The quantification of mechanical loading and its association with bone adaptation.

Reece Scott

Thesis submitted in fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

September 2024

Copyright Statement

Acknowledgements

I would like to thank Dr Ian Varley for allowing me to fulfil this work alongside him and the excellent guidance, support and advice throughout the PhD. I would also like to thank the rest of my supervisory team in Professor Craig Sale, Dr Cleveland Barnett and Dr Ruth James for their support and guidance. Dr Livia Santos and Dr Janelle Tarum for their help and guidance during my *in vitro* work. I thank many more staff and fellow PhD candidates within the Sports Science Department for that I do not just call colleagues but also call friends during my time at NTU. I would like to highlight my appreciation for MRes students that have worked alongside me during the intervention study, making recruitment for the study a lot smoother: Peter Voase, Jordyn Jones, Tom Sharp, Jack Hopewell, Elisa Bronzini and Scott Winton.

I'm appreciative to my participants that took part in my studies, without their time and coherence this thesis would not be possible.

I would also like to thank my family and friends for their support whilst conducting my PhD, without their incredible understanding and support this process would have been even more difficult. I will forever be grateful to everybody who has been a part of this process.

Abstract

Weight-bearing exercise, and resultant high-impact loading, is known to cause positive bone adaptations. The optimal magnitude, frequency and duration of load required to produce an osteogenic response, however, remain unclear. Furthermore, there is a lack of applied research investigating if measurements of external load can be associated to bone adaptation. This thesis investigated: how bone responds to load; practitioner perceptions and use of mechanical loading as a method of estimating bone load; the association between mechanical load and bone characteristics in professional athletes, and the dose-response effect of drop jump height on bone adaptation in low active, young adults.

An *in vitro* model was developed to analyse the effects of intermittent and continuous load on osteoblast activity over 12 days. Bone formation markers alizarin red (ARS), alkaline phosphatase (ALP) and Procollagen I N-Terminal Propeptide (PINP) were assessed. Secondly, support staff (n=71) were questioned to understand how bone is monitored in sport and what methodologies are used. The perceptions were compared to research to investigate if there are similarities between the academic and applied knowledge. Experimentally, external load training characteristics and bone characteristics were assessed in academy footballers (n=15) over 14 weeks. Whole body measurements were determined using dual-energy x-ray absorptiometry (DXA) and global positioning systems (GPS) were used to measure external load. Additionally, external load and bone characteristics of fast bowlers (n=14) and footballers (n=11) were assessed to observe the contralateral effects of mechanical loading. Whole body DXA scans and Peripheral Quantitative Computed Tomography (pQCT) tibial bone characteristics were analysed. External load was measured using inertial measurement units (IMUs). Finally, young people performed 16 weeks of drop jumps from 60cm (n=11), 40cm (n=11), 0cm (n=11) or no jumps (n=11). Bone adaptations (DXA, pQCT) and external load (IMU, force plates, motion capture) were assessed at multiple time points (pre-intervention, week 6, week 12, post-intervention).

An increase in ALP was observed when rest periods were inserted between loading bouts, however, no changes were observed in PINP and ARS between loading conditions *in vitro*. Mechanical load can be used to promote bone adaptation, however, support staff shared they do not monitor external load as a method of estimating bone load due to a

lack of knowledge, indicating there is a disconnect between research and the applied environment. In academy footballers, GPS-derived high metabolic load distance (HMLD), accelerations and decelerations were associated with increases in leg BMC (P=0.008). When measuring site-specific load using IMUs, cumulative load was associated with greater axial and polar tibial strength in professional fast bowlers (P=0.035). Low active, young adults performing 60cm diagonal drop jumps showed an increase in tibial axial strength compared to jumping from 0cm over 16 weeks. Diagonal drop jumps from 40cm, however, produced a greater increase in cortical density than jumping from 60cm. The findings suggest monitoring external load during interventions targeting bone accrual may advance our understanding of mechanical load experienced by individuals. The relationship between external load and bone adaptation may then be used to identify loading thresholds and optimise impact exercise for bone health.

List of Abbreviations

ALP – Alkaline phosphatase

ARS – Alizarin Red Staining

BMC – Bone Mineral Content

BMD – Bone Mineral Density

BSI – Bone stress injury

CMJ – Countermovement jump

CSA – Cross Sectional Area

CTX – C-terminal Telopeptide of type 1 collagen

CV – Coefficients of variation

DM – Differentiation Medium

DXA – Dual-energy x-ray absorptiometry

g – Gravitational unit (1g = 9.81 m/s)

GM – Growth Medium

GPS – Global Positioning System

GRF – Ground Reaction Force

HMLD - High Metabolic Load Distance

I - Impulse

ICC - Interclass correlation coefficient

IMU – Inertial Measurement Unit

IMTP – Isometric mid-thigh pull

IP – Impact peak

LR – Loading Rate

NTX - N-terminal Telopeptide

OC – Osteocalcin

PINP – Procollagen 1 N-Terminal Propeptide

PA – Peak acceleration

PPA – Peak Positive Acceleration

pQCT - Peripheral Quantitative Computed Tomography

RFD – Rate of Force Development

Manuscripts

Scott, R., James, R., Barnett, C.T., Sale, C. and Varley, I., 2023. Perspectives from research and practice: A survey on external load monitoring and bone in sport. *Frontiers in Sports and Active Living*, *5*, p.1150052.

Scott, R., Varley, I., Sale, C., Tarum, J., James, R., Barnett, C. and Santos, L., 2024. Intermittent tensile strain induces an increased response in bone formation markers compared to continuous load in mouse pre-osteoblasts when loading magnitude is matched. *Journal of the Mechanical Behavior of Biomedical Materials*, p.106683.

Conference communications

Scott R. Quantifying load and its association to changes in bone characteristics. Nottingham Trent University, School of Science and Technology STAR conference. 2022

Scott, R., Santos, L., James, R., Barnett, C.T., Sale, C. and Varley, I. Intermittent loading induces an increased bone formation marker response in mouse osteoblasts. Best oral communication prize. Endocrinology society. 2022.

Scott, R., Santos, L., James, R., Barnett, C.T., Sale, C. and Varley, I. Intermittent loading induces an increased bone formation marker response in mouse osteoblasts. Bone Research Society. 2022

Scott, R., James, R., Barnett, C.T., Sale, C. and Varley, I. The association between measurements of external load and bone structural characteristics. European College of Sport Science. 2022

Contents Page

List of Tables

List of Figures

Impact of the Covid-19 pandemic on the PhD thesis

The COVID-19 pandemic had an impact on this programme of work. Access to participants and facilities was shut off for a large proportion of 2020, during which I was not able to conduct human studies. Further challenges were presented as this reset ethical applications that were being processed before the lockdown and restricted the research planned using elite athletes. Specifically, the pandemic considerably influenced the research process for chapter 7, subsequently limiting the possibility of performing repeat measures within the sample and necessitating a cross-sectional design. Prior to the COVID-19 pandemic, ethical approval had been granted for chapter 7, and research was organised to begin within professional sports clubs. As the pandemic developed and restrictions were put into place, this restricted access to the training sessions. As professional sports restarted, the university required a resubmission of the ethical application that provided additional safeguarding practices surrounding COVID-19. Once ethical approval was accepted and clubs were happy to comply with the regulations, I was able to gain access to training sessions albeit with restricted access to the minimum required to collect data. Although this did not influence the study's ability to monitor load in an applied setting using IMUs, it did prevent the possibility of performing repeat measures. Therefore, I was not able to assess the inter and intra-unit variability which would have provided a useful insight into the IMU metrics alongside chapter 7's results.

To combat the restrictions and delays to human studies, I conducted an *in vitro* experiment looking into osteoblast activity in response to intermittent loading (Chapter 4), alongside my other studies. There was a strong rationale to complete this work, owing to previous research on load application and bone formation, and it was feasible to access the required facilities to perform the experiments without violating any COVID-related restrictions. This opportunity allowed me to develop an additional skill set, including cell culture and performing *in vitro* research using a Flexcell system.

1 General introduction

Bone is a dynamic tissue that continually adapts to its surroundings, responding to physical stresses and chemical reactions induced by mechanical loading. It can be categorised into cortical and trabecular bone morphologies, with each being influenced by mechanical stimuli. The integrity of bone structure is sustained through bone remodelling, which involves the orchestrated actions of osteoblasts (bone formation) and osteoclasts (bone resorption). Maintaining a delicate balance between formation and resorption is important for optimal bone health. Disturbances in this equilibrium can lead to bone weakness and the subsequent occurrence of osteoporosis or stress fracture injury (Sozen et al., 2017).

Osteoporosis is a condition of bone deterioration which affects ~3.2 million people (5.2%) in the UK, with females over 50 years of age having an increased risk to the disease compared to age-matched males (females 21.9%, males 6.7%) (Hernlund et al., 2013). Globally, the prevalence of osteoporosis is 18.3% (Salari et al., 2021) and the cost within Europe is reported to amount to ~ €37 billion (Clynes et al., 2020). The risk factors of osteoporosis can be categorised as modifiable and nonmodifiable (Heinemann, 2000). Nonmodifiable risk factors include genetics, ageing and sex, whereas modifiable risk factors include smoking, body mass, physical activity status and diet (Lin & Lane, 2004). The age when we perform exercise influences the bone response to mechanical loading; bone accretion occurs throughout childhood and adolescence with ~90% of bone mineral density (BMD) being acquired by 20 years old (Henry et al., 2004). Engaging in regular impact exercise during childhood and adolescence correlates with enhanced bone mass, BMD, and bone geometry in later years (Chen et al., 2023), attributable to the heightened mechanical loading placed on bone. This premise was observed over 12 years, between the ages of 17 and 29 years old, where current and former athletes displayed higher femoral neck, lumbar spine and whole-body BMD compared to non-athletic individuals (Tervo et al., 2008). This heightened bone response in former athletes is shown to lower the risk of sustaining fragility fractures by up to 50% compared to age-matched non-athletic subjects (Tveit et al., 2013), exemplifying the importance of accruing bone mass in the early years for later life. Between the age of late 30s and early 40s, BMD begins to decline as bone resorption becomes greater than bone formation, caused by oxidative stress creating greater osteoclast-mediated activity (Santos et al., 2017). Age-related bone reduction is also susceptible to variations in mechanosensitivity as aged bone develops a reduced attenuation of strain signal due to increased stiffness (Javaheri & Pitsillides,

2019). The likelihood of suffering a fracture is also increased in osteoporotic bone with one in two females, and one in five males over the age of 50 hypothesised to suffer an osteoporotic fracture in the UK (Clynes et al., 2020). Stress fracture injuries, however, can also occur as a result of bone weakness. Stress fractures occur when the bone is repetitively stressed beyond its capacity to bear load (Moreira & Bilezikian, 2017). These are not only present in those with reduced skeletal mass and quality (Moreira & Bilezikian, 2017), as they can also occur in those with high skeletal mass, such as competitive athletes (Alway et al., 2019). Since repetitive, high load exercise can result in stress fractures it would be beneficial to quantify exercise and assess if an optimised loading regime for good bone health exists.

Mechanical load, using impact exercise, is a well-established method of improving bone mass. This was proposed by Wolff (1982) and updated by Frost (1987), theorising that bone adapts proportionally in response to the mechanical stimuli it experiences. Exercise characteristics (magnitude, frequency and duration) for optimal bone accrual, however, are not clear. Non-weight bearing exercise, such as swimming and cycling, are shown to create a minimal osteogenic response (Morel et al., 2001). The load during these activities creates low bone tension, meaning there is a lack of stimulation to incite an osteogenic reaction (Gómez-Bruton et al., 2015). Impact-based exercise, however, is shown to increase BMD and bone mineral content (BMC) (Simoes et al., 2021 - team sports; Yu et al., 2019 - dance; Allison et al., 2015 - hopping; Vainionpää et al., 2005 - jumping). Increases in bone geometry, such as bone thickness (Allison et al., 2013 - hopping), cross-sectional moment of inertia and section modulus (Ferry et al., 2013 - football), and total area and cortical area (Ducher et al., 2011 - tennis) are also evidenced following impact exercise. This is due to high loads stimulating changes in bone macro- and microstructure. Rest periods between loading bouts have also been found to influence the osteogenic response (Burr et al., 2002; Srinivasan et al., 2002; Robling et al., 2001). Introducing loading-unloading cycles during activity disrupts the negative effects of monotonous load that bone cells become accustomed to, thereby promoting osteoblast activity and subsequent bone formation (Klein-Nuland et al., 2015). Animal models have observed increased relative bone formation rate (Burr et al., 2002), increased periosteal bone formation (Srinivasan et al., 2002), and increased areal BMD and content (Robling et al., 2001) in response to exercise protocols incorporating rest periods compared to continuous loading. The duration of rest cycles required to cause optimal bone accrual, however,

remains unclear due to variations in the cumulative load experienced (LaMothe & Zernicke, 2004). Therefore, habitual impact exercise promotes bone accretion, yet the specific exercise characteristics required to optimise bone adaptation remains somewhat ambiguous.

