic or clinical cases of immature bone trauma (Jalkanen *et al.* 2001; Hajdú *et al.* 2011). Rather, growth plate injuries are often observed in conjunction with other fracture types, such as spiral or buckle fractures, or in compression based injuries (Hajdú *et al.* 2011). This suggests that the experimental setup, particularly the lack of physiological constraints, may have significantly influenced the observed failure patterns. Further research is necessary to determine if these results are representative of in-vivo torsional injuries or an artifact of the testing methodology.

7.4.2 Hypothesis 2 - the role of bone type

Hypothesis 2 tested whether there are any age-related differences in the way fracture patterns may vary across the femora and humeri in conjunction with age. It was initially hypothesized that the humerus and femur are both long bones, similar in their mechanical role as levers and support. Furthermore, there is a species-related difference in the ultimate purpose of the femur and humerus in porcines as opposed to humans, given that humans are bipeds and porcines are quadrupeds. If the general function of the femur and humerus in porcine is the same, it may suggest that the porcine femur and humerus could exhibit similar fracture patterns when fractured. However, despite potentially performing the same function, external physical comparisons reveal that the porcine femur is slimmer and longer than the porcine humerus.

Interestingly, the results, specifically under three-point bending, indicated that no significant differences were found in the exhibited fracture morphologies by bone type or age in the humeral specimens. All humeral specimens exhibited transverse fractures regardless of age category, suggesting that age-related trends in fracture patterns are not present in the humerus. This uniformity in fracture type may reflect the mechanical properties of the humerus, which appears to respond consistently to the applied loads across different age groups. In contrast, the femoral specimens exhibited a notable age-related trend, with oblique fractures increasing in frequency from Age Category 1 (n=0) to Age Category 3 (n=2). This suggests that as the specimens aged, their fracture patterns became more complex, potentially due to changes in bone structure and mechanical properties associated with maturation. These findings do not indicate that factors other than fracture morphologies are better indicators of whether differences exist. This can entail measuring the samples' bone integrity, bone mass, geometry, and material properties (van der Meulen *et al.* 2001; Turner, 2006).

Accounting for bone geometry is difficult to test in a controlled environment since no bone is identical, but bone mass is measurable and has been documented by Koo *et al.* (2001) using immature porcine femora and humeri. Specifically, Koo *et al.* (2001) documented an equivalence in bone mass between not only the immature porcine femora and humeri but also the anatomical sides. While the data produced from the three-point bending tests indicate no difference in behaviour by fracture morphology alone, the literature suggests that anatomic features, such as bone geometry, can affect how immature bone fractures under three-point bending. It is acknowledged that bone mass is a critical factor influencing fracture risk and behaviour and would have been of major assistance in further clarifying the results. However, due to circumstances in which the bone was pre-maturely discarded, this project was unable measure bone mass through techniques such as bone mineral density assessment and ash weight analysis

Similarly, while the compression tests found no discernible differences in the types of fracture patterns exhibited by the femora and humerii specimens by age, it has been documented that older bone tends to be very stiff, but also very brittle, resulting in a reduced capacity for energy absorption and therefore increased risk of fracture (Currey and Butler, 1975; Burstein *et al.* 1976; McCalden *et al.* 1993; Tommasini *et al.* 2007). However, whether this potential factor affects what type of fracture morphology is generated requires further research. As previously mentioned, the compression tests resulted in a varying degree of visually extrinsic behaviours. As such the test set-up cannot be discounted as a potential reason for the results. It may therefore be suggested before an overall conclusion can be made that any potential differences between the immature porcine femur and humerus is drawn once further controlled testing can be conducted.

7.4.3 Hypothesis 3 - loading type and fracture pattern

Hypothesis 3 tested whether there is a relationship between fracture pattern and loading type. It was hypothesised that there will be a difference in fracture pattern depending on loading type. Based on the results derived from section 7.3.3 it can be suggested that Hypothesis 3 is true. Specifically, all fracture patterns produced in this study were particular to one type of loading mechanism. It may be further noted that despite the resultant growth plate fractures and failures generated through compression and torsion, the three-point bending, compression, and torsion tests did produce fracture patterns consistently referenced by the literature (Pierce *et al.* 2004). Both diaphyseal transverse and oblique fractures were generated through the three-point bending tests and are commonly reported in accidental and non-accidental scenarios involving children (Scherl *et al.* 2000; Pierce *et al.* 2004; Haney *et al.* 2009; Bilo *et al.* 2010).

Multiple buckle fractures were recorded from the compression tests and are also commonly observed in paediatric cases where axial loading has been reported (Pierce *et al.* 2004). In clinical cases, distal end fractures such as buckle fractures, are often an observed result from an accidental fall (Sepúlveda *et al.* 2022). Highlighting further the potential prevalence of buckle fractures as a result of falling, Raspopovic (2024) found that falls from a flat surface accounted for 22.4% of accidental fractures, while falls from a height contributed to 48.2% of such injuries. However, buckle fractures in any child under 9 months of age should be of clinical concern, primarily because the mobility and protective reflexes required to stave off an accidental fall (in which the result is generally a buckle fracture) is lacking in a child that young (Bilo *et al.* 2010). Abusive scenarios which may result in a buckle fracture is when a child or infant is thrown or slammed down onto a hard surface (Pierce *et al.* 2004). Cases also exist where a caretaker will intentionally bend a child’s extremity backward to cause pain (Pierce *et al.* 2004).

Whilst the torsion tests resulted in primarily growth plate failures, five of the tested specimens resulted in spiral fractures. In the experimental testing of human long bones, only spiral fractures have been observed as resultant of torsional loading (Kress *et al.* 1995). Spiral fractures were considered to be highly associated with abusive trauma due to the mechanism of twisting. However, more recent studies have since reported other fracture types, such as transverse, to be more common than previously thought, and that a spiral fracture in itself is not a definite suspicion of abuse (Thomas *et al.*1991; Leventhal *et al.* 1993; Scherl *et al.* 2000; Frasier, 2003, Pierce *et al.* 2004). Schwend *et al.* (2000) reported that spiral fractures can occur from seemingly innocuous trauma such as tripping while running.

In regard to growth plate failures observed by the literature, Ranson (2014) estimated the amount to approximately 25% of all fractures in children. In cases of non-accidental trauma, growth plate injuries often manifest as Condylar fractures, which are classified based on their involvement of the growth plate (Ranson, 2014). However, in the context of non-accidental cases the presence of growth plate injuries is primarily reported in conjunction with other fracture types, such as spiral or buckle fractures. These additional fracture morphologies were not observed in this experiment. Similarly, the literature indicates that growth plate injuries in the proximal humerus are relatively rare, constituting about 3% of all growth plate injuries in children (Asai et al. 2021) leaving further opportunity to research why growth plate failure might have been an issue in the torsion testing portion of this experiment.

7.5 Limitations

Naturally, the sample size in this study was limited, and constraints on resources restricted the acquisition of additional samples. While additional loading rates in accordance with Chapters 5 and 6 could have been attempted, it was deemed more prudent to maintain a single loading rate given the limited sample size. It is important to understand that while there is always a desire to test in as many conditions as possible, foundational research is primarily built on evaluating each component of the problem fully. Therefore, it was decided before testing that the behaviour of bone under traditional mechanisms of loading and quasi-static loading rates had to be fully understood first. In building a foundational knowledge base, consistency in testing, verification, and a larger sample size are key. Consequently, an argument could be made for limiting the testing to one form of mechanical test to ensure reliability and validity in the findings.

This chapter, and indeed this thesis as a whole, is based on a small sample of porcine specimens that were available at the time of study. This approach, while necessitated by practical constraints, significantly limits the statistical power of the analyses, particularly given the drastic reduction in sample sizes when transitioning to whole bone testing. Consequently, the findings presented in this chapter must be interpreted with caution, as the limitations in statistical power severely impact the generalisability of the results.

To take this research forward and to increase the reliability of the experiments and the findings, a larger, more systematically resourced sample would be highly desirable. In future studies, it would be useful to conduct formal procedures upfront of the experiment, such as a power analysis, to assess the sample size that would be necessary to detect different percentage variations in the outcome variables. For example, a power analysis could determine the required sample size to detect a clinically significant percent difference in the energy absorbed across different sample types, such as femur versus humerus. The absence of such a prior power analysis in this study raises serious concerns about the validity of the statistical tests performed, potentially rendering even the robust Fisher's Exact test unreliable. Therefore, the findings presented in this chapter should be interpreted with extreme caution.

This study also faced certain methodological decisions that may have limited the interpretation of the results. Steps undertaken during the compression tests could have included a more accurate method of bone alignment and a more stable manner of preventing shearing loads. This includes the use of clamps; other studies have utilised a combination of a fixed platen paired with an adjustable, rotating platen (Badiei *et al.* 2007; Bosisio et al. 2007; Bourgnon *et al.* 2014). Grooved platens have also proven to be a viable option (Balsly *et al.* 2008). The torsion tests resulted in a portion of the bones failing at the growth plates, which can be traced back to the decision not to stabilize the distal and proximal ends of the specimens using insertion pins. While insertion pins in conjunction with potting would likely prevent failure at the growth plates, the realism of the test setup is a concern. The ability to continuously generate spiral fractures is important, particularly in evaluating the mechanisms behind the trauma, but there is also the risk of manipulating the bone and test setup to such a degree that it is no longer attributable to in vivo situations. It is worth mentioning that certain mechanical factors contribute to the rarity of in vivo growth plate fractures; for example, the knee ligaments, capsules, and meniscus all provide restraints against shearing of the growth plates in vivo. A suggestion for future research is therefore the torsion testing of immature bone similarly to Thompson *et al.* (2015), in which the knee joint was left intact and attached to the test specimen.

Additionally, the inability to measure bone mass through techniques such as bone mineral density assessment and ash weight analysis represents a significant limitation of this study. The assessment of bone mass is crucial, as it has been shown to correlate with fracture risk and mechanical properties (Koo *et al.* 2001). Unfortunately, unforeseen circumstances prevented the collection of this data, which could have provided valuable insights into the relationship between bone mass and fracture patterns. Future studies should prioritize the inclusion of bone mass measurements to enhance the understanding of how bone density and geometry influence fracture behaviour.

Lastly, one limitation of this experimental study is that it doesn't fully capture the structure-function relationship of bone, as it fails to replicate the realistic context in which bones are utilised during natural movement and balance. Laboratory testing often involves static loading conditions that do not account for the dynamic forces and complex loading patterns experienced by bones in vivo. For instance, during activities such as walking, running, or jumping, bones are subjected to a combination of compressive, tensile, and torsional forces that vary in magnitude and direction based on the individual's movement patterns (Biewener, 1991). Furthermore, the presence of surrounding soft tissues, such as muscles, tendons, and ligaments, plays a crucial role in distributing forces and providing stability, which is often absent in a controlled laboratory setting (Singh and Chanda 2021). While this study investigates the mechanical function of bone, it does not fully incorporate the complex interplay of bone structure and function as it occurs in vivo. Consequently, while the findings from this study provide valuable insights into the mechanical behaviour of immature porcine bones under specific loading conditions, it is not fully representative of the complexities of bone function in a living organism. Future research should aim to incorporate more dynamic testing methodologies that better simulate the in vivo environment to enhance the ecological validity of the findings and provide a more complete understanding of the structure-function relationship in bone.