A recent consensus statement recommended moderate to high-impact exercise as the most effective way to accumulate bone strength (Brooke-Wavell et al., 2022). Jumping interventions produce impact forces and are often used to demonstrate the benefits of impact exercise on bone mass as load magnitude, duration and frequency can be manipulated. For example, Kato et al. (2006) found a six month jumping protocol consisting of ten maximal vertical jumps on three days/wk increased lumbar spine and femoral neck BMD in young women. Similarly, Weeks et al. (2008) showed that ten minutes of jumping performed twice a week, over eight months induced an increase in femoral neck, trochanter and whole-body BMC, and lumbar spine strength in adolescent boys and girls compared to controls. Furthermore, jump height (Wang & Dueball, 2018 - if performing drop jumps) and increased mass (Vlachopoulos et al., 2018 - adding additional mass) can influence load magnitude. This is shown by Vlachopoulos et al. (2018) where jump magnitude was adjusted every twelve weeks, over nine months, from bodyweight countermovement jumps (CMJ) to 2 kg and 5 kg weighted vest CMJs. The findings showed that leg BMC and bone stiffness increased in participants who performed the jumps compared to a non-jumping control group. A limitation of many studies investigating high-impact exercise, and the subsequent bone response, is the absence of quantitative data to assess load magnitude (Varley et al., 2017; Rogers & Hinton, 2010; Turner & Robling, 2003). Studies instead report data such as the number of jumps (Vlachopoulos et al., 2018) or jumping duration (Weeks et al., 2008), however, these metrics do not quantify mechanical load. Therefore, loading characteristics, such as magnitude, frequency and duration, should be analysed and associated with bone adaptation to help understand the effects of mechanical load on bone accrual.

Studies have attempted to quantify bone load through various means, such as training duration, volume, and mode of exercise. Varley et al. (2017) used training volume as a predictor of external load and found that increasing training volume over twelve weeks improved BMD and tibial cortical area in academy football players. However, training time is an imprecise measure of external load, therefore this study was unable to allude

to the specific type and magnitude of mechanical load distribution that contributed to the response in bone. The lack of quantified load limits the ability of others to replicate the loading characteristics conducted and offers little insight into the mechanisms of bone adaptation. Turner & Robling (2003) proposed the osteogenic index as measurement of bone load using load magnitude, duration and frequency to determine the osteogenic response. By separating exercise sessions into multiple bouts (*e.g.,* 3 x 40 jumps), as opposed to one extended session (*e.g.,* 1 x 120 jumps), the osteogenic potential of exercise increases when using the osteogenic index. This in line with the literature that shows rest periods can enhance mechanosensitivity (Burr et al., 2002; Robling et al., 2001). However, this measure does not directly assess loading cycles during activity, which is a factor known to influence bone response to loading (Burr et al., 2002). Similarly, Rogers & Hinton (2010) categorised bone load using activity recall, based on proxy ground reaction force (GRF) data from other studies. This method lacks validation and practicality and relies on the assumption that participants can accurately recall physical activity, which is subject to recall bias that is known to be inaccurate (Althubaiti, 2016). Therefore, an accurate measurement of external load associated to bone adaptation needs to be established to advance the monitoring of bone load in applied environments.

Establishing an association between quantitative external load and bone accrual is vital for our understanding of exercise and bone interactions. Athletes represent a unique population to study in this regard due to performing habitual and repetitive movements. Studies have assessed habitual activity in the upper extremities of professional athletes and in doing so offer a unique insight into the bone-load interaction, as external forces are limited on the upper extremity in daily life (Krahl et al., 1994; Warden et al., 2009; Bogenschutz et al., 2011). Professional tennis players were shown to have longer ulnas and second metacarpal in the racket arm compared to the non-racket arm (Krahl et al., 1994), whilst baseball and softball pitchers showed greater bone mass, cortical area, cortical thickness and BMC in the humerus of the throwing arm compared to the non-throwing arm (Warden et al., 2009; Bogenschutz et al., 2011; Warden et al., 2019). Although these studies are of interest, they lack quantified load and therefore it cannot be concluded how much load is required to promote bone accrual. Field-based, measurements of external load may offer an applied approach to assessing external load and understanding its association with bone adaptation.

Various measurement tools, such as accelerometry, GPS, inertial measurement units (IMU), force plates, and motion capture have been used to try and quantify the mechanical load exerted on bone. The disparity between methods, however, hinder the efforts to understand the association between load and bone. Higher GRF have been associated with greater BMD accrual in older women (Kohrt et al., 1997), middle-aged men (Rogers & Hinton, 2010), and gymnasts (Wu et al., 1998), supporting the premise that high-impact exercise is an osteogenic stimulus. Although associations are shown between GRF-derived load and bone adaptation, there is evidence arguing GRF's cannot be used as predictors of bone load. Matijevich et al. (2019) argue tibial load cannot be associated to GRF as force data does not consider muscular contraction. The functional muscle-bone unit is an important mechanism, whereby increases in bone strength are preceded by increases in muscle mass (Montgomery et al., 2019; Tagliaferri et al., 2015). Therefore, including strength measurements within exercise characteristics may provide a more complete image of bone adaptation to mechanical loading. Motion capture can be used to create musculoskeletal models to examine the effects of gravity-derived and muscle-derived loads. It has been observed that motion capture metrics, such as joint moments and power, are associated with BMD (Choi et al., 2021; El Deeb & Khodair, 2014) and increase the predictability of BMD and BMC during gait analysis in healthy adults (Moisio et al., 2004). Kinematic data provides estimated internal loads such as moments, stiffness, and torque that kinetic measures do not produce during dynamic tests. This could be useful in understanding site-specific models of load (*e.g.,* tibial load), but due to the time-consuming nature of collecting and processing the data, it is limited to laboratory settings, limiting their practicality in applied exercise scenarios.

It could be suggested the most viable route to practically associate external load and bone is through wearable technology that is less restricted in its application. Higher accelerations, derived from hip-mounted accelerometers, are shown to be positive predictors of bone development during adolescence (Gabel et al., 2017; Tobias et al., 2007) and young adulthood (Janz et al., 2014). Increased participation in moderate-to-vigorous physical activity also results in positive changes in tibial characteristics in accelerometer studies (Gabel et al., 2017; Janz et al., 2014). However, categorising accelerometer-derived load into predetermined thresholds hinders the load specificity and limits the insight into the load effect on bone as the mechanical load is generalised. In addition to using hip-mount accelerometers, external load metrics such as accelerations,

decelerations, and total distance have been measured by GPS and subsequently associated with tibial strength and BMC in professional footballers (Varley et al., 2023). As hip-mounted accelerometers and GPS are attached away from the tibia it is questioned if they are capable of measuring distal load, or informing on tibial adaptation, as it is known sensor placement is integral to load estimation. Due to factors such as absorption of load in the spine and load dispersion through the kinetic chain, it is difficult to assume that the load experienced at the tibia uniformly translates to accelerations at the hip or upper back. To understand site-specific bone adaptation, measurements should be taken at anatomical sites relevant to the area of interest. Therefore, the most practical method of measuring site-specific bone load may be by using IMUs at specific anatomical locations. IMUs are a reliable measurement of site-specific load in an applied environment (Armitage et al., 2021; Doyle et al., 2024) and Besier. (2019) has purported the method can estimate bone load. However, there is currently no data that confers a relationship between IMUs and bone characteristics. As there is no consensus on which external load method is optimal to be a proxy measure of bone load, applied practitioners may be misinformed about monitoring external load as an estimate of bone load.

To summarise, bone is responsive to mechanical loading and positive adaptation is often cited from habitual impact-based exercise. Load magnitude, frequency and duration are all determinants of bone adaptation, as are the rest periods between loading bouts. There are a variety of methods available to monitor external load during activity, but there is limited knowledge on the practicality of these methods and the extent to which they are being used to estimate bone load in an applied environment. Knowledge of how external load relates to bone accrual is important as it would contribute to understanding how we can best prescribe exercise to promote good bone health. This would not only be beneficial to clinical exercise as a preventative measure to combat bone conditions, such as osteoporosis, but also for athletes who can experience bone stress injuries from training load errors (Kelley et al., 2014).

This thesis aims to assess mechanical loading and its association with bone adaptation by (Figure 1.1):

1) Measuring how bone formation responds to intermittent load, through the use of rest periods, in comparison to continuous load.

2) Understanding what measures are currently used to estimate bone load by support staff in an applied setting.
3) Assessing the relationship between mechanical load and bone characteristics.
4) Using IMUs to assess if habitual, high-impact, asymmetrical load is associated with increased bone characteristics.
5) Investigating if there is a dose-response relationship between external load and bone adaptation, using a 16-week drop jump intervention in low active, young adults.

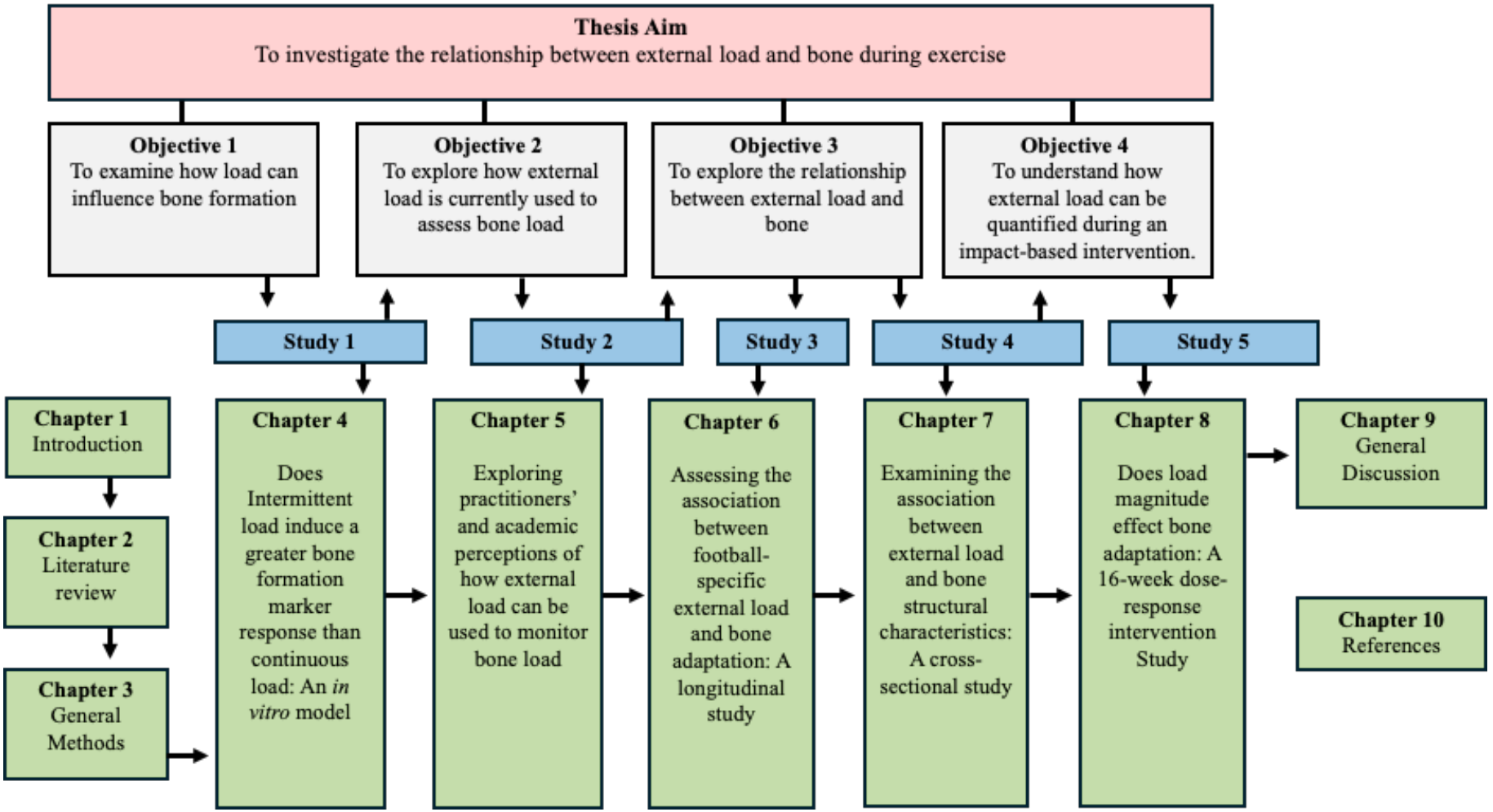


Figure 1.1. Illustration of the aim, objectives, and framework applied to this thesis