7.6 Conclusion

The aim of this study was to evaluate the fracture morphologies generated through the mechanical testing of whole immature porcine femora and humeri. A specific focus was given to the analysis of potential fracture pattern changes across age, bone type, and loading type. The data acquired in this study primarily showed that immature porcine whole bones, depending on the loading type, generate fracture morphologies consistent with the literature. Transverse and oblique fractures were primarily observed under three-point bending, constituting fracture patterns reported in similar real-life conditions. Buckle and condylar fractures were consistently generated under compression tests, which aligns with documented medical cases and the literature.

While the five resultant torsion specimens displayed oblique fractures, seven of the original thirteen specimens failed at the distal ends, raising serious questions about the testing methods, and the structural composition of developing bone. The high number of growth plate failures during the torsion tests raises serious concerns regarding the testing method, and the structural integrity of the immature bone. The same preparation should also be undertaken in compression testing, where a failure to position the specimens may have generated excessive shearing loads, thus affecting the aim of producing fractures under pure compression. Particularly, condylar fractures exhibited during the compression tests may suggest that the specimens had not fully fused at the distal and proximal ends, making these regions sites of stress concentration. In conjunction with considerations for test setups, this area warrants further exploration to understand the mechanisms behind immature bone behaviour.

Importantly, the results indicated some evidence of age-related trends, particularly in the femoral specimens, where oblique fractures increased in frequency with age. This initial finding suggests that age may play a role in influencing fracture patterns, warranting further investigation to explore these trends in greater depth. The lack of significant associations in the humeral specimens, where all fractures were transverse, contrasts with the observed trends in the femora and highlights the need for additional research to clarify these relationships. The lack of bone density measurements also limits the interpretability of the results, as this data would have added to the understanding of the fracture patterns observed.

In conclusion, this study has successfully highlighted potential factors for future research into immature porcine bone models, particularly the influence of age on fracture morphology. Understanding these dynamics is crucial for developing more effective strategies for assessing bone health and injury risk in both animal models and clinical settings. However, due to the small sample sizes used in this study, and the limitations of the testing methods used, the results of this chapter should be interpreted with caution. The limited sample size, particularly in the torsion tests, and the potential for methodological errors in compression testing, impact the generalisability of these findings. To validate the observations made in this study and fully elucidate the complex interplay of age, bone type, and loading type on fracture patterns in immature porcine bones, further research with larger sample sizes and refined testing protocols is recommended. Moreover, future studies should incorporate dynamic testing methodologies to better reflect physiological loading conditions.

Chapter 8 Discussion

8.1 Theoretical implications of empirical findings

The studies summarised in sections 8.1.1 through 8.1.3 have been guided by a theoretical underpinning. This theoretical underpinning has primarily been derived from the past and current research being conducted on the mechanical behaviour of immature bone and its potential implications on research on the formation of paediatric fractures. To better understand those implications, and how the mechanical behaviour of immature porcine bone can contribute to addressing challenges in forensic enquiry, this thesis has focused on the mechanical evaluation and fracture responses of immature porcine bone. A core theme has been the acceptance that the mechanical behaviour of a whole bone is a complex "integration" of the mechanical behaviour of the "intrinsic" bone material with the behaviour of the overall 'structure' of the whole bone, i.e., the extrinsic properties. The work described here has been concerned with both. The following section is a discussion on those evaluations and details how the findings of this thesis can better inform future research.

8.1.1 Theoretical implications regarding immature porcine bone

In the first study (Chapter 5) of this thesis, three-point bending tests were conducted on immature porcine cortical bone samples at a loading rate of 5 mm/min. As the literature has suggested certain mechanical correlations between immature porcine cranial bone and human infant cranial bone (Baumer *et al.* 2009) this thesis has observed some suggestive similarities between immature porcine cortical bone and the results from the singular study which has analysed human infant cortical bone (Currey and Butler, 1975). This similarity is foremost an age related trend, not in values but in direction.

Immature porcine cortical bone under high-energy loading rates (Chapter 6) was descriptively observed to exhibit energy absorptive outputs higher than outputs generated in studies employing low-energy loading rates (Currey and Butler, 1975). This aligns with the understanding that energy absorption generally increases with strain rate. However, it's important to note that at very high strain rates, energy absorption may decrease, suggesting a more complex relationship between strain rate and bone behaviour (Punjabi *et al.* 1973; Margulies and Thibault, 2000). However, in a field requiring specificity, the lack of comparable and continued research at current makes Chapter 6's findings interesting but requiring of further research.

Additional similarities were identified in Chapter 7, where this thesis' research moved from the mechanical behaviour of immature porcine cortical bone to whole immature porcine femora and humerii. The tested immature porcine femora and humerii were found to display several of the same fracture morphologies observed by the literature and clinical case studies. This foremost suggests that despite certain variations in the material properties and physical geometry between immature porcine and human infant long bones the fracture behaviour is similar. An important observation from Chapter 7 is the possibility that under compression and torsion the distal and proximal ends of immature porcine bone may be areas of stress concentration. Whilst spiral fractures are 'desired' during torsion tests (Cheong, 2015; Vaughan, 2017; Pierce *et al*. 2000; Pierce *et al.* 2004) due to its frequency in child abusive cases, the finding that immature bone may be more susceptible to growth plate failures raise questions as to whether the starting site of the torsion-based mechanisms is the factor which determines a distal end or centre of the diaphysis oriented fracture.

To address the primary research question - 'What is the mechanical behaviour of immature porcine bone and how can understanding this assist with forensic enquiry?' - the mechanical properties of immature porcine cortical bone cannot, at this point, be directly compared to human infant cortical bone. However, this is not to suggest that immature porcine bone is to be discounted as a potential proxy only that more information is required. If considering some of the findings from a 'look-see' perspective then yes, an age-related trend has been observed While this needs to tempered to the unknown of what may be affecting that trend it does imply that if we are to model paediatric bone behaviour, we should be concerned about age aspects (given the similarities that have been noted to exist between humans and pigs). As such, it may be suggested that the mechanical behaviour of immature bone does change with age. However, can these values, at present, be directly transferred to human infants? **No**. While the research presented in the three empirical chapters provides reliable evidence derived from mechanical testing of immature porcine bone samples, including three-point bending, impact tests, and whole bone assessments (bending, compression, and torsion), It is important to acknowledge the strengths and limitations of the testing methodologies employed when interpreting these findings.

The data generated from these tests are fundamentally empirical, derived directly from experimental observations rather than theoretical extrapolations or calculations based on intrinsic material properties. This empirical approach is essential in the context of immature porcine bone, as it allows for the assessment of mechanical properties under conditions that closely mimic physiological loading scenarios. The reliance on direct experimentation has enhanced the credibility of the results, as they reflect the actual performance of the immature bone samples when subjected to mechanical stress, but the truth is, that these tests were conducted completely without context. Specifically, while the empirical data obtained from these tests provides valuable insights into the mechanical behaviour of immature porcine bone under specific loading conditions, it must be acknowledged that this approach does not fully integrate the structure-function relationship as it exists in a living organism. The absence of surrounding soft tissues, physiological loading patterns, and the dynamic interplay of biological factors limits the extent to which these results can be directly extrapolated to *in vivo* bone function. Consequently, while the observed fracture patterns and mechanical properties are empirically valid, their interpretation must be tempered by the understanding that they represent a first step. Therefore, while the mechanical properties of the bone were measured, the lack of the simulation of the *in vivo* environment, impacts the ability to fully understand the structure-function relationship. Future studies should aim to bridge this gap by incorporating methodologies that more accurately replicate the complex biological context of bone function, such as dynamic loading protocols and computational modelling that accounts for soft tissue interactions.

Furthermore, the tests primarily yielded information regarding the structural behaviour and performance of the immature bone, which can be categorized as extrinsic properties. These properties, as has been noted, are influenced by various external factors, including the size and shape of the bone samples, as well as the specific testing conditions such as the orientation of the bone samples and the fixing of the whole bones. The focus on extrinsic properties is particularly relevant in understanding how immature bone behaves under different loading conditions, as these factors can significantly affect the material's performance. However, it is important to note that while extrinsic properties provide valuable insights, they do not fully encapsulate the intrinsic characteristics of the bone material itself.
A notable limitation of such as force at fracture, flexural strength, and flexural modulus. These properties are inherent to the material and should remain constant regardless of external dimensions or configurations. The discrepancies observed in the intrinsic property measurements suggest that the testing methodologies may not have adequately captured the true nature of the immature bone. This raises concerns regarding the accuracy of the results and their implications for understanding the mechanical properties of immature bone.

One of the primary factors contributing to the inaccuracies in measuring intrinsic properties is the use of non-standard sample dimensions. The test samples did not conform to established standards, which is critical for ensuring consistency and reliability in mechanical property assessments. Non-standard dimensions can introduce significant errors and inconsistencies in both calculations and measurements, particularly for intrinsic properties. This limitation emphasises the necessity of adhering to standardised protocols in future studies to enhance the accuracy and comparability of mechanical property measurements in immature bone.

In conclusion, while the tests conducted on immature porcine bone samples provided reliable empirical and structural (extrinsic) data, they were less exact in yielding accurate measurements of intrinsic material properties. The inaccuracies observed are likely attributable to the non-standard dimensions of the test samples, which hindered the reliability of the intrinsic property assessments. This discussion highlights the critical need for standardised testing methodologies to ensure the validity of mechanical property evaluations in immature bone and underscores the importance of further research to refine these methodologies.

The findings of this thesis contribute to the ongoing discussion surrounding the mechanical behaviour of paediatric cortical bone. While some results align with established trends, discrepancies were observed when compared to findings from some previous studies. For instance, a notable difference was seen in the magnitude of flexural modulus when compared with the results reported by Currey and Butler (1975), a foundational study due to its mechanical testing of paediatric cortical bone. These variations, along with differences observed in comparison to studies such as McPherson and Kriewall (1980) and Margulies and Thibault, 2000, highlight the complex interplay of factors influencing immature bone mechanics

One of the primary aims of this thesis was the development of an evidence base which would inform realistic experimentation. This included a mechanical evaluation of the immature porcine model, with the goal of providing data relevant to the complex challenge of diagnosing accidental and non-accidental fractures by clinical practitioners. This research provides data relevant to this challenge by characterizing the mechanical properties of immature bone, examining its response to high-impact loads, and documenting the fracture patterns produced under controlled conditions. These findings contribute to a more comprehensive understanding of how immature bone behaves under stress, which is essential for accurate interpretation of injuries in clinical settings. It has become clear throughout this project that the development of an empirical knowledge base is resource and time intensive. However, an evidence base is only an effective tool if it is assembled from the ground up (Morgan *et al.* 2008; Morgan *et al.* 2009). This thesis undertook to take this first step through experimental research contributing valuable data and laying the foundation for future investigations into the mechanical behaviour of immature bone.

8.1.2 Theoretical implications pertaining to forensic pathology

The research conducted in this thesis has been foundational and perhaps therefore not immediately helpful to the physicians, paediatricians, radiologists, and forensic professionals who are currently on staff at an emergency health unit or crime scene. However, in an effort to better assess plausibility of injury and history, the findings in this thesis has shown certain indications which could be useful in a case-based biomechanical investigations. This includes the primary finding that despite widespread use of the immature porcine model on a material level immature porcine cortical bone is not comparable to human infant cortical bone. However, general similarities in whole bone fracture behaviour has been observed by this thesis and several other studies (Baumer *et al.* 2009; Bilo *et al.* 2009; Vaughan, 2017; This thesis Chapter 7). Therefore, a recommendation by this thesis is any future research endeavour employing immature porcine cortical should offer pause to using the bone data to inform other models (e.g. computational modelling).

A secondary finding which could influence the field of injury biomechanics in relation to use of the immature porcine model is the age factor. Specifically, in younger specimens or samples, age should always be considered. That this thesis found differences even across specimens and samples one to two days apart in age indicates that no liberty with age groups should be taken. While the specific material properties of immature porcine cortical bone were not directly comparable to the human infant cortical bone, age was a significant factor. Even if the immediate data is not comparable between species, the results speak to the importance of accounting for the age of a child admitted with fractures of an uncertain context.

8.2 Practical implications of findings

To address the primary research question of this thesis – 'what is the mechanical behaviour of immature porcine bone and how can understanding this assist with forensic enquiry?' - the next section of the general discussion will describe how the mechanical tests conducted in this thesis inform the field and future research.

Although the various experimental studies in this thesis were not perfect, they provided valuable insight into mechanically testing immature bone. The following section provides an overview and discussion of the practical implications of these findings, particularly for conducting future biomechanical experiments.

8.2.1 Validation and specificity of method

The scientific method is grounded in the concept that the validation of a theory can only be achieved through rigorous testing (Popper, 1968). While the experiments conducted in this thesis were extensive, the results from these studies require further validation. It is not only a question of the quantity of tested samples but the manner of the testing as well. Just as each aspect of a research study needs to be confirmed and validated, so too does each aspect of a child abuse case in the course of reaching either conviction or acquittal of a suspected perpetrator. Such assessments require an adherence to patient-specific data, including the age of the individual, skeletal development, nutritional status, and the presence of any chronic or predisposing diseases (Pierce and Bertocci, 2008). It is only when each variable has been examined and understood that a new and perhaps more complex experiment can be undertaken (Morgan et al. 2008; Morgan et al. 2009).

Biomechanical research requires equipment and material, none of which is easy to prepare or acquire. The harvesting and preparation of organic tissue is not only a question of funds, but the networking and physical locations required to acquire and store organic tissue. This thesis is in part a microcosm of the obstacles encountered in conducting experimental research in the field of paediatric biomechanics. As highlighted in Chapters 5, 6, and 7, challenges such as limited sample sizes, potential inconsistencies in sample preparation, the use of non-standard test setups, and the inherent variability of biological samples were encountered. Paediatric biomechanics is a field which is driven by technology (e.g. computational modelling, test alignment method; Li *et al.* 2018; Cheong, 2015). In consideration to the results of this thesis, the author has come to consider transparency in method as one of the more important aspects of conducting research but also future experimentation.

Specifically, when replication and validation is important to the omission of method, the scarcity of specifics is especially a hindrance.

The importance of method became especially clear in the evaluation and diagnosis of fracture patterns in Chapter 7. As discussed, the descriptive nature of fracture pattern identification in Chapter 7 introduced a degree of subjectivity. Furthermore, limitations in Chapters 5 and 6, such as the use of custom-built rigs, non-standard sample dimensions, potential inconsistencies in sample preparation, and the challenges of comparing results across different studies, further highlight the importance of methodological transparency. Errors in the identification of fractures as abusive or accidental are often diminished by the aid of experts in the field, but the lack of an empirical standard still highlights that the method is not objective-based. Efforts have been made by Cheong (2017) and Vaughan (2017) in recording fracture propagation through whole bone, which could help provide a more detailed understanding of fractures in children. However, while such advancements are crucial, it's important to acknowledge that when dealing with complex biological systems, such as how paediatric bone will behave, not everything can be reduced to a single measurement or objective standard. As Bilo *et al.* (2009) stated, no injury is pathognomonic of abuse. The overall medical standard utilised by clinicians is therefore not a single all-encompassing descriptive or quantitative approach, but a combination of various evaluations which informs a decision. Identifying and preventing child abuse requires refining biomechanical research through mechanical testing of immature tissue and the identification of fracture morphologies. This should be combined with the introduction of quantitative measures and measures for preventing bias (Love, 2016; Cheong, 2017; Vaughan, 2017).

8.3 Future research

Considering the overall findings of this thesis, three major areas of future research are suggested below. These include replication of research, comparative studies with human samples, bone mineral tests, further comparison between different loading rates, and the development of larger biological system proxies.

8.3.1 Replication of research

As with any empirical research, it is of the utmost importance to replicate previously conducted studies for the purpose of setting a robust scientific foundation upon which new theories may develop. With regard to the findings of this thesis, it is deemed suitable to conduct further studies which replicate the experimental methods presented here, in order to understand patterns across large sets of data. Indeed, the need for replicable studies to establish sound scientific methods in forensic science has recently been discussed by Morgan (2019), for the purpose of establishing sound scientific methods in forensic science. This holds equally true for research conducted within forensic pathology, forensic anthropology, or immature bone research in the context of forensic enquiry.

Based on the literature review for this thesis, it was noted that published studies looking at mechanical bone behaviour often present challenges for replication. Variations in test set-up and methodology across different studies, as well as limitations in data presentation, were identified. This can hinder the ability to reproduce and compare research findings. It is important to acknowledge that this thesis also faces some of these challenges due to the specific methodological choices made, as discussed in detail in the limitations section. Thus, it is recommended that future research adopts an open science approach, where methods and data are published in order to prompt replication studies.

Future research would also be advised to replicate the methods presented in this thesis. However, considering the challenges encountered in this thesis, such as scattered data, non-standardised sample dimensions, and bespoke equipment that prevents comparisons, future replication studies should prioritise methodological standardisation. This includes the use of larger sample sizes, standardised sample preparation, and consistent testing protocols to enhance the reliability and comparability of results.

While the studies in this thesis presented results from tests on femoral and humeral bones, which leave a gap of understanding in other types of bone, introducing additional complexity by focusing on trabecular bone may not be the most logical next step. Instead, the focus should first be on addressing the identified methodological limitations to create a more robust foundation for future research

8.3.2 Comparative studies from human samples

This thesis utilised immature porcine bone as a surrogate for human infant bone, a common practice in biomechanical research due to the limited availability of human paediatric tissue. While the use of animal models offers valuable insights, the thesis acknowledges the inherent limitations of this approach. Key differences exist between porcine and human bone, including morphological and developmental variations, raising questions about the direct translatability of the findings. Therefore, future research must prioritise comparative studies with human samples to validate the applicability of the current findings and determine the accuracy of using porcine models in this context.

Such comparative research is essential for several reasons. Firstly, it would allow for a direct comparison of mechanical properties and fracture behaviour between immature porcine and human infant bone, providing a more accurate assessment of the porcine model's biofidelity. This would help refine the use of porcine bone as a surrogate and improve the reliability of future studies that utilise this model. Secondly, comparative studies could investigate the specific differences in bone development and maturation between porcine and human samples. This would contribute to a deeper understanding of the factors influencing bone behaviour in children and inform the development of more accurate predictive models.

To overcome the challenges associated with obtaining human paediatric bone samples, future research could explore alternative avenues such as utilising medical imaging data from paediatric fracture cases. By analysing fracture patterns and bone morphology in clinical data, researchers could establish a valuable comparative dataset. This approach, combined with advanced imaging techniques and computational modelling, could provide a powerful tool for validating animal model findings and improving our understanding of paediatric bone trauma.

8.3.3 Bone Mineral Density tests

The findings of this thesis highlight the complex interplay of factors influencing immature bone behaviour. While the mechanical testing conducted provided valuable data on the extrinsic properties of bone, a comprehensive understanding necessitates further investigation into the intrinsic properties, specifically the structure-function relationship. The thesis acknowledges that a microstructural analysis, including BMD assessment, was not possible in the current study due to the unavailability of the original bone samples. Future research should prioritize the inclusion of Bone Mineral Density (BMD) tests to address this limitation.

BMD, a measure of bone mineral content per unit area, is a crucial determinant of bone strength and an important factor in understanding fracture risk. By quantifying BMD in conjunction with mechanical testing, future studies can establish clearer correlations between bone composition and its mechanical behaviour in immature porcine bone. This would provide valuable insights into how mineralization influences bone strength, stiffness, and toughness across different developmental stages. Such research could also explore how BMD relates to the observed variations in fracture patterns under different loading conditions.

Furthermore, incorporating BMD analysis would address a key challenge in the current research: the variability in mechanical properties observed across samples. While factors such as age and loading rate were investigated, differences in BMD could explain some of the scatter in the data. By accounting for BMD, future studies could potentially reduce unexplained variability and isolate the specific effects of other variables on bone behaviour.

Moreover, exploring the relationship between BMD and microstructural characteristics would further enhance our understanding of the factors influencing bone behaviour. Techniques such as micro-CT imaging could be used alongside

BMD assessment to provide a more detailed analysis of bone microstructure and its connection to mechanical properties. This multi-scale approach would offer a more comprehensive view of the structure-function relationship in immature bone.

8.3.4 Strain-rate dependency of immature porcine bone

The influence of loading rate on bone behaviour is a critical consideration, particularly in the context of forensic investigations involving trauma. As highlighted in Chapter 6, this thesis encountered limitations in making definitive comparisons regarding the effect of loading rate on immature porcine bone. Specifically, challenges in data output and comparability hindered a comprehensive analysis of how bone responds to varying speeds of applied force. Therefore, further research is essential to address this gap in understanding.

Future studies should prioritise a systematic investigation of the strain-rate dependency of immature porcine bone. This necessitates a more robust experimental design that incorporates both low and high impact testing methodologies. To facilitate meaningful comparisons, these studies must utilise similar sample groups of immature porcine cortical bone, ideally spanning a relevant age range such as newborn to 40 days. This focus on a specific age range allows for a targeted analysis of how strain-rate effects may evolve during early development.

Furthermore, it is crucial that future research expands the range of loading rates investigated. Rather than focusing on only two extremes, employing a spectrum of intermediate loading rates will provide a more nuanced understanding of bone's response to dynamic loading. This approach will help to identify potential thresholds or transitions in bone behaviour as loading rate increases.

To ensure the reliability and generalisability of findings, future studies must also address limitations related to sample size and statistical analysis. Larger sample sets are necessary to account for biological variability and to increase the statistical power of the analysis. Appropriate statistical tests should be employed to rigorously compare the data obtained from different loading rates and age groups. This will enable researchers to determine whether differences in bone behaviour are statistically significant and not simply due to chance.

Ultimately, a comprehensive investigation of the strain-rate dependency of immature porcine bone will contribute significantly to our understanding of paediatric bone trauma. By elucidating how bone responds to different loading speeds, this research will provide valuable insights for forensic investigations, injury biomechanics, and the development of more accurate models of bone behaviour.

8.3.5 Larger biological systems

While this thesis provides valuable data on the mechanical behaviour of isolated immature bone, a crucial next step, following validation, is to investigate how these properties, which are inherently linked to bone's underlying structure, translate to more complex, integrated systems. The simplified loading conditions used in this thesis, while necessary for controlled experimentation, do not fully replicate the complex forces acting on bones within the body during trauma, where the interaction of multiple structural components plays a critical role. Therefore, future research should focus on integrating the material properties of immature bone into biomechanical models of specific anatomical regions. Such models are crucial for improving the accuracy of injury reconstruction in cases of suspected child abuse, where understanding, how structural variations between different types of tissues influence their functional response to loading, is essential.