2 Literature review

2.1 Bone

Bone is a multifunctional, dynamic tissue comprised of minerals (~65%), type 1 collagen (~25%) and water (~10%) that supports and protects the body. Bone mineral is formed from hydroxyapatite crystals aligned along the collagen fibril axis, composed of ions such as calcium, magnesium and phosphate and their solubility play a prominent role in ion homeostasis (Gokhale et al., 2001). Located in the medullary cavity of the diaphysis (mid-section) of long bone is yellow bone marrow which is responsible for fat storage and blood cell production (Figure 2.1). Bone marrow is important for homeostasis as it regulates immune and stromal cell trafficking (Jansen et al., 2015). Mineralisation of bone is accountable for the rigid mechanical strength of the tissue whilst collagenous proteins are key structural molecules that provide tensile strength in mineralised tissue (Parry., 1988). Bone mass is understood to peak around the early 20s and gradually declines with ageing (Santos et al., 2017). In females, bone mass peaks at a lower rate than in males and the decline is greater due to the hormonal response during menopause (Hernandez et al., 2003). The mechanical function of bone is served by trabecular and cortical bone. Cortical bone is a hard outer layer, made up of the periosteum (connective tissue layer), that offers protection and structure to the skeleton. The articular cartilage is a thin layer of connective tissue found at the proximal and distant ends of bone to form joints acting as facilitators of skeletal movement. Trabecular bone (also referred to as cancellous or spongey bone) is mostly present in long bones within the epiphysis and is structured as a trabeculae matrix network.

Cortical and trabecular bone are both influenced by mechanical load. Cortical bone is stronger under compression, whereas trabecular bone is less predictable to load due to its varied lamellar and architecture (Hart et al., 2017). The combination of cortical and trabecular components allows bone strength and stiffness to be maintained whilst aiding its flexibility, thus avoiding stress-related fractures and bone disorders which can occur through high-magnitude, repetitive loading. When bone is subjected to loading, water is distributed through canalicular and vascular channels which may be linked to mechanotransduction signalling in bone (Section 2.3; Burr & Akkus, 2014). As mineralisation increases, water volume lowers, which is critical for the mechanical properties of bone stiffness. The formation also permits deformability for energy absorption, allowing blood vessels and nutrient arteries to pass through and provide

metabolic and endocrine functions, such as calcium facilitation and blood transportation (Burr & Akkus, 2014).

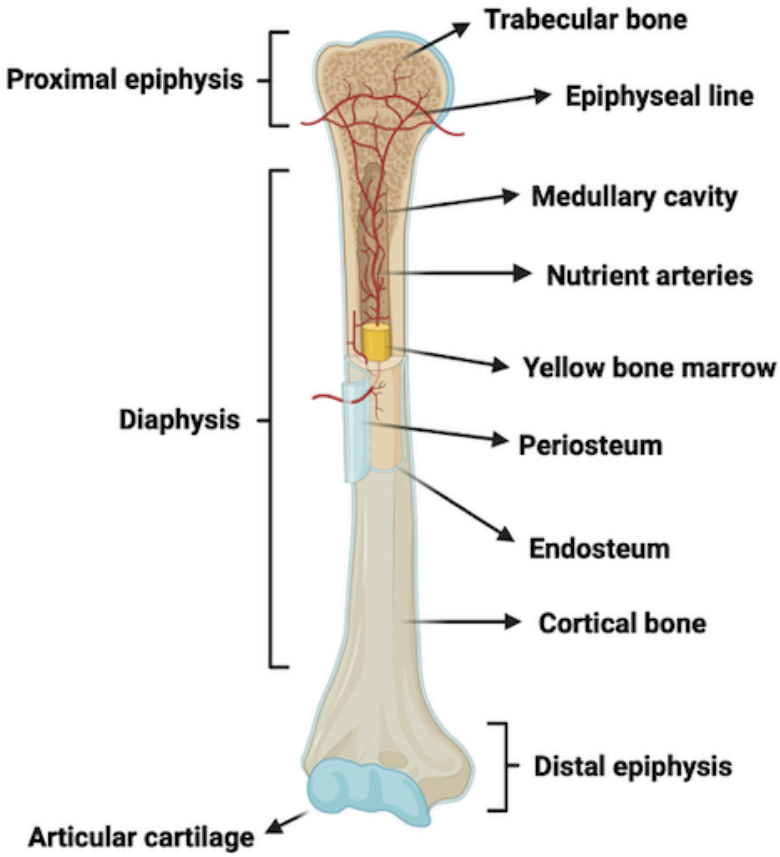


Figure 2.1. Anatomical structure of long bone (created with BioRender.com).

2.1.1 Bone modelling cycle

Bone modelling, not to be confused with remodelling, occurs during early skeletal development. Most bone modelling is accomplished by skeletal maturity during the growing years (Burr et al., 1989). During the modelling process, bone resorption and formation act independently, which is not the case during bone remodelling. The primary roles of bone modelling are (1) to preserve skeletal shape during growth and (2) to encourage radial growth of the diaphysis in long bones (Kenkre & Bassett, 2018). To preserve skeletal shape during growth, osteoclastic resorption occurs on the periosteal surface of the metaphysis whilst osteoblastic formation occurs on the inner endosteal surface to shape the epiphysis into the diaphysis (Allen & Burr, 2014). If the process is disrupted, for example by conditions such as osteoporosis, normal modelling of the metaphysis is inhibited (Grissom & Harcke., 2003).

2.1.2 Osteoclasts

Responsible for bone resorption, osteoclasts are unique cells that dictate skeletal mass. Bone-weakening diseases, such as osteoporosis, reflect enhanced osteoclast activity relative to osteoblasts (Teitelbaum, 2007). The unique cytoskeleton of osteoclasts, particularly the ruffled membrane and actin rings, enables them to polarize on bone and degrade the mineralised surface. They contain numerous vesicle-bearing pumps (H^+ATPase) that attach to bone to secrete acid and degrade the microenvironment (Teitelbaum, 2007). H^+ATPase proton pumps transport H+ into lacunae coupled alongside Cl^- from chloride channels to maintain electroneutrality (Tolar et al., 2004) resulting in the bone matrix being degraded by proteases (matrix metalloproteinases) until osteoclasts die, terminating the resorption phase. Upon osteoclast apoptosis, cytokines are secreted *e.g.,* interleukin 6 (IL-6) or a membrane-bound ligand (Ephrin) that is present in osteoblasts (Zhao et al., 2006). The formation of osteoclasts occurs from precursor cells regulated by the key osteoclastogenic cytokine, receptor activator of nuclear factor-KB ligand (RANKL). This protein resides on osteoblasts and recognises RANK located on marrow macrophages to bind to and activate various signalling pathways to transform cells into osteoclasts (Udagawa., 1990). Therefore, the formation process may be stimulated by the suppression of osteoclast differentiation. The cycle is regulated to attain a balance between resorption and formation with local regulation being critical to the process.

2.1.3 Osteoblasts

Osteoblasts are primarily involved in bone formation and have an important role in creating and maintaining skeletal architecture. They are specialized mononuclear cells that are responsible for osteoclast regulation and acquire the ability to secrete bone matrix as they differentiate from mesenchymal cells (Caetano-Lopes et al., 2007). The osteoblasts' main function is to secrete osteoid, therefore they have a prominent Golgi complex and endoplasmic reticulum that allows them to form tight junctions with adjacent osteoblasts (Mackie, 2003). Osteoprogenitors, also known as osteogenic cells, within the periosteum, engage in osteoblastogenesis by which Runx2 and osterix necessitate sequential action (Karsenty et al., 2009). For osteoblasts to create new bone, a range of hormones (Parathyroid Hormone (PTH), osteocalcin), growth factors (TGF β, IGFs) and enzymes (ALP, collagenase) are produced (Karsenty et al., 2009). Wnt has

been identified as a major signalling pathway for regulating bone formation (Kenkre & Basset., 2018). Wnt facilitates the binding to a dual receptor complex to inhibit the process of β-catenin being degraded by a multisubunit destruction complex. This allows β-catenin to relocate to the nucleus and activate gene transcription to allow osteoblast differentiation (Clevers & Nusse, 2012; Figure 2.2). During bone remodelling Wnt inhibitor expression declines to aid bone formation. The differentiation of osteoblasts to osteocytes occurs as the bone matrix calcifies the cells trapping them within the resulting lacunae and creating osteocytes (Ottewell, 2016). Other osteoblasts become flattened and line the surface of bone cells or die via apoptosis (Ottewell, 2016). Osteoblast activity is responsive to mechanical loading with muscle-derived and GRF-derived force both contributing to increases in bone strength (Yuan et al., 2016).

2.1.4 Osteocytes

Osteocytes are the most abundant bone cells (~90%) and are widely regarded as being responsible for sensing mechanical strain (Bonnet & Ferrari, 2010). Transitioning from osteoblasts, they transform their shape and function from cuboidal into a dendritic cell aimed to connect to nearby osteocytes and bone surface cells. Broadly distributed between trabecular and cortical compartments within calcified tissue, the mechanoreceptors present in these cells detect small changes in the electrical charge to activate calcium channels and regulate molecular mediators (Bonnet & Ferrari, 2010). Substrate deformation, hydrostatic pressure and fluid shear produced by mechanical loading cause stress to osteocyte membranes, predominantly from the fluid flow in the lacuna-canicular system (Weinbaum et al., 1994). A key signalling pathway that has a regulatory role in bone (re)modelling is the RANKL pathway. Macrophage colony-stimulating factor (M-CSF) stimulates RANK expression to bind to its receptor on osteoclast precursor cells. This reaction activates signalling molecules such as tumour necrosis factor (TNF)-receptor-associated factor 6, and transcription factors such as NFATc1, that regulate osteoclast genes (Takayanagi et al., 2002). Although RANKL can be created by osteoblasts and chondrocytes, it is the osteocytes within the bone matrix that initiate osteoclastogenesis via the production of RANKL. Osteoprotegerin (OPG), secreted by osteoblasts and osteocytes, hinders RANKL as a decoy receptor acting as an inhibitor of bone resorption by attaching to RANKL and preventing it from attaching to RANK (Kenkre & Basset., 2018). Therefore, the ratio of RANKL:OPG is fundamental for bone

turnover and skeletal development (see Figure 2.2). PTH can help modify the OPG-RANKL-RANK signalling system. Continuous PTH increases RANKL and reduces OPG to incite osteoclastogenesis, however, intermittent PTH increases bone formation using Wnt signalling. This reduces the expression of sclerostin and upregulates Wnt, leading to an increase in osteoblastogenesis that enhances bone formation (O'Brien et al., 2008). Due to the structure of the canicular walls and bone matrix, the cell membranes can experience drag forces and radially directed strains that are much higher than tissue-level strains (Schaffler et al., 2014). In response to mechanical load osteocytes communicate with surface cells to relay sensory information to initiate bone resorption or formation (Schaffler et al., 2014). The mechanosensory ability of the cells allows osteocytes to possess the unique capacity to regulate all phases of the bone remodelling cycle.

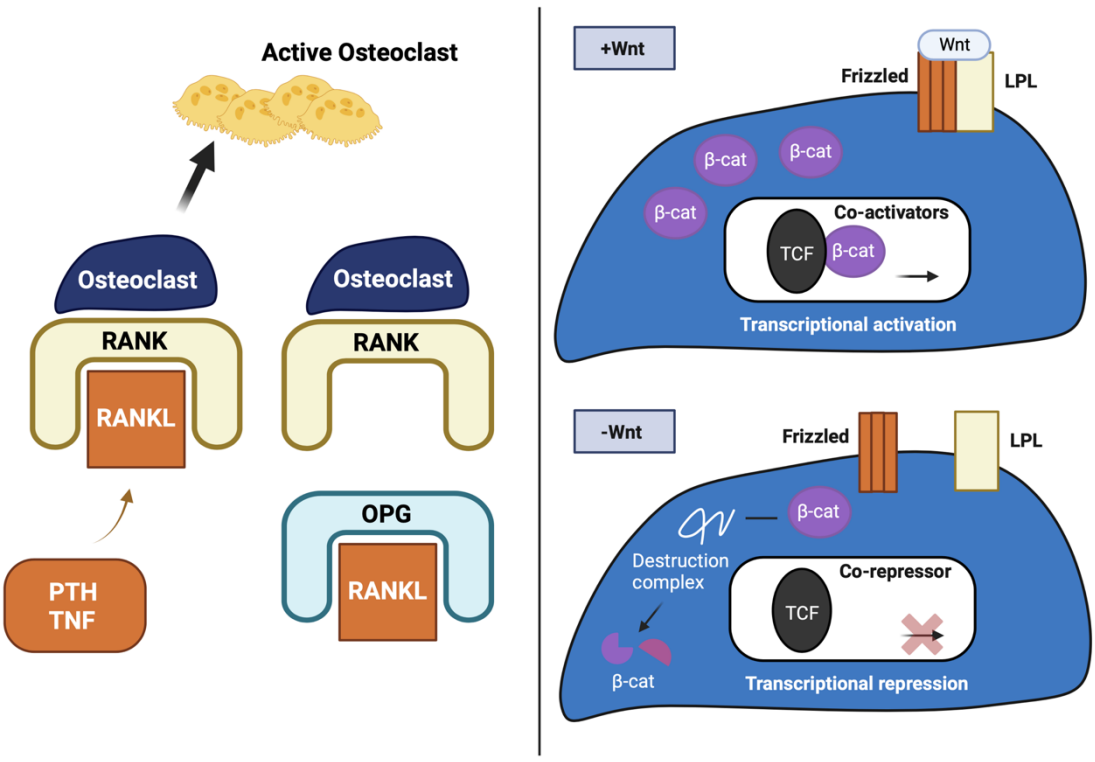


Figure 2.2. a) RANK/RANKL/OPG signalling pathway b) Wnt signalling pathway (created with BioRender.com).

2.1.5 Bone remodelling cycle

The purpose of the remodelling cycle is to replace old and damaged bone with new bone during adulthood. Unlike bone modelling, the remodelling cycle involves osteoclasts and

-blasts working sequentially at the same location. The process occurs simultaneously throughout the body and can be separated into four main stages; activation, resorption, reversal/formation and quiescence (Kenkre & Bassett, 2018; Figure 2.3). The cycle is initiated by the recruitment of precursor osteoclasts onto the surface that differentiate into osteoclasts. Once the osteoclasts bind to the surface of the bone, they actively dissolve the mineral and fragment collagen by acidic secretion (this can provide valuable biomarkers). The number of osteoclasts recruited for resorption is dependent on the response to the mechanical environment. After resorption, bone formation is initiated to reverse the damage of the osteoclast activity. The arrival of osteoblasts secretes an unmineralised organic matrix (osteoid) to the resorbed bone lining to create osteoid mineralisation. Bone matrix protein expression may be stage dependent as specific bone cells are not capable of producing all of the processes of bone maturation. The crossover of cells (preosteoblasts, osteoblasts and sometimes osteocytes) between the stages of bone remodelling suggests cooperativity must occur between bone cells (Gokhale et al., 2001). Throughout the formation stage, the osteoblasts either die via apoptosis, become incorporated into bone to form osteocytes or remain at the bone surface as lining cells. This process is continually renewed throughout an individual's life with a cycle taking 4 – 6 months to complete (Allen & Burr, 2014). In order to monitor bone adaptation there are numerous bone measurement techniques.

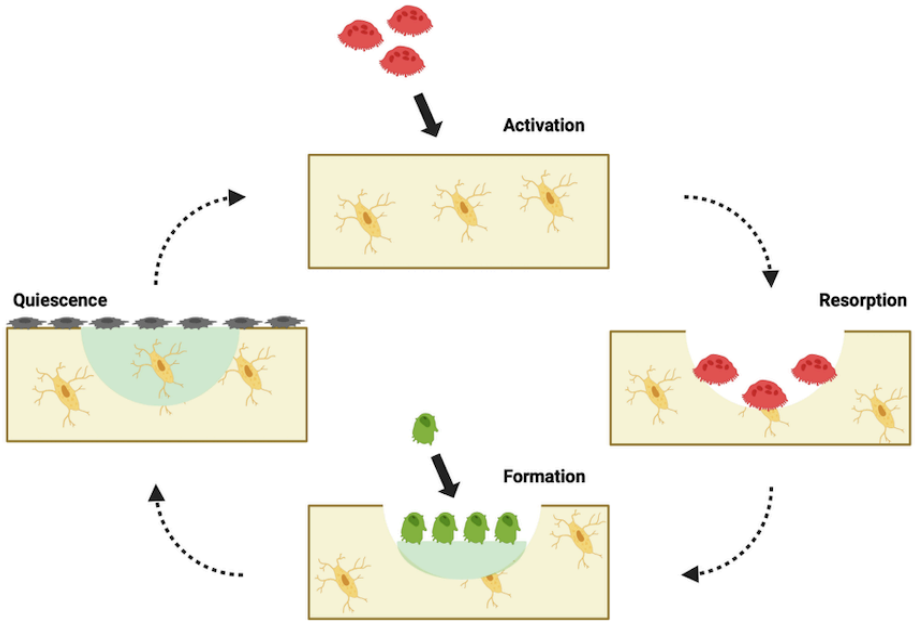


Figure 2.3. The main phases of the bone remodelling cycle (created with BioRender.com).

2.2 Bone measurements

To understand the properties of bone and how they are influenced by exercise and lifestyle factors, bone turnover markers (BTMs) and imaging techniques are performed (see general methodology for more detailed explanations of the principles).

2.2.1 Bone turnover markers

BTMs play a crucial role in bone research by providing insights into the dynamic processes of bone remodelling. Unlike bone imaging techniques, such as DXA or pQCT that assess bone structure and density, BTMs offer an assessment of bone metabolism. These markers can reflect osteoblast and -clast activity, providing valuable information on the rate of bone turnover. An advantage of BTMs is their ability to detect changes in bone metabolism in response to various stimuli, including exercise, in a relatively short period. For instance, studies have shown that weight-bearing exercise and resistance training can increase bone formation markers (Wen et al., 2017; Lin et al., 2012; Rantalainen et al., 2009), producing a positive effect on bone health. Many BTMs contribute to bone formation or bone resorption, the below information provides details on commonly used markers (Table 2.1).

2.2.2 Bone formation markers

Alkaline phosphatase (ALP) is an enzyme that is closely linked to the osteoblast cell cycle (Gokhale et al., 2001). The presence of ALP in uncommitted progenitors signals the beginning of a cell differentiating into an osteoblast. During the osteogenesis process, ALP is present in areas of maturing bone, demonstrating its necessity in cell development over bone maturation (Gokhale et al., 2001). The Wnt signalling pathway (see section 2.1.3) is a major regulator for ALP expression during mineralisation and is highly articulated in the extracellular space (Salazar et al., 2016). During mineralisation the ratio of phosphate to inorganic pyrophosphate is critical. ALP hydrolyses inorganic pyrophosphate to create phosphate for hydroxyapatite production which helps maintain the ratio required for mineralisation (Vimalraj., 2020). There is research on bone-specific ALP, but non-specific ALP can also heighten during osteoid development (Fedde et al., 1999; Narisawa et al., 1997). Procollagen I N-Terminal Propeptide (PINP) is another important marker of bone formation. Type I collagen composes 90% of bone matrix proteins and PINP levels correlate with BMD and histomorphometric bone formation

(Eriksen et al., 1993). The acute secretion of PINP may be due to the stimulation of developing osteoblastic cells that express type I collagen (Szulc., 2018). Type I collagen triple helices spontaneously compile into collagen fibrils which are mineralization sites (Hale et al., 2007). An increase in PINP levels is shown to project an increase in other bone formation markers such as carboxy-terminal extension peptide of procollagen type I (PICP) and ALP (Chen et al., 2005). PINP assays reflect the synthesis of the most abundant protein in bone tissue and is produced mainly during bone formation (Vasikaran et al., 2011). A limitation of PINP and ALP is that they are not exclusively derived from bone. PINP can also be synthesized in other connective tissues meaning it is not certain where PINP originates (Szulc et al., 2017) whereas ALP can be produced by the liver and kidney meaning there is a possibility of misinterpreting the marker from bone activity (Gokhale et al., 2001).

2.2.3 Bone resorption markers

C-terminal telopeptide of type I collagen (CTX) is a recommended National Bone Health Alliance marker of bone resorption (Szulc et al., 2017). Osteoclasts resorb bone by secreting acid and digesting collagen which may contain CTX (Bhattoa et al., 2021). It is a product of the breakdown of type I collagen, a main component of the bone matrix. The marker is observed to increase in those with osteoporosis and post-menopause (Park et al., 2018). CTX assays are measured via urinary or blood samples although the variability within blood serum samples is understood to improve the practicality and variability of the marker (Herrmann & Seibel. 2008). Serum levels of CTX are associated with histomorphometric measures of resorption (Chavassieux et al., 2015). N-terminal telopeptide of type I collagen (NTX), like CTX, is released during collagen degradation suggesting it may also be a bone resorption marker. It is shown to increase during bone metastasis (López-Carrizosa et al., 2010) and within postmenopausal women (Kanterewicz et al., 2013) and is negatively correlated with total body BMD (Ryan & Elahi., 1998). Like CTX it can be measured within urinary and blood samples, however, urinary samples are a more robust (Di Medio & Brandi., 2021) and sensitive (Civitelli et al., 2009) method of measuring changes in NTX. Although BTMs are good for detecting acute activity in bone, to measure long term adaptation imaging techniques are often used.

Table 2.1. Description of BTM characteristics and properties of analysis

Bone Turnover Marker	Full Name	Characteristics	Analysis
Bone formation			
PINP	Procollagen I N Propeptide	Mostly derived from Type I collagen following secretion into the extracellular space.	International reference formation marker for clinical and research use due to stability of measurement.
ALP	Alkaline Phosphatase	Bone specific and non-specific variations. Main function in formation of calcium hydroxyapatite.	Cross reactivity with liver isoform (20% for bone specific, 50% for non-specific). Can be irreproducible and variable.
OC	Osteocalcin	Marker of osteoblast function and liberated from bone matrix.	Can be released from kidneys therefore analysis may not reflect bone activity limiting its usefulness. Great sensitivity in measurement.
Bone resorption			
CTX	C-terminal telopeptide of type I collagen	Mostly derived from type I collagen and specific to collagen degradation in bone.	Influenced by circadian rhythm and diet therefore analysis requires standardisation.
NTX	N-terminal telopeptide of type I collagen	Mostly derived from type I collagen and specific to mature collagen degradation.	Influenced by circadian rhythm. Measured commonly as urinary assay.
OPG	Osteoprotegerin	Expressed in osteoblasts and -clasts. Decoy receptor for RANKL so important role in bone resorption.	Difficult to measure due to lack of knowledge on molecular form.
TRAP5b	Tartrate-resistant acid phosphatase	Produced by osteoclasts therefore reflects osteoclast number.	Sensitive and specific marker to measure resorption.

2.2.4 *Dual-energy x-ray absorptiometry*

Imaging techniques are used to measure whole body and/or site-specific bone properties. These are mainly performed as Dual-energy x-ray absorptiometry (DXA) and Peripheral Quantitative Computed Tomography (pQCT). DXA scans are considered a gold standard method (Bazzocchi et al., 2016) to assess bone and body composition due to its precision and ease of measuring bone and soft tissue (Toombs et al., 2012). The technique generates high and low levels of energy where high-level materials (*e.g.,* bone) attenuate the X-ray beam more and low-level materials (*e.g.,* soft tissues) attenuate the beam less (Bazzocchi et al., 2016). The technique can measure BMC, BMD, fat mass and lean mass at a whole-body and site-specific level. The variability (CV: 0.84 – 2.25%) and reliability (ICC: 0.99) of bone measurements during DXA are shown to be excellent (Lodder et al., 2004), as well as producing low radiation exposure (Toombs et al., 2012). This makes it a popular scanning option compared to alternatives, such as quantitative computer tomography, broadband ultrasonic attenuation and magnetic resonance imaging (Pennington et al., 2021). Although DXA is a gold standard method to observe whole body bone and body composition values it is restricted beyond measuring BMD and BMC which pQCT is able to provide.