The development of pneumatic artificial muscle systems, such as those being initiated at the Department of Mechanical Engineering at University College London, offers a promising avenue for creating more realistic simulations of muscle-driven bone loading. Combining this technology with a more validated form of data from this thesis in computational models would allow for a more accurate representation of dynamic loading scenarios, where muscle forces (a functional component) interact with bone structure. Integrating bone material properties with realistic muscle simulations will allow for a more comprehensive understanding of how different loading scenarios, involving complex interplay between structure and function, lead to specific fracture patterns, ultimately aiding in the differentiation between accidental and non-accidental injuries. This approach will improve the predictive power of injury assessments and contribute to a more comprehensive understanding of paediatric bone trauma

Chapter 9 Conclusion

The mechanical evaluation of immature bone, specifically immature porcine bone, is crucial in the research field of paediatric skeletal injuries. For medical clinicians and forensic professionals, a greater understanding of immature bone behaviour has the potential to aid the differentiation between accidental and non-accidental fractures in children. This is particularly important considering the significant developmental changes that bone undergoes from newborn to 3 years of age (Altai et al. 2018).

However, research in this area is limited by the scarcity of available and testable paediatric tissue. To address this, immature porcine bone has been proposed and utilised as a suitable alternative model (Baumer *et al.* 2009; Margulies and Thibault 2000; Coats and Margulies, 2003). As such, the overarching research question leading this thesis is: *what is the mechanical behaviour of immature porcine bone?* It was determined that should such an overarching research question be answered, there would be a need for three intermediate questions. These intermediate research questions were the following:

1) What are the basic underlying mechanical properties of immature porcine bone as a material?
2) As a material, how does immature porcine bone material act under high impact loads?
3) What type of fracture patterns can be generated through the mechanical testing of whole immature porcine long bones?

Each intermediate research question has in turn been answered through three empirical studies which are detailed in Chapters 5, 6, and 7. However, in an effort to summarise the findings in regard to each intermediate research question and the overall primary research question this chapter will provide that overview. Each intermediate research question will therefore be summarised and discussed below (Section 9.1.1, 9.1.2, and 9.1.3).

9.1 What are the basic underlying mechanical properties of immature porcine bone as a material?

The mechanical properties of immature bone are governed by a complex interplay of factors, of which age is a critical consideration due to the continuous developmental state of immature bone (Suresh, 2001; Wu *et al.* 2012; Altai *et al.* 2018). The first three years of life are particularly significant, characterised by rapid bone development (Huelke, 1998; Altai *et al.* 2018). This period is also clinically crucial, as it coincides with increased infant mobility and reliance on adult care, making the interpretation of fractures challenging (Worlock *et al.* 1986; Lyons *et al.* 1999; Khoriati *et al.* 2016).

Currently, Currey and Butler (1975) provide the sole investigation into the age-related intrinsic properties of human infant cortical bone under three years of age. Their study, employing three-point bending at 5 mm/min on machined femoral specimens aged 2 to 4 years, revealed a decrease in flexural stress and an increase in elastic modulus with age. Given the scarcity of human infant tissue, Chapter 5 of this thesis explored an alternative approach using immature porcine cortical bone as a proxy. While the test protocol and results diverge from Currey and Butler (1975), this thesis has offered insights into immature bone behaviour and whilst having facilitated a descriptive comparison. Drawing upon Baumer *et al.* (2009)'s correlation of porcine days to human months, 90 machined porcine cortical bone samples, aged newborn to 40 days, were subjected to three-point bending at 5 mm/min. This age range corresponds to approximately newborn to three years in humans. Recognising the strain-rate dependence of bone, additional samples were tested at 50 mm/min (n=78) and 100 mm/min (n=92). Furthermore, given that samples were derived from both humeral and femoral bones, the study investigated potential differences in mechanical properties between these bone types, which, despite their shared classification as long bones, differ in function and anatomical location (Currey, 2002).

The results from these experiments have presented a range of findings that will offer new insights into immature porcine mechanical behaviour. Specifically, the study highlighted that immature porcine cortical bone does exhibit an age-related mechanical behaviour similar in trend to Currey and Butler (1975). This included an upwards trend in force at fracture, flexural strength, and flexural modulus with age. Whilst certain mechanical behaviours correspond between the immature porcine bone samples and the paediatric samples derived from Currey and Butler (1975), each measured mechanical property is a puzzle piece which upon completion determines the overall material behaviour between immature porcine bone and human paediatric bone. Thus, while certain mechanical properties between immature porcine bone and human paediatric bone show a similar age-related trajectory, the statistical tests and comparative values does indicate that immature porcine cortical bone does not correspond to paediatric cortical bone on a mechanical level.

While these findings provide a foundation for understanding immature bone behaviour, it is crucial to acknowledge several methodological limitations that could have influenced the interpretation of these results. Firstly, the potential for misalignment of the impact head during three-point bending was not explicitly addressed. Any deviation from perfect alignment could have introduced extraneous forces, affecting the accuracy of the measurements. Secondly, while age was considered, the confounding effect of bone size was not adequately accounted for. However, it is crucial to acknowledge several methodological limitations that could influence the interpretation of these results. Future research should address these limitations by implementing rigorous alignment procedures, accounting for bone size variations, and providing detailed sample preparation protocols would enhance the robustness and applicability of the results.

9.2 As a material, how does immature porcine bone act under high impact loads?

A factor which can begin to help determine how children's bone may mechanically react under different conditions is examining such behaviour under various loading rates. Specifically, loading rate has been observed to be a deciding factor on bone's mechanical behaviour (Hansen *et* al. 2008; Zioupos *et al.* 2008; Cheong *et al.* 2017;

Bertocci *et al.* 2017; Vaughan 2017). McElhaney (1966) tested 24-year-old human cortical bone samples in compression and observed the elastic modulus and ultimate compressive stress to increase comparatively with strain rate. The strength and energy absorption of bone have also been found to increase under increasing torsion (Punjabi *et al.* 1973) and tension (Crowninshield and Pope, 1974; Hansen *et al.* 2008). Margulies and Thibault (2000) in turn three-point bend tested machined cranial bone from both paediatric specimens (25 weeks of gestation to 6 months of age) and immature porcines (2 to 3 days of age) and found the flexural modulus and energy absorbed to differ depending on a loading rate of 2.54 mm/min and 2540 mm/min.

Therefore, the second intermediate research question posed by this thesis was 'how does immature porcine bone material mechanically act under high impact loads?'. This research question was answered through a series of experiments detailed in Chapter 5 and 6. The results from Chapter 5 indicate a more complex relationship between loading rates and the mechanical behaviour of immature porcine bone than initially hypothesised. While descriptive trends were observed across the three loading rates (5 mm/min, 50 mm/min, and 100 mm/min), differences in age-related mechanical properties were primarily limited to force at fracture. Specifically, force at fracture showed differences between age category 1 and age category 2.

Descriptive observations revealed that at the slowest loading rate of 5 mm/min, a more gradual age-related increase in force at fracture was observed, with the peak force occurring in the 29-31 day age group. This trend, however, was accompanied by fluctuations in flexural strength and modulus across the various age groups. In contrast, the higher loading rates of 50 mm/min and 100 mm/min exhibited greater variability in all measured mechanical properties, including force at fracture, flexural strength, and flexural modulus. Notably, the peak values for force at fracture and flexural strength were generally higher at the 100 mm/min loading rate compared to both 50 mm/min and 5 mm/min.

These observations highlight that while some trends in mechanical properties can be identified across the loading rates used, the differences were not consistently observed across all measures. This suggests that the magnitude of the loading rates used in Chapter 5 may not have been sufficient to consistently elicit a clear strain-dependent response across all measured mechanical properties, as has been observed and documented in the literature (Cheong *et al.* 2017).

For Chapter 6, 40 immature humeral and femoral cortical bone samples (age range: newborn to 40 days) were subjected to a loading rate of 2.93 m/s using the Charpy impact method (Chapter 4; Chapter 6). This method measures energy absorbed. An important consideration is that variations in the impact location on the bone samples could have influenced the energy absorbed measurements, a factor we took into account as we analysed these results in terms of sample age, bone type, and the energy absorbed results from Chapter 5.

A significant change in energy absorbed across age was observed. Bone type was also found to be statistically significant across age. This suggests that bone type is a factor when mechanically testing immature porcine cortical bone. However, the results from both Chapter 5 and Chapter 6 indicate that this difference only becomes apparent when using high-energy loading rates. That this behavioural difference would exist between femoral and humeral samples at specifically high energy loading was unexpected. Currently, no other study has detailed the material differences between immature femoral and humeral cortical bone across age. It is therefore recommended that validation of the results should be conducted on the future.

In order to overcome the limitations of this study and further our understanding of immature bone behaviour, future research should prioritise a systematic investigation of the strain-rate dependency of immature porcine bone. This necessitates a more robust experimental design, incorporating a spectrum of intermediate loading rates, larger sample sets, and similar sample groups. By clarifying how bone responds to different loading speeds, such research will provide valuable insights for forensic investigations, injury biomechanics, and the development of more accurate models of bone behaviour, ultimately contributing to a more comprehensive understanding of paediatric bone trauma

9.3 What are the fracture characteristics of whole immature porcine long bones?

Forensic professionals and clinicians frequently evaluate fracture patterns in whole bones to differentiate between accidental and non-accidental injuries in children. These patterns can be influenced by developmental changes in bone, such as growth in width, length, and shape (Naughton *et al.* 2018; Kress *et al.* 1995; Ouyang *et al.* 2003). The benefits of understanding how certain failure mechanisms may generate certain fracture patterns trough early life would allow for the prevention of bone fractures through better design of equipment, improvement in treatment processes, and forensic characterisation of the causes of injuries, but also the opportunity for investigating whether and how fracture morphologies may change with age (Kress et al. 1995; Ouyang *et al.* 2003; Ebacher *et al.* 2007; Cheong, 2015). However, whilst the research has provided several references to the mechanisms required to induce certain fracture patterns little comparative data still relates to the earliest age groups: birth to 3 years of age. In humans, this age group is particularly challenging and of interest due to an accelerated growth rate and change in anatomy and functional capability (Altai *et al.* 2018) To investigate the use of the immature porcine as a paediatric model only Thompson et al. (2015) have employed whole porcine bones under the age of three months (3- and 7-day old specimens).

The third and final study of this thesis, detailed in Chapter 7, evaluated fracture characteristics of immature porcine long bones. This included the mechanical testing of 41 whole immature porcine femora and humerii (age range of newborn to 40 days) under three-point bending, compression, and torsion. Specifically, the three-point bending and compression tests were conducted at a loading rate of 50 mm/min. The specific aims of the experiment included the analysis of any fracture pattern differences across age, and type of loading (three-point bending, compression, and torsion).

Observations found fracture pattern to be specific to certain loading types. Transverse and oblique fractures were exclusively generated under three-point bending. Condylar and buckle fractures were exclusively observed under compression testing. Whilst 5 of the successfully tested torsion specimens displayed spiral fractures, the remaining 9 specimens failed at the growth plates.

The frequent condylar fractures and growth plate failures in compression and torsion tests raise questions about the vulnerability of immature porcine bone in these areas. As has been noted by the literature, at this age of the specimens, bone fusion is still ongoing (Wang *et al.* 2010). Furthermore, as the growth plates in particular are composed of cartilage they are more compliant than bone (Aguel, 2004). It could therefore be reasoned that cartilage, due to being more compliant would fail at strains smaller than the bone. However, an additional possibility was the presence of shearing forces which, as noted by the literature, can contribute to condylar fractures under compression, and growth plate failures in torsion (Pierce *et al.* 2001). As such, these specific results may indicate either suggest that immature porcine is more susceptible to fracture in certain anatomical areas or that unexpected shearing forces unaccounted for during the compression and torsion tests was a contributing factor.