2.2.5 Peripheral Quantitative Computed Tomography

pQCT is a technique permitting the selective analysis of different bone compartments which make it advantageous to DXA. It is, however, limited to measuring peripheral sites in the lower and upper extremities. The 3-dimensional application of the method allows for the measurement of cortical and trabecular bone, and predictive mechanical properties to gain a detailed analysis of peripheral bone. Due to its analysis, it has been suggested that pQCT may be a reasonable surrogate measure of bone histomorphometry (Rosen et al., 1995). The precision of the technique is shown to be excellent in studies of rat tibiae (CV: 0.3 – 1.6%; Gasser. 1995) and femur (CV: 1.2 – 2.3%; Horton et al., 2003), and upper and lower human long bones (CV: 0.3 – 1.6%; Szabo et al., 2011; CV: 0.8 – 7.5%; Sievänen et al., 1998). Unlike DXA scans, pQCT provides segmental analysis that allows the user to analyse bone at various sites (*e.g.,* anterior, posterior, medial, lateral), providing insight into site-specific bone adaptation. Therefore, using a combination of DXA and pQCT is recommended to retrieve an extensive insight into bone response to mechanical load.

2.3 Mechanotransduction

Mechanotransduction is the ability for a cell to actively sense, integrate and convert mechanical stimuli into biochemical signals that produce intracellular changes. Cells are sensitive to forces that regulate a large response in physiological processes, from feedback mechanisms within proteins to the activation of signalling pathways (Orr et al., 2006). How cells convert mechanical energy into electrical and biochemical signals is important for maintaining good bone health. Mechanosensors can be defined as any cellular structure capable of detecting changes in external or internal forces (Yavropoulou & Yovos., 2016). For example, the sensitivity of bone cells (osteoblasts, -clasts, -cytes) enables them to respond to mechanical forces that regulate the bone remodelling cycle. Osteocytes are believed to play a pivotal role in mechanotransduction, responding to deformations in the fluid surrounding their structure. This responsiveness enables them to swiftly adapt to environmental changes by signalling either osteoclasts for bone resorption or osteoblasts for bone formation, thereby regulating the bone remodelling cycle (Santos et al., 2009). While it remains unclear which specific component of the osteocyte is primarily responsible for its mechanosensory properties, there is a suggestion that fluid flow can induce strains in the actin filament of the cytoskeleton at a magnitude greater than tissue-level strains (Han et al., 2004).

The significance of osteocytes to bone mechanosensitivity is evidenced *in vitro*, where mechanical loading has been shown to upregulate nitric oxide, a key mediator of osteocyte response leading to bone formation (Vatsa et al., 2006). The relationship between bone and mechanical load is postulated by Wolff's Law (Wolff, 1892) and developed further by the mechanostat theory (Frost, 1987). In the simplest of terms, bone adapts to the physical needs of its mechanical environment. The mechanostat theory further hypothesises that bone structure optimises to prevent microdamage and best suit the functional needs of the tissue by adapting its strength to the habitual load it experiences. Therefore, when bone is subjected to increased loading, the remodelling process will adapt to increase bone's ability to withstand higher loads. Conversely, when loading decreases, the adaptive mechanisms in place adjust catabolically to the reduced loads (Figure 2.4). This concept is exemplified at the cellular level by studies that demonstrate faster shear stress and vibration stress elicits stronger cellular responses, thereby promoting bone growth and mitigating osteoporosis (Santos et al., 2009; Bacabac

et al., 2005; Bacabac et al., 2004). Human research further elucidates the impact of mechanical loading on bone accrual, revealing that high-impact loading leads to greater BMD compared to low-impact loading among athletes (Bass et al., 2005; Morel et al., 2001), collegiate athletes (Tenforde et al., 2018; Carbuhn et al., 2010), adolescents (Weeks et al., 2008), and youth (Ubago-Guisado et al., 2015; Courteix et al., 1998). Additionally, investigations into bilateral differences among athletes highlight the effects of strain on bone adaptation (Warden et al., 2019; Bogenschutz et al., 2011; Warden et al., 2009; Krahl et al., 1994).

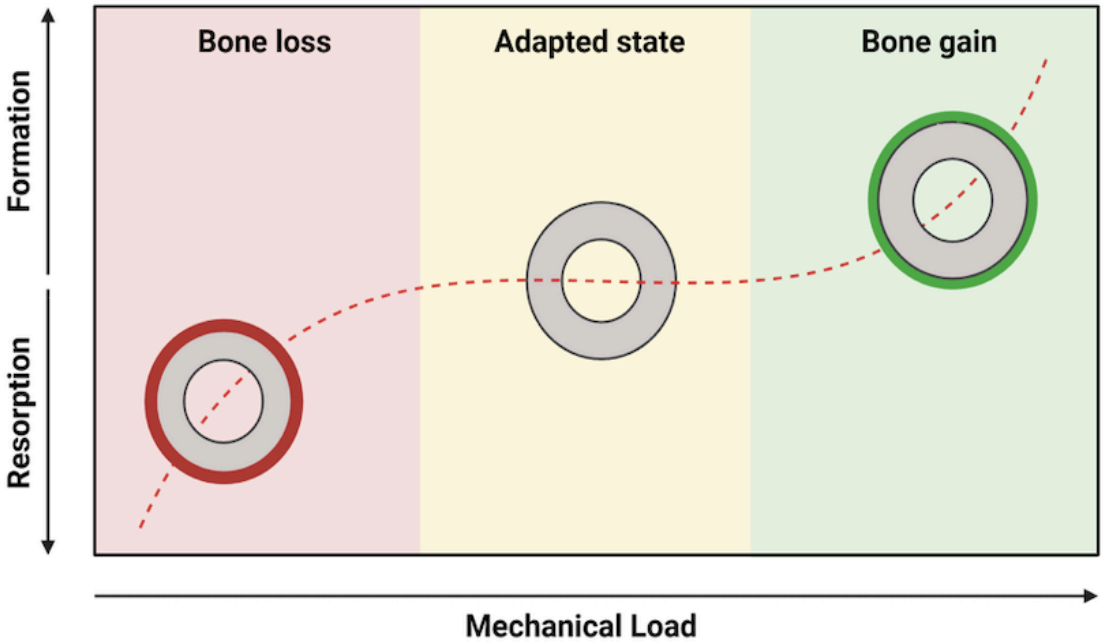


Figure 2.4. Wolff's law and Mechanostat theory. As mechanical load increases bone adapts to strengthen. Bone resorption (loss) occurs when there is a low mechanical stimulus whereas bone formation (gain) occurs when there is a high mechanical stimulus (created with BioRender.com).

The effects of loading have been demonstrated during *in vitro* models (Zhong et al., 2013; Plunkett et al., 2010; Murray & Rushton, 1990). For instance, tension has been shown to enhance the quantity and functionality of osteoblasts (Zhong et al., 2013). However, determining an optimal loading protocol (*e.g.,* load magnitude, frequency, duration, and type) to promote osteogenesis remains a subject of ongoing research. Research indicates that the magnitude of mechanical load plays a significant role in influencing bone formation, with higher loads correlating with increased osteoblastic activity (Jaasma et al., 2007). ALP has been identified as a facilitator of mineralization, as it increases local

inorganic phosphate rates while reducing extracellular pyrophosphate concentrations, which inhibit bone formation (Vimalraj, 2020). Studies have demonstrated that dynamic loading over a period of two hours leads to an upregulation of ALP activity in human bone marrow mononuclear cells (Sittichokechaiwut et al., 2010). Furthermore, PINP levels have been correlated with parameters of bone formation, such as osteoid thickness and volume (Chavassieux et al., 2015). Studies have also shown that PINP increases the protein content in mechanically loaded osteocytes and osteoblasts after short-term (one hour) and long-term (five days) loading periods (Vazquez et al., 2014; Wu et al., 2016).

2.3.1 Loading types

The capacity of bone to respond to mechanical load and strains is imperative for the success of a functional skeleton. The type of load that is subjected to bone is shown to have diverging effects on bone response. For example, higher vertebra BMD and BMC are shown in fast bowlers compared to other cricketers and rugby players as the higher forces experienced initiate a site-specific remodelling response (Alway et al., 2019). As well as load magnitude, the rotation and angles of the pelvis during a bowling action are large predictors for an increase in BMD on the contralateral side of the vertebra (Keylock et al., 2022). The rotational strain experienced at the lumbopelvic joint highlights force magnitude is not the only mechanism to initiate a positive response, but the direction of loading may also be important. During daily activities, bone can undergo load in the forms of torsional, tensile, compressional, and axial loading (George & Vashishth., 2005) (Figure 2.5).

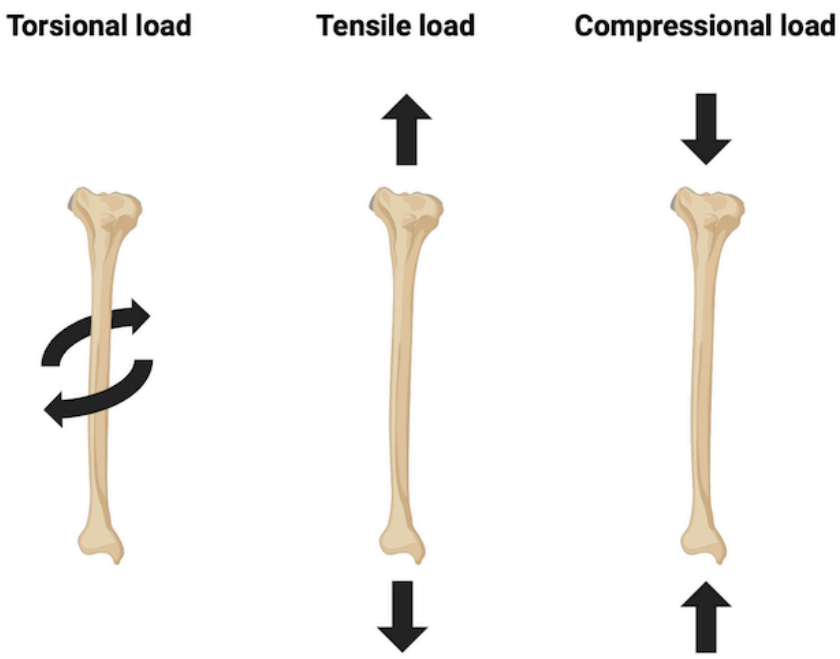


Figure 2.5. Types of load that can be applied to bone during daily activity and exercise (created with BioRender.com).

2.3.2 Torsional load

Torsional load is the twisting of a body about the transverse axis due to an applied torque. When investigating torsional resistance in birds, De Margerie et al. (2005) suggested torsion plays a significant contribution in tissue organisation and when combined with axial load this balance is advantageous to the biomechanics of the structure-function relationship of long bone. The application of torsional load has been shown to induce a sufficient contribution to bone strength and maintain bone mass that compressional load cannot do alone (Mittag et al., 2018; Rubin et al., 1996). Within sports, torsional load has been shown to incite greater bone strength in racquet players (Kontulainen et al., 2002), baseball pitchers (Warden et al., 2009) and BMD in fast bowlers (Alway et al., 2019). These studies, however, lack any load measurements and only refer to the training status of the subjects as a means of quantifying load. This is an issue as there is no quantitative data for load magnitude to detail how bone responds to load. The positive effects of torsion are conflicted in experimental studies reporting torsional load does not have an osteogenic effect on bone (Pead & Lanyon., 1990) but can have a detrimental effect on the trabeculae from imposed bending (Fatihhi et al., 2016). The fatigue life of bone is reduced under torsional load in comparison to axial and compressional loading (George & Vashishth., 2006) which can be explained by mixed-mode loading, meaning the

application of torsion creates strain across numerous axes simultaneously. It is therefore not uncommon for stress fractures to occur from the perilous effects of shear stress through torsion (Burr., 1997).