In addition to the loading-specific fracture patterns, the study also revealed some evidence of age-related trends. This included the femoral specimens, where oblique fractures appeared to increase in frequency with age. Simultaneously, under compression buckle fractures were observed in the youngest specimens while condylar fractures were generated more frequently in older specimens. While a descriptive observation these initial finding may suggest that age may play a role in influencing fracture patterns, warranting further investigation to explore these trends in greater depth. The lack of significant associations in the humeral specimens, where all fractures were transverse, contrasts with the observed trends in the femora and highlights the need for additional research to clarify these relationships

9.4 Concluding remarks

This thesis sought to answer research question “what is the mechanical behaviour of immature porcine bone?”. Through a series of material and whole bone mechanical tests, the studies of this thesis have provided a clear but unfinished view of the topic.

Firstly, the findings indicate that immature porcine cortical bone exhibits key differences from human infant bone, suggesting it may not be a directly comparable surrogate in all contexts. However, in addressing gaps in the literature these studies have identified that immature porcine cortical bone does exhibit age-related trends in regard to the mechanical properties of force at fracture, flexural strength, and flexural modulus. Immature porcine cortical bone has also shown indications of exhibiting strain-rate dependent trends which may be affected by age. Bone type was identified to be a non-factor for the material testing and showcased the importance of test considerations such as bone size and sample size when testing.

Immature porcine long bones generated fracture patterns under three-point bending, compression, and torsion that align with those documented in literature and paediatric case studies. Notably, condylar fractures and growth plate failures were observed in compression and torsion tests. The prevalence of condylar fractures and growth plate failures, especially in compression and torsion tests, may be attributed to both the ongoing fusion process in immature bone at the distal ends and potential limitations in the test setup. These limitations might have introduced shearing forces or instability, influencing the observed fracture patterns. Therefore, while the observed fracture patterns align with those documented in the literature and clinical cases involving children, the extent to which they accurately reflect in-vivo fracture mechanisms remains unclear

In the context of the high prevalence of child abuse and the urgent need for reliable evidential data towards its investigation this thesis has provided valuable data on immature porcine bone mechanics, highlighting the influence of age and loading type on fracture patterns. To build on these findings and address the study’s limitations, future research needs to prioritise replication using standardised methods and larger sample sizes to establish a robust evidence base. Furthermore, comparative studies with human infant bone to validate the porcine model's applicability, inclusion of Bone Mineral Density (BMD) analysis to better understand the structure-function relationship, further investigation of strain-rate dependency to improve understanding of bone response to dynamic loading, and integration of results into biomechanical models to simulate realistic loading and enhance injury reconstruction accuracy are also necessary. Pursuing these directions will lead to a more comprehensive and clinically relevant understanding of immature bone behaviour.

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Appendix A

Appendix A: Source porcine characteristics

Specimen Number	Date of Birth	Age (Days)	Breed	Sex
1	05/10/2017	29-30d	Large White	Male
2	05/10/2017	29-30d	Large White	Male
3	29/09/2017	35-37d	Large White	Male
4	29/09/2017	35-37d	Large White	Male
5	01/10/2017	32-34d	Large White	Male
6	01/10/2017	32-34d	Large White	Male
7	26/09/2017	38-40d	Large White	Male
8	26/09/2017	38-40d	Large White	Male
9	27/10/2017	08-10d	Large White	Male
10	27/10/2017	08-10d	Large White	Male
11	29/10/2017	05-07d	Large White	Male
12	29/10/2017	05-07d	Large White	Male
13	01/11/2017	03-04d	Large White	Male
14	01/11/2017	03-04d	Large White	Male
15	02/11/2017	01-02d	Large White	Male
16	02/11/2017	01-02d	Large White	Male
17	27/10/2017	11-13d	Large White	Male
18	27/10/2017	11-13d	Large White	Male
19	23/10/2017	14-16d	Large White	Male
20	23/10/2017	14-16d	Large White	Male
21	20/10/2017	17-19d	Large White	Male
22	20/10/2017	17-19d	Large White	Male
23	19/10/2017	20-22d	Large White	Male
24	19/10/2017	20-22d	Large White	Male
25	16/10/2017	23-25d	Large White	Male
26	16/10/2017	23-25d	Large White	Male

27	12/10/2017	26-28d	Large White	Male
28	12/10/2017	26-28d	Large White	Male
29	03/11/2017	00-01d	Large White	Male
30	03/11/2017	00-01d	Large White	Male

Appendix B

Three-point bending material tests

Legend

RF - Right femur
LF - Left femur
RH - Right humerus
LH - Left humerus

Appendix B: Individual immature porcine cortical bone samples at 5 mm/min.

Age Category	Age	Specimen Name	Side and Bone Type	Test	Force at fracture (N)	Deflection (mm)	Flexural Strength (MPa)	Flexural modulus (GPa)	Cross-Sectional Area (mm^2)	Depth (mm)	Width (mm)	Length (mm)	Span (mm)
1	00-01d	MATS-29-RF	RF	#1	21	0.168	6.33	1.664	1.5	0.94	1.6	6.2	4
1	00-01d	MATS-29-RF	RF	#2	76	0.025	51.47	1.618	6.65	2.51	2.65	6.88	4
1	00-01d	MATS-29-LH	LH	#1	116	0.050	41.65	1.019	5.47	2.08	2.63	7.03	4
1	00-01d	MATS-29-LH	LH	#2	107	0.041	56.32	1.283	5.49	1.77	3.1	6.43	4
1	00-01d	MATS-29-LF	LF	#2	35	0.086	152.21	0.684	4.97	1.78	2.79	6.83	4
1	00-01d	MATS-29-RH	RH	#1	65	0.050	117.03	2.784	2.25	1.5	1.5	6.5	4
1	00-01d	MATS-29-RH	RH	#2	76	0.054	140	2.569	2.25	1.5	1.5	6.5	4
1	01-02d	MATS-15-RH	RH	#1	55	0.061	24.82	2.373	2.24	1.4	1.6	6.4	4
1	01-02d	MATS-15-RF	RF	#1	85	0.066	45.94	0.885	4.81	2.03	2.37	6.11	4

1	01-02d	MATS-15-LH	LH	#1	92	0.036	56.42	1.716	4.54	2.1	2.16	6.34	4
1	01-02d	MATS-15-RF	RF	#2	57	0.146	29.34	0.368	5.12	2.16	2.37	6.71	4
1	01-02d	MATS-15-RH	RH	#2	100	0.046	75.6	1.153	5.18	2.25	2.3	6.89	4
1	01-02d	MATS-15-LH	LH	#2	73	0.036	45.98	1.205	6.51	2.04	3.19	6.85	4
1	01-02d	MATS-15-LF	LF	#2	43	0.129	82.9	0.525	4.23	1.87	2.26	6.88	4
1	03-04d	MATS-13-LH	LH	#2	78	0.058	59.2	1.447	3.45	1.79	1.93	6.85	4
1	03-04d	MATS-13-LH	LH	#3	78	0.066	209.48	1.140	3.78	1.87	2.02	6.93	4
1	03-04d	MATS-13-LH	LH	#1	45	0.087	38.37	2.049	2.24	1	2.24	6.85	4
1	03-04d	MATS-13-RF	RF	#2	57	0.029	54.15	2.837	3.56	1.77	2.01	6.94	4
1	03-04d	MATS-13-LF	LF	#2	48	0.138	46.32	0.999	2.25	1.5	1.5	6.2	4
1	03-04d	MATS-13-LF	LF	#1	63	0.050	72.29	1.030	5.38	2.12	2.54	5.66	4
1	03-04d	MATS-13-RH	RH	#2	27	0.180	47.56	0.511	3.37	1.51	2.23	7.39	4
1	05-07d	MATS-11-RH	RH	#2	87	0.048	41.36	2.980	2.34	1.3	1.8	6.5	4
1	05-07d	MATS-11-RH	RH	#1	116	0.048	48.54	3.352	2.08	1.3	1.6	6.5	4
1	05-07d	MATS-11-RF	RF	#1	48	0.101	37.55	0.645	4.53	1.69	2.68	6.69	4
1	05-07d	MATS-12-LF	LH	#1	71	0.090	52.61	0.473	6.37	2.25	2.83	6.84	4
1	05-07d	MATS-11-LF	LF	#2	45	0.071	101.34	0.635	6.24	2.02	3.09	6.79	4
1	08-10d	MATS-09-RF	RF	#1	72	0.066	34.53	0.966	4.59	1.78	2.58	7.18	4
1	08-10d	MATS-09-LF	LF	#2	75	0.116	35.56	0.443	5.34	2.19	2.44	5.73	4
1	08-10d	MATS-09-LF	LF	#1	83	0.078	42.39	0.624	5.61	2.27	2.47	6.04	4
1	08-10d	MATS-09-RH	RH	#1	94	0.061	51.1	0.798	5.65	2.2	2.57	6.36	4
1	08-10d	MATS-09-RF	RF	#2	69	0.086	58.37	0.878	3.81	1.86	2.05	5.79	4
1	08-10d	MATS-09-RH	RH	#2	74	0.021	69.48	2.621	5.1	2.17	2.35	6.26	4
2	11-13d	MATS-17-RH	RH	#2	129	0.016	105.71	5.201	3.61	1.77	2.04	6.1	4
2	11-13d	MATS-17-LF	LF	#1	92	0.033	44.78	1.458	5.7	2.16	2.64	6.88	4
2	11-13d	MATS-17-LH	LH	#1	138	0.016	74.63	3.977	4.49	2.08	2.16	7.22	4
2	11-13d	MATS-17-LH	LH	#3	123	0.021	67.11	3.760	3.9	1.62	2.41	6.68	4