2.3.3 Compressional load

Compressional load is when physical forces press inward on an object causing it to become compact. Compression is a widely used technique for observing the mechanical properties of bone strength in research (Zhao et al., 2018). When loaded under compression, bone offers a high load-bearing capacity of strength and toughness which can be double that created from tension (Li et al., 2013). Unlike tensile load, bone under compression can carry load after deformation (Currey., 2012). Mice models have observed that low-magnitude compression can augment healing determined by increased callus strength (Gardner et al., 2006). Compressional load occurs in the lower extremities during daily activity, therefore the function to withstand this type of loading is important for human locomotion. Sporting movements such as powerlifting result in higher compressional forces than those found in footballers and triple jumpers, however, bone strength is shown to be lower in powerlifters emphasising the effect dynamic movements have on bone adaptation (Niinimäki et al., 2017).

2.3.4 Tensile load

Tensile load elongates or stretches the object, increasing its length. Bone routinely withstands tensile load from mechanical loading and muscular contractions (Hart et al., 2017) but has a relatively poor ability to withstand tensile load (Augat & Schorlemmer., 2006). When bone is exposed to tensile stresses the organic matrix consisting of Type I collagen is subjected to normal and shear stress that can cause damage in the organic matrix of bone (Havaldar et al., 2014). When stresses go beyond the yield point tensile failure can cause microcracks to appear throughout bone, predominantly from the tissue being subjected to normal and shear stress under tensile load (Kotha & Guzelsu., 2003). *In vitro* research, however, has observed tensile load improves osteoblast function important for bone formation (Zhong et al., 2013). As *in vitro* studies remove systemic response they are useful to understand the effects of load in an isolated model. For this purpose, tensile load is a common and simple method of triggering cell response (Baudequin et al., 2019).

2.3.5 Axial load

Axial loads, also known as thrust loading, are forces applied in the same direction as the axis of the object. For example, the tibia receives axial force along the shaft of the long bone during locomotion. Axial loading can be applied in various ways; longitudinally (running proximally or distally towards the epiphysis), transversely (horizontally fully encircling the diaphysis) or oblique (running at an angle to the long axis of the bone). The direction of load is a key principle for bone response as fracture patterns are more prominent under the combination of torsional and axial loading. Lateral loading on bone creates the most prominent fracture lines whereas posterior loading results in the lowest number of fracture patterns (Cohen et al., 2017). *In vivo* experiments have shown osteogenic responses in cortical and trabecular bone as a result of axial loading (Pereira et al., 2015; De Souza et al., 2005). Endosteal, periosteal and trabecular bone characteristics have also been shown to strengthen due to repetitive axial loading (De Souza et al., 2005) and are expressed as a major contributor to the adaptive response in cortical thickness (Pereira et al., 2015). Measuring load is therefore important to understand the effects of mechanical load on bone accretion.

2.4 Internal and External Load

Internal loads are how the body reacts physiologically to physical activity. Primarily associated with exercise, it is the biological stress imposed on an individual during training or competition, *e.g.,* blood lactate or heart rate (Bourdon et al., 2017). External loads are the work performed during exercise measured independently of internal characteristics (Impellizzeri et al., 2019). External loads can include power, acceleration, and force. It is difficult to associate external load with the internal structures of the body (bone, muscle, joints) as it is not possible to directly measure the forces acting upon biological tissue without invasive techniques. To measure external load in an applied setting, the affordability of the technology and usefulness of the equipment is often considered. Some organisations may have better access to external load equipment, however, the application of the data and its interpretation is most important when integrating technology with exercise.

The methods of quantifying load are largely measured externally making it difficult to accurately quantify bone load. The mechanical stresses on soft tissue come from the external demands of impact forces created from pushing off against the ground or another body, meaning non-invasive procedures (Figure 2.6), especially in the context of sport, may be used as a superficial representative of what the athletes are encountering internally (Vanrenterghem et al., 2017). Currently, there is no consensus or gold standard for quantifying external load and bone, therefore we rely on a range of non-invasive methods to inform us on bone adaptation to exercise. It is important to gain an understanding of the relationship between external load and bone so we can inform practitioners (sport or clinical) on relevant measures to maintain and optimise bone health.

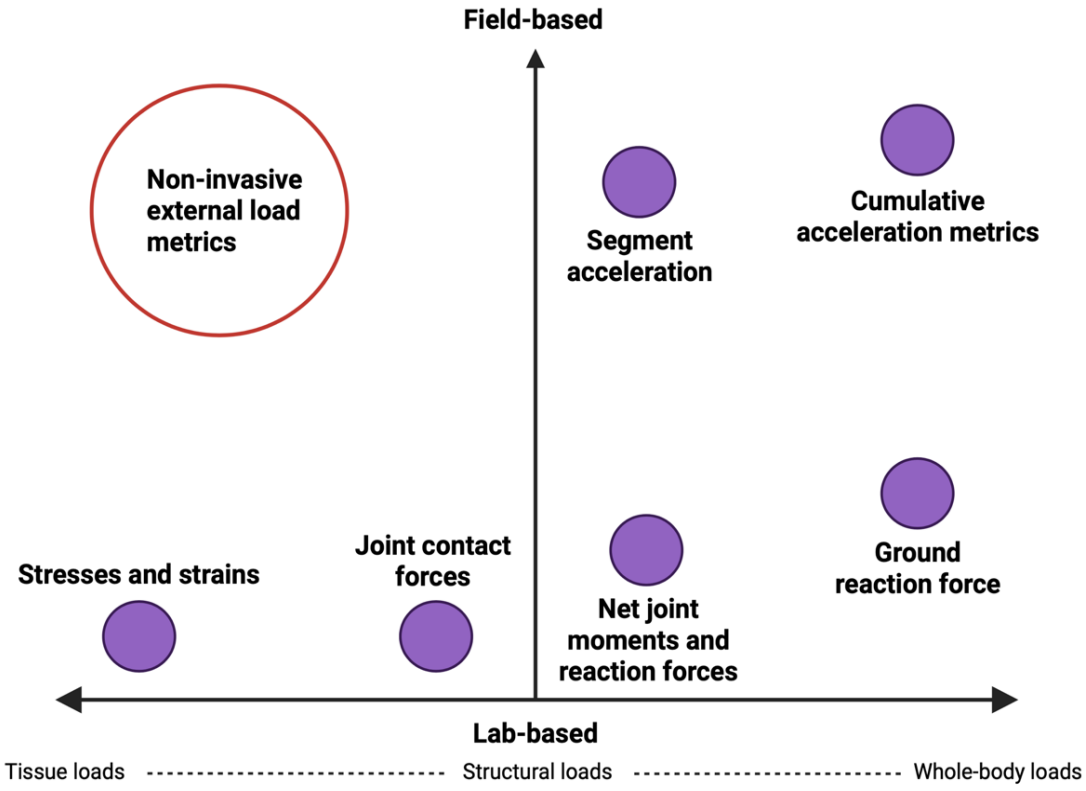


Figure 2.6. Adaptation of Verheul et al. (2020) schematic. Overview of biomechanical load metrics, area of applicability and location measurement for bone (created with BioRender.com).

2.5 Skeletal unloading

The absence of gravitational loading is shown to have detrimental effects on the skeletal system. Prolonged bed rest (12 weeks) has been observed to decrease bone mass at the femoral neck, lumbar spine, and trochanter (Zerwekh et al., 1998), whereas cortical bone

has been shown to amass greater bone loss than trabecular bone from 35 days of bed rest (Rittweger et al., 2009). Furthermore, bone resorption markers, CTX and NTX, can increase after as little as 2 days of bed rest (Baecker et al., 2003). This is shown in exercise where low-impact activities, such as swimming and cycling, do not offer any osteogenic benefits (Morel et al., 2001). If bone is not regularly subjected to impact load (*e.g.,* swimmers or cyclists) it will become accustomed to the mechanical environment and adapt accordingly to the lower loads.

2.6 Skeletal loading

Impact-based exercise (Du et al., 2021; Varley et al., 2017; Vicente-Rodriguez et al., 2007) induces an osteogenic response due to the heightened load imposed on bone. Factors such as magnitude, frequency, duration, and load type all contribute to bone load, and their application dictates bone adaptation (Robling et al., 2019). The beneficial effects of using impact-based interventions on bone accrual are shown in youth (Gunter et al., 2008; Heinonen et al., 2000; Weeks et al., 2008), middle aged (Hinton et al., 2015; Bailey & Brooke-Wavell., 2010; Vainionpää et al., 2005) and older populations (Allison et al., 2015; Allison et al., 2013; Kemmler & Von Stengel., 2013; Cussler et al., 2005). Increases in bone size and strength in the dominant arm of tennis players and baseball pitchers compared to the contralateral limb suggest increased forces and acceleration/decelerations are contributing factors for bone accrual (Bogenschutz et al., 2011; Warden et al., 2009; Bass et al., 2005; Krahl et al., 1994). This consensus is supported by studies implementing impact-based exercise where increases in BMD (Simoes et al., 2021; Yu et al., 2019; Vainionpää et al., 2005; Bassey & Ramsdale, 1994) and bone geometry (Lambert et al., 2020; Allison et al., 2013; Ferry et al., 2013; Ducher et al., 2011) are higher than inactive controls. Guidelines suggest performing 3-5 sets, 10-20 repetitions of impact exercise, that produce >2 body weights (BW), separated by 1-2 minute rest periods and performed 4-7 times a week are recommended for the prevention of osteoporosis in postmenopausal women (Daly et al., 2019). Similarly, Min et al. (2019) produced guidelines recommending performing impact exercise 50 jumps/min, >10 minutes a day, at least 2 times a week to improve peak bone mass in adolescents. It is clear impact exercise is a way to increase bone strength throughout life, however, external

load characteristics during interventions warrant further investigation as current guidelines are ambiguous.

2.6.1 Load Magnitude

Animal models have shown load magnitude is a significant mechanical factor for bone accrual, with higher loads inciting more positive bone adaptations (Guadalupe-Grau et al., 2009). Axial loading in mice tibiae above 10N increases cortical bone formation, endosteal formation and periosteal inter-label (De Souza et al., 2005). The osteogenic effects of high-impact exercise (stamping, jumping, running) on bone mass and site-specific BMD have been demonstrated by intervention studies (Allison et al., 2013; Jämsä et al., 2006; Vainionpää et al., 2005). BMD is shown to be greater in the femoral neck, trochanter, and Ward's triangle when daily impacts and accelerations over 12 months exceed 3.6 g's in comparison to accelerations below this threshold (Jämsä et al., 2006). This is supported by Vlachopoulos et al. (2018), where a nine month jump intervention progressing every twelve weeks from 20 x 3 counter movement jumps (CMJ), 3 days/wk to 20 x 4 CMJ's with a 2 kg weighted vest, 3 days/wk and then 20 x 4 CMJ's with a 5 kg weighted vest, 4 days/wk produced an increase in leg BMC and bone stiffness in adolescent athletes compared to sport specific non-jumping controls. Allison et al. (2013) demonstrated unilateral, multidirectional hopping over twelve months caused increases in femoral neck BMD, BMC and cross-sectional area post-intervention, whilst the control leg decreased in the same metrics. Furthermore, Varley et al. (2022) showed tibial strength and BMC were positively correlated to GPS external load metrics, such as accelerations and decelerations, in elite male footballers across a season. Rowlands et al. (2020) also showed young adults that produce high magnitude loading during daily living, monitored by accelerometers (Section 2.7.1), resulted in greater BMD and BMC compared to medium and low magnitude load.

Jump interventions producing GRF values between 2 and 5 BWs have reported increased BMD and BMC (Bolam et al., 2015; McKay et al., 2005; Heinonen et al., 1996). Unilateral jumping interventions comparing exercise legs to control legs have also reported GRFs between 2 and 3 BWs applied to a single leg create an osteogenic response (Hartley et al., 2020; Allison et al., 2015; Allison et al., 2013). Although there may be a minimal magnitude threshold required to initiate bone accrual, no intervention studies

examine the possibility of an upper threshold that may cause detrimental effects on bone adaptation. This is due to the current interventions comparing a single load magnitude to a control group. There is a gap in current research to examine the dose-response effect of impact exercise from multiple magnitudes and assess if there is an upper loading threshold that causes detrimental effects in bone. Load magnitude, therefore, influences bone adaptation, whereby higher loads produce greater bone accrual. Load frequency and duration, however, are also understood to affect bone adaptation.