2	11-13d	MATS-17-LF	LF	#3	96	0.031	91.54	1.699	5.35	2.08	2.57	7.46	4
2	11-13d	MATS-17-LF	LF	#2	70	0.071	45	0.757	5.15	2.13	2.42	7.06	4
2	11-13d	MATS-17-RH	RH	#1	123	0.021	99.52	3.582	3.86	1.92	2.01	5.88	4
2	14-16d	MATS-20-RH	RH	#1	135	0.012	62.97	5.433	4.58	1.6	2.86	6.3	4
2	14-16d	MATS-20-LH	LH	#2	40	0.040	44.92	3.182	2.81	1.17	2.4	6.71	4
2	14-16d	MATS-20-RH	RH	#2	160	0.018	66.76	2.923	5.28	2.19	2.41	5.47	4
2	14-16d	MATS-20-RF	RF	#2	86	0.051	55.55	1.648	3.84	1.39	2.76	5.18	4
2	14-16d	MATS-20-LH	LH	#1	90	0.034	46.81	1.849	4.37	2.06	2.12	6.44	4
2	14-16d	MATS-20-LF	LF	#2	16	0.296	28.45	0.198	4.84	1.92	2.52	6.75	4
2	14-16d	MATS-20-RF	RF	#1	102	0.048	67.89	0.946	6.05	2.1	2.88	7.11	4
2	17-19d	MATS-21-RF	RF	#2	110	0.021	8.37	2.112	6.24	2.31	2.7	6.84	4
2	17-19d	MATS-21-RF	RF	#1	106	0.034	10.86	4.085	2.32	1.38	1.68	6.72	4
2	17-19d	MATS-21-RH	RH	#1	196	0.016	67.45	4.944	3.72	1.88	1.98	6.39	4
2	17-19d	MATS-21-LH	LH	#2	138	0.048	86.72	1.052	5.38	2.25	2.39	5.22	4
2	17-19d	MATS-21-RH	RH	#2	25	0.296	65.78	0.193	4.97	1.92	2.59	5.61	4
2	17-19d	MATS-21-LH	LH	#1	65	0.103	32.07	0.426	6.28	2.15	2.92	6.41	4
2	17-19d	MATS-21-LF	LF	#2	98	0.031	83.95	1.146	7.64	2.44	3.13	6.56	4
2	17-19d	MATS-21-LF	LF	#1	84	0.036	156.91	1.096	6.79	2.58	2.63	7.04	4
2	20-22d	MATS-23-RH	RH	#2	89	0.034	64.74	5.038	2.37	0.97	2.44	6.9	4
2	20-22d	MATS-23-RH	RH	#1	171	0.023	97.04	4.065	3.17	1.64	1.93	7.49	4
3	20-22d	MATS-23-LF	LF	#2	98	0.054	53.37	0.942	5.53	1.99	2.78	7.17	4
3	20-22d	MATS-23-RF	RF	#2	110	0.064	68.6	0.968	4.47	2.05	2.18	7.29	4
3	20-22d	MATS-23-RF	RF	#1	98	0.040	55.03	2.220	3.45	1.56	2.21	6.93	4
2	20-22d	MATS-23-LH	LH	#2	22	0.179	24.91	0.311	4.93	2.18	2.26	5.95	4
2	20-22d	MATS-23-LH	LH	#1	85	0.042	55.78	1.371	4.9	1.96	2.5	6.13	4
3	20-22d	MATS-23-LF	LF	#1	69	0.040	79.27	1.274	5.59	1.89	2.96	5.15	4
3	23-25d	MATS-25-LF	LF	#1	148	0.029	45.47	2.024	5.18	1.6	3.24	5.73	4

2	23-25d	MATS-25-RH	RH	#1	144	0.029	80.69	1.093	8.6	2.27	3.79	6.97	4
2	23-25d	MATS-25-RH	RH	#2	87	0.024	41.73	1.674	6.67	2.35	2.84	7.49	4
3	23-25d	MATS-25-LF	LF	#2	138	0.053	52.91	0.746	6.77	2.48	2.73	8.6	4
3	23-25d	MATS-25-RF	RF	#1	141	0.017	50.49	3.056	5.27	2.01	2.62	8.02	4
3	23-25d	MATS-25-RF	RF	#2	95	0.049	53.99	1.059	5.28	2.2	2.4	7.93	4
3	23-25d	MATS-25-RF	RF	#3	127	0.036	65.55	1.300	6.29	1.7	3.7	5.3	4
3	26-28d	MATS-27-RF	RF	#1	170	0.015	97.96	5.924	3.33	1.79	1.86	4.48	4
3	26-28d	MATS-27-RH	RH	#1	230	0.026	63.67	1.948	5.86	1.77	3.31	5.41	4
3	26-28d	MATS-27-RH	RH	#2	136	0.016	72.79	1.923	8.43	2.57	3.28	7.67	4
3	26-28d	MATS-28-LF	LF	#2	54	0.062	44.14	0.819	5.31	2.29	2.32	7.54	4
3	26-28d	MATS-28-LF	LF	#1	124	0.020	61.1	3.356	4.33	1.71	2.53	7.21	4
3	26-28d	MATS-28-LF	LF	#3	92	0.016	96.44	5.407	3.34	1.72	1.94	7.39	4
3	26-28d	MATS-27-LH	LH	#1	40	0.040	40.44	0.774	8.56	2.54	3.37	6	4
3	29-31d	MATS-01-RH	RH	#3	80	0.042	28.6	1.027	6.26	2.22	2.82	5.84	4
3	29-31d	MATS-01-RH	RH	#2	130	0.096	54.57	0.428	6.66	2.22	3	8.91	4
3	29-31d	MATS-01-RF	RF	#1	270	0.011	109.28	4.147	6.19	2.32	2.67	9.03	4
3	29-31d	MATS-01-RF	RF	#2	227	0.016	94.98	2.596	6.9	2.06	3.35	7.78	4
3	32-34d	MATS-05-RF	RF	#1	134	0.012	119.2	2.882	7.87	2.11	3.73	6.56	4
3	32-34d	MATS-05-RH	RH	#1	158	0.018	78.9	3.051	5	2	2.5	6.5	4
3	35-37d	MATS-03-RH	RH	#1	239	0.022	59.55	3.119	4.5	1.5	3	7	4
3	35-37d	MATS-04-LF	LF	#1	142	0.022	66.89	2.105	6	2	3	6.5	4
3	35-37d	MATS-04-LF	LF	#2	70	0.060	33.27	0.454	9.88	2.44	4.05	6.44	4
3	38-40d	MATS-07-RF	RF	#3	118	0.017	69.34	2.687	6.09	2.09	2.91	6.33	4
3	38-40d	MATS-07-RF	RF	#2	122	0.022	50.88	2.089	5.91	2.14	2.76	6.56	4
3	38-40d	MATS-07-RF	RF	#1	42	0.121	36.29	0.613	3.97	1.74	2.28	6.78	4
3	38-40d	MATS-08-RH	RH	#2	93	0.049	54.35	0.889	6.17	2.33	2.65	6.45	4
3	38-40d	MATS-08-RH	RH	#1	121	0.016	96.81	3.278	5.28	1.94	2.72	6.72	4

Appendix B: Individual immature porcine cortical bone samples at 50 mm/min.

Age Category	Age (Days)	Specimen Name	Side and Bone Type	Test	Force at Fracture (N)	Deflection (mm)	Flexural strength (MPa)	Flexural modulus (GPa)	Cross-Sectional Area (mm)	Depth (mm)	Width (mm)	Length (mm)	Span (mm)
1	00-01d	MATS-29-LH	LH	#1	95	0.046	35.68	3.039	2.25	1.5	1.5	6.4	4
1	00-01d	MATS-29-LH	LH	#2	134	0.021	87.18	1.912	6.87	2.33	2.95	6.77	4
1	00-01d	MATS-29-LF	LF	#2	38	0.103	67.25	0.572	4.83	1.91	2.53	6.88	4
1	00-01d	MATS-29-RH	RH	#1	35	0.149	62.19	0.926	2.25	1.5	1.5	6.3	4
1	01-02d	MATS-15-RF	RF	#1	134	0.029	55.46	2.650	3.92	1.64	2.39	6.18	4
1	01-02d	MATS-15-LH	LH	#1	82	0.043	77.08	0.901	7.2	2.01	3.58	6.89	4
1	01-02d	MATS-15-RH	RH	#1	78	0.029	78.02	2.988	3.37	1.79	1.88	7.09	4
1	03-04d	MATS-13-LF	LF	#1	84	0.037	41.25	1.161	6.68	1.82	3.67	6.79	4
1	03-04d	MATS-13-LH	LH	#2	51	0.046	43.3	1.979	3.28	1.7	1.93	7	4
1	03-04d	MATS-13-RF	RF	#1	92	0.023	97.62	3.011	4.23	1.68	2.52	7.1	4
1	03-04d	MATS-13-LH	LH	#1	62	0.096	66.78	0.907	3.45	1.65	2.09	6.54	4
1	03-04d	MATS-13-RH	RH	#2	40	0.096	70.52	1.448	2.25	1.5	1.5	6.4	4
1	05-07d	MATS-12-LF	LF	#1	46	0.058	33.59	0.843	5.7	2.03	2.81	6.46	4
1	05-07d	MATS-11-RH	RH	#1	66	0.058	34.42	1.048	4.89	1.67	2.93	7.13	4
1	08-10d	MATS-09-RH	RH	#1	61	0.046	46.97	1.750	3.65	1.78	2.05	5.73	4
1	08-10d	MATS-09-RF	RF	#1	65	0.072	47.57	0.920	4.26	1.91	2.23	6.05	4
1	08-10d	MATS-09-LF	LF	#1	73	0.023	67.36	2.860	4.37	1.77	2.47	6.27	4
2	11-13d	MATS-17-LH	LH	#2	21	0.246	24.68	0.422	2.95	1.56	1.89	6.64	4
2	11-13d	MATS-17-RH	RH	#1	114	0.017	83.81	5.143	3.64	1.44	2.53	6.97	4
2	11-13d	MATS-17-LF	LF	#1	44	0.129	57.72	0.528	4.12	1.99	2.07	5.94	4
2	14-16d	MATS-20-LF	LF	#1	81	0.054	26.51	0.579	8.9	2.07	4.3	6.77	4

2	14-16d	MATS-20-RF	RF	#1	105	0.054	72.8	1.189	4.39	1.97	2.23	6.9	4
2	14-16d	MATS-20-RF	RF	#2	100	0.037	98.56	2.202	3.62	1.69	2.14	6.89	4
2	14-16d	MATS-20-RH	RH	#1	84	0.029	83.71	2.970	3.4	1.77	1.92	6.55	4
2	17-19d	MATS-21-RF	RF	#1	256	0.013	117.1	4.997	4.22	2	2.11	6.74	4
2	17-19d	MATS-21-LH	LH	#1	83	0.096	40.76	0.515	5.56	2.19	2.54	5.09	4
2	17-19d	MATS-21-RF	RF	#2	173	0.016	82.14	2.620	7.05	1.86	3.79	6.37	4
2	17-19d	MATS-21-LF	LF	#1	101	0.044	71.87	1.125	5.52	2.3	2.4	6.35	4
2	17-19d	MATS-21-RH	RH	#1	62	0.053	53.78	1.444	3.8	1.81	2.1	7.08	4
2	20-22d	MATS-23-RF	RF	#1	185	0.021	95.24	11.682	1.83	0.9	2.03	7.45	4
2	20-22d	MATS-23-RH	RH	#1	110	0.040	73.42	1.989	3.66	1.77	2.07	6.99	4
2	20-22d	MATS-23-RF	RF	#2	105	0.038	74.59	2.250	3.51	1.59	2.21	7.2	4
2	20-22d	MATS-23-RH	RH	#2	95	0.029	232.86	1.590	6.22	1.88	3.31	6.15	4
2	20-22d	MATS-23-LH	LH	#2	74	0.023	79.55	2.669	4.64	1.82	2.55	6.28	4
2	20-22d	MATS-23-LH	LH	#1	118	0.014	109.73	3.981	5.17	1.73	2.99	5.31	4
2	20-22d	MATS-23-LF	LF	#2	20	0.121	69.95	1.360	2.44	1	2.44	5.35	4
2	23-25d	MATS-25-LF	LF	#1	170	0.017	77.54	2.440	6.85	1.93	3.55	7.3	4
2	23-25d	MATS-25-RH	RH	#2	159	0.046	59.54	1.240	4.91	2.08	2.36	7.78	4
2	23-25d	MATS-25-RF	RF	#1	141	0.034	83.27	4.415	2.48	1.08	2.3	7.68	4
2	23-25d	MATS-25-RF	RF	#2	78	0.046	175.39	0.891	6.59	2.43	2.71	5.2	4
3	26-28d	MATS-27-RH	RH	#2	157	0.013	123.29	3.748	5.69	2.07	2.75	7.54	4
3	26-28d	MATS-28-RH	RH	#1	147	0.021	63.67	3.143	4.64	1.65	2.81	7.6	4
3	26-28d	MATS-27-RH	RH	#1	204	0.013	104.23	5.026	4.28	1.87	2.29	7.46	4
3	26-28d	MATS-28-LF	LF	#2	45	0.056	60.59	1.808	3.23	1.38	2.34	7.19	4
3	26-28d	MATS-28-LF	LF	#1	113	0.022	84.89	1.977	6.2	2.24	2.77	6.11	4
3	26-28d	MATS-28-RH	RH	#2	137	0.020	98.48	3.463	4.14	2.01	2.06	5.86	4