2.6.2 Load Frequency

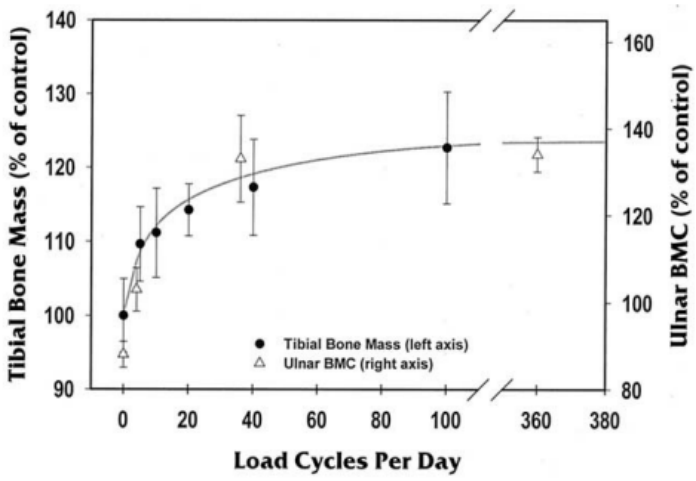
Exercise frequency is positively associated with bone accrual (Daly et al., 2021). The notion that frequent loading can improve bone health has led to studies investigating frequency thresholds. For example, protocols comprised of >50-100 load cycles in a single bout did not produce additional bone adaptations, whilst few load cycles (36 cycles) are needed to initiate a positive response (Burr et al., 2002; Figure 2.7). Beyond the 100 cycle range, the response plateaus due to mechanosensory saturation. This suggests increasing load cycles lessens the return in bone formation, meaning cells become "bored" of repeated mechanical load (Burr et al., 2002). Mice exposed to short, frequent periods of vigorous fighting have shown increases in tibiae bone mass as a result of increased load compared to mice not engaging in fighting (Meakin et al., 2013). This is supported by avian models where free-range chickens had significantly larger cortical area and thickness, and greater trabecular thickness compared to chickens that move less frequently (Shipov et al., 2010). However, exercise was not quantified and was only assumed within these studies, therefore a comparison of load magnitude between groups is not known. In rats, it has been observed that 5 jumps/day from a 40cm platform causes an increase in tibial and femur bone mass over 8 weeks as bending moments and stress increase during jumping (Umemura et al., 1997). The acute response is also shown in rooster ulnae where as little as 4 consecutive loading cycles/day of 0.5 Hz are effective for increasing bone mass (Rubin & Lanyon., 1985), emphasising bone accrual can be gained shortly into loading cycles. *In vivo* studies are important to understand bone adaptation using invasive methods beyond the means that are possible in human studies. Although bone adaptation is similar in humans and animal models, the functional properties may differ between species (Troy et al., 2013). Therefore, to understand bone adaptation in humans applied studies are more informative.

High-impact exercise performed ≥2 sessions/wk during an 18-month intervention in 50-79 year old men is positively associated with increases in femoral neck BMD and lumbar spine trabecular BMD compared to those exercising <2 sessions (Daly et al., 2021). This study suggests for each additional training session performed across the intervention there was a 0.7% increase in femoral neck BMD and a 3.1% increase in lumbar spine trabecular BMD. This demonstrates increasing the frequency of high-impact exercise can promote bone adaptation. This is further shown in studies on pre/postmenopausal women who have an increased risk of fracture. Premenopausal women performing frequent hopping (50 hops, 7 days/wk) are shown to increase femoral neck BMD by 1.7% whereas less frequent hopping (50 hops, 4 days/wk and 50 hops, 2 days/wk) results in smaller increases of 0.9% and 0.2% across six months, respectively (Bailey & Brooke-Wavell., 2010). This is replicated in research conducted in postmenopausal women where partaking in high frequency jumping (>2 days/wk) and resistance exercise across twelve years showed greater lumbar spine BMD and hip BMD in those that performed the intervention less frequently (<2 days/wk; Kemmler & Von Stengel., 2014). Similarly, postmenopausal women partaking in moderate impact exercise frequently (~2 days/wk) are associated with a 1.5% increase in BMD, whilst less frequent exercisers (<1 day/month) showed bone loss (Cussler et al., 2005). The evidence suggests load frequency influences bone's adaptive response, but the way in which load cycles can be conducted (*e.g.,* rest periods) can also have a significant effect on bone activity.

2.6.3 Rest periods

Rest periods inserted between loading bouts have been shown to promote bone accretion (Burr et al., 2002). If bone response diminishes quickly, it is important to understand how rest periods may be applied to recover the mechanical sensitivity. This is due to the notion that osteoblast mechanosensitivity desensitises to regular cyclic loading, meaning bone cells become accustomed to monotonous load (Robling et al., 2001). Therefore, to improve bone accrual, separating loading bouts with rest periods is suggested to increase the effectiveness of the loading stimulus (Robling et al., 2002). Rodent models have shown rest periods incorporated into loading bouts heighten the effectiveness of the bone response by necessitating the restoration of mechanosensitivity (Srinivasan et al., 2007; Srinivasan et al., 2003; Robling et al., 2002; Srinivasan et al., 2002; Raab-Cullen et al., 1994). Turkey ulnae exposed to intermittent load (rest periods) have also displayed larger

periosteum compared to continuous load with no rest (Srinivasan et al., 2002). Introducing rest periods of 0.5, 3.5, 7 and 14 seconds between loading cycles found greater bone formation and mineralisation than no rest periods and non-loaded groups (Burr et al., 2002). The group with 14 second rest periods, however, was also shown to have a ~50% greater increase in mineralisation compared to the other rest-inserted groups, demonstrating longer spacing between loads can improve bone response (Burr et al., 2002; Figure 2.7). This is supported by Srinivasan et al. (2002) showing 10 second rest periods between high magnitude load cycles (0.5N) can enhance bone's adaptive response compared to loading without rest intervals. A similar response is observed when investigating time between loading bouts. The data suggest a rest period between 4-8 hours can optimise bone response, whilst no extended advantage is seen beyond ~8 to 10 hours (Robling et al., 2001; Figure 2.7). Furthermore, studies have determined that ~98% of mechanosensitivity is restored ~24 hours following a loading event (Robling et al., 2006). The alteration of signalling molecules (Wnt, bone morphogenetic proteins) responsible for bone cell activity is adaptable to mechanical stimulation and an anabolic response can increase with loading-unloading (Klein-Nuland et al., 2015). Therefore, the inclusion of rest periods between loading bouts has the potential to optimise the adaptive response of bone induced by mechanical loading. It is clear frequent exercise, incorporated with rest periods, increases bone accrual, however, exercise duration can affect bone adaptation.



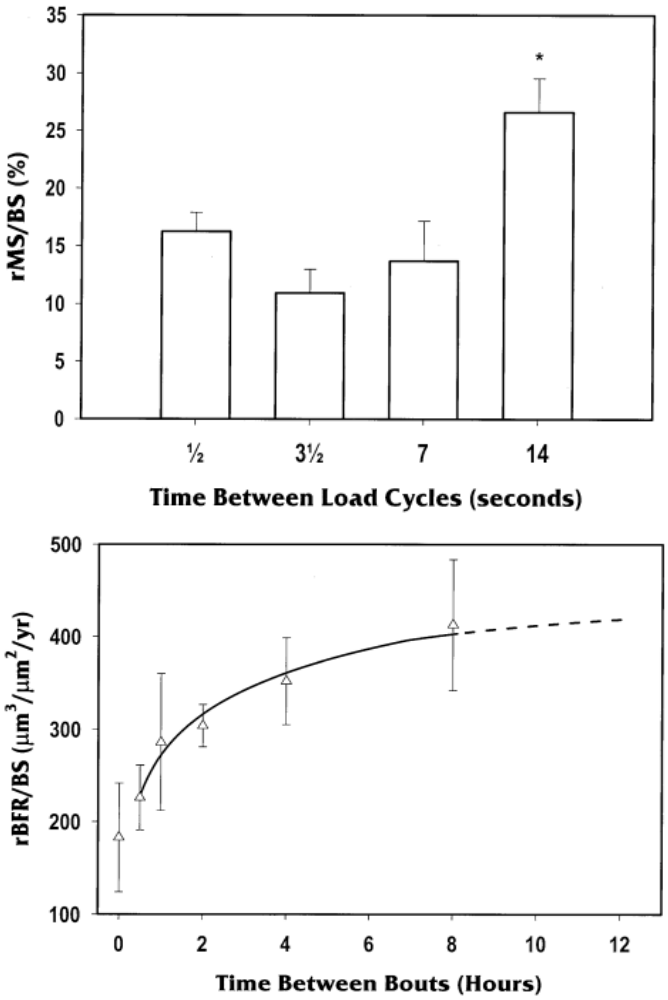


Figure 2.7. Load frequency and rest periods for restoring mechanosensitivity in turkey and rat models taken from Burr et al. (2002).

2.6.4 Load Duration

There are limited studies surrounding optimal load duration and bone adaptation. However, load duration has been highlighted as a strong predictor of bone strength (SSI) and geometry (cortical area), with the notion that prolonged loading stimulates bone as long as the distribution of the strain is altered i.e., not repetitious (Farr et al., 2011). It is understood from *in vivo* research how many cycles (36 load cycles) are required to initiate bone adaptation during exercise (Burr et al., 2002), whilst human interventions have shown the effects of exercise duration on bone. A cross-sectional study by Marin-Puyalto et al. (2019) showed ≥15 minutes of impact exercise was associated to higher bone mass and density at the femoral neck and whole body in male adolescents compared to those that engaged in the same magnitude of loading for <15 minutes. However, the quantitative data in this study grouped load into generalised cut points making it difficult to understand

the specific duration there was a significant effect. Furthermore, impact exercise performed 10 minutes, 3 days/wk showed a significant improvement in BMC at the proximal femur and total body over 7 months in schoolboys compared to controls (MacKelvie et al., 2002). This is supported among footballers whereby increasing training volume to 12 hr/wk initiated a higher BMD and cortical area response than training 6 hr/wk (Varley et al., 2017). This demonstrates increasing the duration of impact exercise can promote bone adaptation.

2.6.5 Bone turnover response to exercise

The response of bone turnover markers to exercise is shown to be variable. The varied response in BTMs to exercise are likely caused by the differences in methodologies, such as diet, population, marker cross-reactivity and historical physical activity (Hutson & Varley, 2024). A systematic review and meta-analysis conducted by Civil et al. (2023) showed no changes in PINP following running. There was also no evidence of any changes in CTX in the hours or days post-running (Civil et al., 2023). Although there were no effects on BTMs in running, acute increases in PINP levels during (Bowtell et al., 2016; Scott et al., 2011) and immediately post impact exercise (Prawiradilaga et al., 2020) have been observed. This response is different from that of ALP, which only shown to increase 24 hours post-exercise (Kish et al., 2015). Impact exercise is also observed to create a decrease or have no effect on bone resorption marker CTX (Hilkens et al., 2023; Yusni & Rahman., 2019), whereas low-impact exercise, such as cycling, has been shown to induce a moderate increase in the bone resorption marker CTX (Dolan et al., 2022). There is also evidence of bone resorption marker (CTX) being downregulated and bone formation marker (PINP) being upregulated as a result of impact-based exercise (Wen et al., 2017; Lin et al., 2012; Rantalainen et al., 2009). Therefore, based on current research high-impact exercise may elicit acute responses in bone markers as highlighted by increases in PINP. The dose-response relationship between load and BTMs promotes the ideology that jumping interventions may be a method to promote bone formation and reduce the risk of developing osteoporosis (Prawiradilaga et al., 2020).