3	29-31d	MATS-01-RH	RH	#1	205	0.018	39.63	1.211	11.84	2.63	4.5	6.91	4
3	29-31d	MATS-02-RF	RF	#1	123	0.021	59.52	2.979	4.69	1.86	2.52	8.45	4
3	29-31d	MATS-01-RH	RH	#2	92	0.042	63.32	1.113	5.87	2.12	2.77	7.02	4
3	29-31d	MATS-02-RF	RF	#2	60	0.036	58.46	2.236	3.79	1.62	2.34	7.2	4
3	32-34d	MATS-05-RF	RF	#2	137	0.046	103.05	1.538	4	2	2	7	4
3	35-37d	MATS-03-RH	RH	#2	143	0.018	64.46	2.375	6.33	2.11	3	6.39	4
3	35-37d	MATS-04-LF	LF	#2	167	0.026	93.07	2.996	3.91	1.65	2.37	6.6	4
3	35-37d	MATS-04-LF	LF	#1	80	0.031	74.55	1.743	5.22	2.07	2.52	6.43	4
3	38-40d	MATS-07-RF	RF	#1	117	0.053	49.15	0.820	6.27	2.28	2.75	6.75	4
3	38-40d	MATS-08-RH	RH	#2	145	0.021	74.19	3.488	4.07	1.77	2.3	6.92	4
3	38-40d	MATS-07-RF	RF	#2	93	0.046	77.44	1.400	4.37	2.05	2.13	7.57	4
3	38-40d	MATS-08-RH	RH	#1	133	0.021	109.17	3.807	3.69	1.82	2.03	7.62	4
3	38-40d	MATS-08-LF	LF	#1	85	0.037	57.26	1.929	4.02	1.82	2.21	10.44	4
3	38-40d	MATS-08-LF	LF	#2	57	0.053	50.86	0.930	5.66	2.08	2.72	9.91	4

Appendix B: Individual immature porcine cortical bone samples at 100 mm/min.

Age Category	Age (Days)	Specimen Name	Side and Bone Type	Test	Force at Fracture (N)	Deflection (mm)	Original Flexural Strength (MPa)	Flexural modulus (GPa)	Cross-Sectional Area (mm)	Depth (mm)	Width (mm)	Length (mm)	Span (mm)
1	00-01d	MATS-29-LH	LH	#1	172	0.029	76.27	5.952	1.95	1.3	1.5	6.3	4
1	00-01d	MATS-29-LH	LH	#2	210.9	0.026	86.67	1.717	6.26	2.16	2.9	6.93	4
1	00-01d	MATS-29-RH	RH	#2	68.67	0.053	100.59	0.809	6.35	2.3	2.76	6.78	4

1	00-01d	MATS-29-LF	LF	#2	59.17	0.076	140.05	1.565	2.56	1.6	1.6	6.3	4
1	01-02d	MATS-15-LH	LH	#1	86.83	0.042	59.39	3.447	2.24	1.4	1.6	6.5	4
1	01-02d	MATS-15-LH	LH	#2	54.58	0.071	38.93	2.037	2.24	1.4	1.6	6.4	4
1	01-02d	MATS-15-RH	RH	#1	42.42	0.083	39.49	0.742	4.62	1.9	2.43	6.3	4
1	01-02d	MATS-15-RH	RH	#2	43.71	0.046	37.58	1.401	4.47	1.88	2.38	6.47	4
1	01-02d	MATS-15-LF	LF	#1	58.67	0.076	112.25	1.032	3.79	1.7	2.23	6.79	4
1	01-02d	MATS-15-LF	LF	#2	24.33	0.079	46.55	0.950	3.86	1.81	2.13	7.74	4
1	03-04d	MATS-13-RF	RF	#1	117	0.037	37.54	2.408	3.24	1.79	1.81	6.96	4
1	03-04d	MATS-13-LH	LH	#2	19.17	0.115	28.09	0.696	3.59	1.86	1.93	7.38	4
1	03-04d	MATS-13-LH	LH	#1	39.83	0.067	35.79	1.632	2.97	1.38	2.15	6.89	4
1	03-04d	MATS-13-LF	LF	#1	85.67	0.037	88.63	0.943	7.6	2.46	3.09	7.04	4
1	03-04d	MATS-13-RH	RH	#2	49.58	0.062	117.35	2.761	1.95	1.3	1.5	6.3	4
1	05-07d	MATS-12-LF	LF	#1	76.25	0.072	23.65	1.790	2.4	1.5	1.6	6.5	4
1	05-07d	MATS-11-RH	RH	#1	124.9	0.046	58.48	0.937	6.64	1.93	3.44	6.57	4
1	05-07d	MATS-11-LF	LF	#2	55	0.067	91.67	0.466	8.75	2.21	3.96	7.42	4
1	08-10d	MATS-09-RH	RH	#2	36.42	0.162	31.09	0.655	2.85	1.6	1.78	5.81	4
1	08-10d	MATS-09-RF	RF	#1	41.75	0.071	40.18	2.282	2.08	1.3	1.6	6.5	4
1	08-10d	MATS-09-LF	LF	#2	50.42	0.086	66.39	0.915	3.78	1.65	2.29	6.24	4
1	08-10d	MATS-09-LH	LH	#1	40.08	0.086	88.93	0.821	4.18	1.68	2.49	6	4
2	11-13d	MATS-17-LH	LH	#1	88.42	0.029	60.11	1.966	5.22	1.69	3.09	7.07	4
2	11-13d	MATS-17-LH	LH	#2	104.2	0.029	70.64	2.004	5.06	1.75	2.89	7.25	4
2	11-13d	MATS-17-RH	RH	#1	133	0.038	66.59	1.253	5.76	2.08	2.77	6.08	4
2	11-13d	MATS-17-RH	RH	#2	104	0.038	56.98	1.386	5.19	2.11	2.46	5.91	4
2	14-16d	MATS-20-LF	LF	#2	45.83	0.076	57.38	1.176	3.55	1.46	2.43	7.16	4
2	14-16d	MATS-20-LF	LF	#1	65.67	0.058	76.07	1.560	3.72	1.29	2.88	6.52	4
2	14-16d	MATS-20-RF	RF	#2	89.92	0.046	86.75	1.559	4.04	1.86	2.17	5.66	4
2	14-16d	MATS-20-RH	RH	#1	48.58	0.096	67.6	0.883	3.54	1.66	2.13	5.57	4
2	14-16d	MATS-20-RF	RF	#1	56.17	0.033	62.15	2.675	3.45	1.57	2.2	6.62	4
2	14-16d	MATS-20-LH	LH	#1	126.5	0.021	101.1	3.895	3.72	1.67	2.23	6.82	4
2	14-16d	MATS-20-LH	LH	#2	77.92	0.067	79.65	1.560	3.08	1.4	2.2	6.76	4
2	17-19d	MATS-21-RF	RF	#1	239.3	0.022	55.35	2.875	4.59	1.75	2.62	6.43	4

2	17-19d	MATS-21-LF	LF	#1	61.5	0.049	45.99	1.456	3.99	1.91	2.09	4.89	4
2	17-19d	MATS-21-LH	LH	#1	65.08	0.049	51.21	0.546	9.86	2.63	3.75	6.36	4
2	20-22d	MATS-23-RF	RF	#1	255.4	0.013	139.64	4.659	4.57	1.93	2.37	7.24	4
2	20-22d	MATS-23-RF	RF	#2	135.8	0.021	91.04	2.700	4.93	2.21	2.23	6	4
2	20-22d	MATS-23-LH	LH	#1	70.25	0.049	49.6	1.288	4.54	1.87	2.43	7	4
2	20-22d	MATS-23-LH	LH	#2	52.58	0.062	57.17	1.365	3.65	1.51	2.42	6.86	4
2	20-22d	MATS-23-RH	RH	#2	79	0.036	69.15	1.261	6.69	1.64	4.08	5.67	4
2	20-22d	MATS-23-LF	LF	#1	97.83	0.053	66.49	1.024	5.63	1.59	3.54	6.22	4
2	20-22d	MATS-23-RH	RH	#1	62.83	0.076	59.36	1.040	3.78	1.68	2.25	5.18	4
2	20-22d	MATS-23-LF	LF	#2	74.92	0.056	41.27	1.315	4.06	1.69	2.4	5.5	4
2	20-22d	MATS-23-RH	RH	#3	55.42	0.041	100.36	3.169	2.69	1.23	2.19	5.56	4
2	23-25d	MATS-25-RF	RF	#2	103.1	0.040	63.87	1.088	6.17	2.39	2.58	7.12	4
2	23-25d	MATS-25-RF	RF	#1	116.1	0.033	103.36	2.184	4.19	1.61	2.6	7.29	4
2	23-25d	MATS-25-LF	LF	#1	188.8	0.031	76.87	1.849	5.02	1.93	2.6	8.04	4
2	23-25d	MATS-25-RH	RH	#2	91	0.038	56.64	1.399	5.08	2.21	2.3	5.4	4
2	23-25d	MATS-25-RH	RH	#1	78.16	0.064	41.75	0.904	4.82	2	2.41	4.88	4
3	26-28d	MATS-27-RF	RF	#1	149.4	0.029	52.14	1.626	5.77	2.29	2.52	4.4	4
3	26-28d	MATS-28-LF	LF	#2	193.9	0.016	104.64	2.364	7.35	2.34	3.14	5.81	4
3	26-28d	MATS-27-LH	LH	#1	147.4	0.023	66.92	2.256	5.25	2.11	2.49	7.67	4
3	26-28d	MATS-27-RH	RH	#1	170.9	0.014	92.5	3.494	5.43	2.3	2.36	7.64	4
3	26-28d	MATS-27-RH	RH	#2	101.6	0.046	48.83	1.383	4.5	1.93	2.33	7.23	4
3	26-28d	MATS-28-LF	LF	#1	95	0.026	65.68	2.018	5.4	2.06	2.62	7.02	4
3	29-31d	MATS-01-RH	RH	#1	149.7	0.016	36.74	3.275	5.73	1.77	3.24	8.91	4
3	29-31d	MATS-01-RH	RH	#3	146.2	0.026	80.41	1.041	9.7	2.52	3.85	6.81	4
3	29-31d	MATS-01-RF	RF	#1	101.5	0.017	60	1.943	8.05	2.54	3.17	7.05	4
3	29-31d	MATS-01-RH	RH	#2	208.8	0.026	61.26	1.824	6.03	1.81	3.33	7.22	4
3	29-31d	MATS-02-RF	RF	#2	117.7	0.016	87.02	4.336	4.16	2.01	2.07	6.84	4
3	29-31d	MATS-02-RF	RF	#1	68.42	0.053	49.09	1.162	4.86	1.67	2.91	6.91	4
3	32-34d	MATS-05-RH	RH	#1	137.6	0.026	122.31	2.632	4.5	1.5	3	6.5	4
3	35-37d	MATS-03-RH	RH	#1	356.8	0.016	79	1.425	12.37	2.19	5.65	6.8	4
3	35-37d	MATS-03-RH	RH	#2	162.6	0.018	61.46	2.088	7.28	2.18	3.34	6.38	4

3	35-37d	MATS-04-LF	LF	#2	80	0.041	44.53	1.375	4.89	2.08	2.35	6.87	4
3	35-37d	MATS-04-LF	LF	#1	91.67	0.030	54.1	1.769	5.28	2.04	2.59	6.75	4
3	38-40d	MATS-08-RH	RH	#2	93.4	0.051	51.63	1.492	3.8	1.8	2.11	6.42	4
3	38-40d	MATS-07-RF	RF	#2	76.42	0.059	48.38	1.018	4.67	2.03	2.3	6.65	4
3	38-40d	MATS-08-LF	LF	#1	55.08	0.056	57.15	1.530	3.53	1.64	2.15	7.49	4
3	38-40d	MATS-07-RF	RF	#1	85.58	0.040	75.11	2.055	3.59	1.71	2.1	7.33	4
3	38-40d	MATS-08-LF	LF	#2	73.58	0.056	71.9	2.326	2.53	1.36	1.86	9.75	4
3	38-40d	MATS-08-RH	RH	#1	49	0.058	85.46	0.935	5.07	2.14	2.37	9.48	4

Appendix C

Material impact test sample specifications

Legend

RF - Right femur
LF - Left femur
RH - Right humerus
LH - Left humerus

Appendix C: Material impact sample dimensions.

Age Category	Age (Days)	Specimen Name	Side and Bone Type	Test	Energy Absorbed (J)	Energy Absorbed across Cross-Sectional Area (J/m^2)	Cross-Sectional Area (m^2)	Depth (mm)	Width (mm)	Length (mm)	Span (mm)
1	00-01d	IMP-30-LF	LF	#1	0.33	33500	0.01	2.5	4	11	6
1	01-02d	IMP-16-LF	LF	#1	0.07	14285.71	0.005	1.5	3.5	8	3
1	01-02d	IMP-16-LF	LF	#2	0.07	15000	0.005	2	2.5	8.5	3.5
1	01-02d	IMP-16-LF	LF	#3	0.13	17333.33	0.0075	2.5	3	7.5	2.5
1	01-02d	IMP-16-LF	LF	#4	0.13	14444.44	0.009	3	3	7	2
1	01-02d	IMP-16-RF	RF	#1	0.06	13333.33	0.0045	1.5	3	13	8
1	01-02d	IMP-16-RF	RF	#2	0.1	10000	0.01	2.5	4	7.5	2.5
1	01-02d	IMP-16-RF	RF	#3	0.06	7500	0.008	2	4	8	3

1	03-04d	IMP-14-LF	LF	#1	0.12	33333.33	0.003	1.5	2.5	8	3
1	03-04d	IMP-14-LF	LF	#2	0.26	57777.78	0.004	1.5	3	6.5	1.5
1	03-04d	IMP-14-LF	LF	#3	0.11	24444.44	0.004	1	1.5	7	2
1	03-04d	IMP-14-LF	LF	#4	0.25	31250	0.008	2	4	7	2
1	03-04d	IMP-14-RH	RH	#1	0.1	33333.33	0.003	1.5	2	14	9
1	03-04d	IMP-14-RH	RH	#2	0.29	48333.33	0.006	1.5	4	13	8
1	03-04d	IMP-14-RH	RH	#3	0.05	6666.67	0.007	2.5	3	14	9
1	05-07d	IMP-12-RH	RH	#1	0.19	19500	0.01	2.5	4	8	3
1	05-07d	IMP-12-LH	LH	#1	0.09	11250	0.008	2	4	8	3
1	05-07d	IMP-12-LH	LH	#2	0.3	18181.82	0.016	3	5.5	9	4
1	08-10d	IMP-10-LF	LF	#1	0.16	35555.56	0.0045	1.5	3	10.5	5.5
1	08-10d	IMP-10-LF	LF	#2	0.07	7000	0.01	2.5	4	10.5	5.5
1	08-10d	IMP-10-LF	LF	#3	0.28	40000	0.007	2	3.5	10.5	5.5
1	08-10d	IMP-9-RF	RF	#1	0.05	6250	0.008	2	4	8	3
1	08-10d	IMP-9-RF	RF	#2	0.12	16666.67	0.007	2.5	3	9.5	4.5
1	08-10d	IMP-9-RF	RF	#3	0.15	51666.67	0.003	1	3	10	5
1	08-10d	IMP-9-RF	RF	#4	0.08	13333.33	0.006	2	3	9	4
1	08-10d	IMP-9-RF	RF	#5	0.08	17000	0.005	2	2.5	10	5
2	11-13d	IMP-18-RH	RH	#1	0.29	32777.78	0.009	2	4.5	13	8
2	11-13d	IMP-18-RH	RH	#2	0.20	16400	0.012	2.5	5	12	7
2	11-13d	IMP-18-RH	RH	#3	0.10	13125	0.008	2	4	12	7
2	11-13d	IMP-18-RH	RH	#4	0.07	8750	0.008	2	4	12	7
2	14-16d	IMP-19-LF	LF	#1	0.25	50000	0.005	2	2.5	8	3
2	17-19d	IMP-22-RF	RF	#1	0.15	37500	0.004	2	2	6.5	1.5
2	17-19d	IMP-22-RH	RH	#1	0.15	20000	0.007	2.5	3	7	2
2	17-19d	IMP-22-RH	RH	#2	0.3	75000	0.004	2	2	7	2
2	17-19d	IMP-22-RH	RH	#3	0.15	25833.33	0.006	3	2	7	2

2	17-19d	IMP-22-RH	RH	#4	0.42	70000	0.006	2	3	9	4
2	17-19d	IMP-22-RH	RH	#5	0.2	40000	0.005	2	2.5	8	3
2	20-22d	IMP-24-LH	LH	#1	0.39	48750	0.008	2	4	7.5	2.5
2	20-22d	IMP-24-LH	LH	#1	0.39	39000	0.01	2.5	4	8.5	3.5
2	20-22d	IMP-24-LH	LH	#2	0.32	26000	0.012	2.5	5	8	3
2	20-22d	IMP-24-LH	LH	#3	0.32	32500	0.01	2.5	4	9	4
2	23-25d	IMP-26-LF	LF	#1	0.45	50000	0.009	3	3	10	5
2	23-25d	IMP-26-LF	LF	#2	0.18	20000	0.009	2	4.5	9.5	4.5
2	23-25d	IMP-26-LF	LF	#3	0.40	50625	0.008	2	4	10	5
2	23-25d	IMP-26-LF	LF	#4	0.40	101250	0.004	2	2	9	4
2	23-25d	IMP-26-LF	LF	#5	0.40	67500	0.006	2	3	9	4
2	23-25d	IMP-26-LF	LF	#6	0.25	42500	0.006	2	3	9.5	4.5
3	29-31d	IMP-2-LF	LF	#1	0.11	13750	0.008	2	4	10	5
3	29-31d	IMP-2-LF	LF	#2	0.12	20000	0.006	1.5	4	10	5
3	29-31d	IMP-2-LF	LF	#3	0.23	32857.14	0.007	2	3.5	10	5
3	29-31d	IMP-2-LF	LF	#4	0.10	8400	0.012	2.5	5	9.5	4.5
3	29-31d	IMP-2-LF	LF	#5	0.3	30000	0.01	2.5	4	10	5
3	35-37d	IMP-3-RF	RF	#1	0.07	8750	0.008	4	2	14	9
3	35-37d	IMP-3-RF	RF	#2	0.18	15000	0.012	4	3	9	4
3	35-37d	IMP-3-RF	RF	#3	0.08	9444.44	0.009	2	4.5	14	9
3	38-40d	IMP-8-RF	RF	#1	0.2	29629.63	0.006	1.5	4.5	8.5	3.5
3	38-40d	IMP-8-RF	RF	#2	0.1	12500	0.008	2	4	9	4
3	38-40d	IMP-8-RF	RF	#3	0.12	24000	0.005	1	5	7.5	2.5
3	38-40d	IMP-8-RF	RF	#4	0.16	20000	0.008	2	4	8.5	3.5
3	38-40d	IMP-8-RF	RF	#5	0.18	20000	0.009	2	4.5	10	5

Appendix D

Whole bone test specifications

Legend

RF - Right femur
LF - Left femur
RH - Right humerus
LH - Left humerus

Appendix D: Whole bone measurements – three-point bending

Specimen Name	Side and Bone Type	Age (Days)	Outer Diameter (mm)	Inner Diameter (mm)	Shaft Length (cm)	Span (cm)
3PB-30-RH	RH	00-01d	7.5	4.5	5.7	1.7
3PB-16-LF	LF	01-02d	7	3	6.07	2.07
3PB-14-LF	LF	03-04d	6.5	4	5.47	1.47
3PB-14-LH	LH	03-04d	6.5	3.5	5.49	1.49
3PB-12-LH	LH	05-07d	8.5	5	6.34	2.34
3PB-10-LF	LF	08-10d	7	4.5	6.66	2.66
3PB-18-RH	RH	11-13d	8.5	5	6.68	2.68
3PB-19-LF	LF	14-16d	8	4.5	6.67	2.67
3PB-22-RF	RF	17-19d	10	5	7.7	3.7

3PB-24-LH	LH	20-22d	12	6	9.2	5.2
3PB-26-LF	LF	23-25d	13	6	8.34	4.34
3PB-06-RH	RH	32-34d	12.5	9	9.04	5.04
3PB-03-RF	RF	35-37d	14	8	9.15	5.15
3PB-08-RF	RF	38-40d	12.5	7	9.7	5.7

Appendix D: Whole bone measurements - compression

Side and Bone Type	Side and Bone Type	Age (Days)	Inner Diameter (mm)	Outer Diameter (mm)	Shaft Length (cm)	Span (cm)
COMP-30-LF	LF	00-01d	5.33	7.09	5.56	1.56
COMP-16-RF	RF	01-02d	6.57	8.51	6.67	2.67
COMP-14-RH	RH	03-04d	5.97	8.41	5.4	1.4
COMP-12-RF	RF	05-07d	6.19	8.34	6.37	2.37
COMP-10-RH	RH	08-10d	6.07	7.95	6.89	2.89
COMP-18-LF	LF	11-13d	6.33	8.25	7.04	3.04
COMP-19-LH	LH	14-16d	7.42	9.52	6.6	2.6
COMP-22-RH	RH	17-19d	7.13	10.98	7.54	3.54
COMP-24-LF	LF	20-22d	8	10	8.6	4.6
COMP-26-LH	LH	23-25d	8.53	11.38	8.9	4.9
COMP-28-RF	RF	26-28d	8.71	13.11	8.3	4.3
COMP-01-LH	LH	29-31d	8.82	12.85	9.5	5.5
COMP-06-LH	LH	32-34d	7.41	9.99	8.56	4.56

Appendix D: Whole bone measurements - torsion

Specimen Name	Side and Bone Type	Age (Days)	Outer Diameter (mm)	Inner Diameter (mm)	Shaft Length (cm)	Span (cm)

TOR-16-LH	LH	01-02d	7	3	6.13	2.13
TOR-16-RH	RH	01-02d	7	3.5	6.21	2.21
TOR-19-RH	RH	14-16d	8	5	6.64	2.64
TOR-25-LF	LF	23-25d	12.5	6	12.5	8.5
TOR-27-LF	LF	26-28d	14	6.5	14	10