2.6.6 Load response to ageing

No exercise guidelines would be relevant without referring to the influence of ageing on the adaptive response of bone to mechanical loading. This is due to the notion that bone

adaptation responds differently across the lifespan. Exercise during childhood maximises the chances of developing stronger bones in later life, whereas exercise during adulthood and older age can help maintain bone mass (Santos et al., 2017). Achieving higher peak bone mass during childhood and adolescence can reduce the risk of developing osteoporosis (Wilsgaard et al., 2009). It is also observed performing mechanical loading during youth, particularly impact exercise, contributes to greater bone strength in later life as the skeleton is more responsive to exercise than in adulthood and old age (Warden et al., 2014). This is evident in older adults that participated in volleyball and basketball in youth having greater BMD at the lumber spine and femoral neck compared to those that did not (Otsuka et al., 2023). Additionally, age-related bone loss is evident in less active and sedentary adults (Rittweger et al., 2009). This is also shown in rodents where younger mice (8 weeks) increased bone volume from axial load, whereas older mice (-10, -12 weeks) showed a detrimental effect, highlighting the age-specific response to load similarly observed in humans (De Souza et al., 2005). Many studies conduct exercise interventions using pre/postmenopausal women due to their risk of fracture/osteoporosis. However, achieving high bone mass in adolescence and early adulthood is a more effective way of preventing osteoporosis since younger adults are more susceptible to larger increases in BMD (Florence et al., 2024). For this reason, exercise should be prescribed to prevent osteoporosis instead of being used as rehabilitation method in older adults (Beck et al., 2017).

2.7 Quantifying exercise

A limitation of current research is the lack of concurrent load and bone measurements (Detter et al., 2014; Duckham et al., 2014; Hagman et al., 2018; Strope et al., 2015). Exercises involving high-impact, multidirectional movements have been associated with greater BMD in football (Varley et al., 2017), gymnastics (Vicente-Rodriguez et al., 2007), speed skating (Varley et al., 2019) and volleyball (Nichols et al., 2007) compared to those that do not partake in activity. Whilst engagement in impact exercise is known to induce increases in bone mass, the specific movement patterns necessary to optimise bone health are not fully established. This gap in the literature is primarily due to studies not accurately quantifying mechanical load. However, studies have attempted to monitor external load and bone characteristics in applied environments in a variety of manners.

For instance, Worthen et al. (2005) associated wrist monitor activity to BMD in stroke patients. They suggested information on the daily loading history of the skeleton (*e.g.,* bone density index) correlated more effectively with BMD than gait-related variables, such as walking speed or steps per day. Although quantifying exercise using participant recall can offer information on the characteristics of exercise (*e.g.,* impact exercise, multi-directional, etc), it does not offer objective data on exercise, such as magnitude and frequency, that is influential in bone adaptation. In addition, Turner & Robling (2003) suggest GRF-derived peak force can be used as a proportional measure of skeletal loading and inform on bone accrual. The development of the osteogenic index is based on GRF-derived peak force, load frequency and recovery periods of exercise, however, assumptions are made to produce the metric as loading cycles are estimated from activity duration. The notion of creating a metric using influential aspects of osteogenic exercise (magnitude, frequency and duration) is well thought, however, the assumptions surrounding the metric (*e.g.,* loading cycles) make it an inaccurate measurement of bone load. Therefore, accurate quantification is needed to understand the effects of mechanical load on bone. This may be achieved through external load measurement tools such as accelerometry, IMU's, force plates and motion capture if they can be shown to be associated with bone load and subsequent adaptation. Although external load can only be used to estimate bone load, the applicability of the measurement tools used to quantify bone load lack association to bone in an applied setting. The following sections will detail the measures used to quantify impact-based exercise and discuss how these factors have been associated to bone accrual.

Table 2.2. Effects of external load on bone from impact-based exercise in humans.

Study	Design/Subjects	Protocol	Load assessment	Bone assessment	Findings
Vainionpää et al 2009	RCT: 60 INT and 60 CON (females 35 – 40 yrs of age).	12-month impact exercise protocol (jumping, running, stamping). Supervised sessions 3 d/wk; 60 min/d. Home sessions 10 min/d.	Daily worn hip accelerometer during all waking hours.	Pre- and Post-intervention DXA, QCT and pre-, post- and 6 month bone turnover markers (PINP, PTH, TRACP5b).	No changes in bone turnover markers. BMD increased in exercise group. Accelerations 2.5 – 5.3 g incited the most changes to PTH levels.
Anliker et al 2012	RCT school based intervention: 30 INT and 30 CON (boys and girls 8 – 12 yrs of age).	9 month high impact exercise (jumping, sprinting). Supervised PE lesson 2 d/wk; 10 min/d. Exercise frequency (number of jumps) increased every 6 weeks.	Force plates used during single legged hopping.	Pre- and Post-intervention pQCT scans of the non-dominant tibia.	No changes in GRF data for either group. No adaptations found between groups, however, increase in INT was greater in BMC, BMD, bone area, SSIPOL.
Johannsen et al 2003	RCT school based intervention: 28 INT and 26 CON (children 3 – 18 yrs of age).	12 week drop jump intervention (45 cm box). Supervised sessions 25 jumps; 5 d/wk.	Pre- and Post-intervention force plate assessment.	Pre- and Post-intervention DXA and left tibia pQCT.	Increase in total and leg BMC of jumpers. No differences in load data.

Macdonald et al 2007	RCT school based intervention: 281 INT and 129 CON (children grades 4 - 5).	16 month supervised intervention (skipping, dancing, circuits, resistance bands) 5 d/wk; 15 min/d. Bounce at the bell (CMJ or side to side jumps) 4 d/wk; 9 min/d.	Vertec device for standing long jump.	Pre- and Post-intervention pQCT of left tibia.	Distal tibia bone strength index greater in INT prepubertal boys. No other significant changes in bone reported. No report of load data.
Nogueira et al 2014	RCT school based intervention: 71 INT and 67 CON (girls 10 yrs of age).	9 month intervention (capoeira – jumps, hops, cartwheels). Supervised 3 d/wk; 10 min/d. Exercise frequency (number of movements) increased gradually.	Yardstick for vertical jump.	Pre- and Post-intervention BUA (n = 10), DXA and pQCT (n = 13).	Vertical jump increased INT group. Calcaneal BUA increased in INT. Greater improvements in INT for LS bone structural strength.
Cheng et al 2002	RCT: 12 INT, 15 HRT, 10 INT & HRT, 15 CON (female 50 – 57 yrs of age).	12 month circuit intervention (skipping, jumping, hopping). Supervised session 2 d/wk. Home session 4 d/wk.	Force plates for jumping and bounding.	Pre- and Post-intervention CT scan of dominant femur and tibia.	Increase in proximal tibia BMD and bone mass spectrum in INT and INT & HRT. Limited report of load data.
Pinho et al 2020	RCT: 21 INT and 17 CON (elderly female 60 – 70 yrs of age).	20 week high impact supervised intervention (jumping) 3 d/wk; 60 min/d.	Pre- and Post-intervention force plates for drop jumps and squat jumps.	Pre- and Post-intervention DXA and dominant limb HR-pQCT.	INT increase in LS Tb.bone score, Tb.thickness. Limited report of load data.

Nikander et al 2012	RCT: 37 INT and 30 CON (breast cancer patients 38 – 66 yrs of age).	12 month intervention (step aerobics or circuits). Supervised session 1 d/wk; 30 – 40 min/d. Home session 3 d/week.	Pre- and Post-intervention CMJ force plate and IKD assessment.	Pre- and Post-intervention DXA and left tibia pQCT scan.	Limited effect of load data on bone characteristics. Small effects in bone mass, CSA and bone structural strength.
Allison et al 2013	RCT: 35 older male (65 – 80 yrs of age). INT and CON leg.	12 month home intervention (unilateral hopping). 3 - 7 d/wk; 5 x 10 hops/d.	Pre-intervention and 6 month force plate assessment of hops.	Pre- and Post-intervention DXA.	Increase in peak GRF following 6 months of intervention. FN BMD, FN BMC and CSA higher in INT leg post-intervention.
Bailey & Brooke-Wavell 2010	RCT: 45 INT and 19 CON (females 18 – 45 yrs of age).	6 month intervention (unilateral hopping). Unsupervised 5 x 10 hop/d; 2, 4 or 7 d/wk.	Pre- and Post-intervention assessment of maximal hop and 10 consecutive hops.	Pre- and Post-intervention DXA.	Greater increase in FN BMD of 7 day hoppers. Hop height and GRF also increased in INT leg compared to CON.
Greenway et al 2015	RCT: 52 INT and 55 CON (premenopausal female).	64 week intervention (step dropping or wall dropping). Unsupervised 6 – 40 reps/d; 4 d/wk.	Force plates used to measure impact forces for upper and lower body programs.	Pre- and Post-intervention DXA.	Increases in radius BMD in upper body INT. Increases in hip and spine BMD in lower body INT. Increase in total body BMD of both INT. Limited report of load data.

Rantalainen et al 2009	Cross-sectional: 15 young males (25 ± 3 yrs of age).	Supervised fatiguing bilateral jumping exercise performed to exhaustion. 65% of target GRF to determine failure.	Force plates to monitor continuous jumping.	Bone turnover markers CTx and P1NP taken pre-, post-, 2 hours post, 24 hours post and 48 hours post jump protocol.	Increase in CTx from baseline to 48 hours post jumps. Positive correlations shown between maximal GRF, slope of acceleration, osteogenic index and PINP.
Varley et al 2023	15 first year academy footballers INT and 13 recreational footballers CON (male 16 yrs of age).	Supervised 12 week training period (running, technique, matches). Increase in training volume from 7 hrs/wk to 11 hrs/wk.	GPS to monitor training across 12 week period.	Baseline and 12 week pQCT scan of dominant leg.	Increase in bone mass, Tb.density, SSIPOL, cortical area, cortical thickness in INT. Positive correlations between run distance and cortical density and peak speed and Tb.density.

Varley et al 2023	Longitudinal perspective study on 20 senior male footballers	Supervised season long perspective.	GPS to monitor training across season.	DXA and pQCT scans performed at start of pre-season, end of pre-season, during season and end of season.	Increases in body BMD, legs BMC, body BMC, tibial mass, strength, density between end of pre-season and during season. Decrease in tibial mass during season to end of season. Positive correlations between leg BMC and total distance, accelerations, decelerations, and tibial strength and accelerations.
Jämsä et al 2006	RCT: 34 INT and 30 CON (premenopausal females 35 – 40 yrs of age).	12 month high impact intervention (stamping, jumping, running). Supervised session 3 d/wk; 60 min/d. Home session 7 d/wk; 10 min/d.	Daily worn hip accelerometer during all waking hours.	Pre- and Post-intervention DXA of left proximal femur.	BMD correlated with accelerations exceeding 3.6 g. Strongest association with 5.6 g. Increase in BMD in INT group.
Rantalainen et al 2008	Cross-sectional: 20 active males and 20 active females (24 ± 3 yrs of age).	Supervised Neuromuscular measurement protocol. IKD 5 – 10 submaximal contractions followed by 10 – 15 bilateral jumps.	Force plates assessing maximal jumps. Torque assessment via IKD.	Tibial pQCT at single time point.	GRF and torque correlated positively with tibial compressive bone strength and cortical moment of inertia.

Rantalainen et al 2010	Cross-sectional: 221 premenopausal (23 ± 5) and 82 postmenopausal females (58 ± 1).	Supervised CMJ performed.	Force platform to assess CMJ.	Dominant tibia pQCT at single time point.	Premenopausal females had higher bending, compressive bone strength, impulse and peak power. Positive association between bone strength and bone loading.
Wu et al 1998	15 INT, 8 substitutes, 10 CON (rhythmic gymnasts 18 – 21 yrs of age).	Cross-sectional study of supervised rhythmic gymnasts compared to healthy CON.	Force plates to assess take-off and landing.	DXA scan at single time point.	Impulse was greater in landing leg of INT. BMD was greater in the left leg of INT.
Choi et al 2021	168 male and 258 female (consecutive hospital patients).	Cross-sectional study of supervised hospital patients.	Motion capture of 9 m long walk at self-selected speed.	DXA scan at single time point.	In males correlations shown between maximum hip power, trochanter BMD and femur BMD. Females found correlations between hip power, trochanter region and total femur.

El Deeb et al 2014	17 normal BMD and 17 low BMD (postmenopausal females 50 – 65 yrs of age).	Cross-sectional study of supervised hospital patients.	Motion capture and force plates used to assess gait pattern at self-selected speed.	DXA scan at single time point.	Low BMD patients produced less hip, knee and ankle power and extension moments. LS and FN BMD positively corelated with extension moments.
Bolam et al 2015	RCT: 13 high dose INT, 15 moderate dose INT and 14 CON (50+ yrs of age).	9 month intervention study (jumping, bounding). Supervised session 2 d/wk; 60 min/d. Home session 2 d/wk; 40 – 80 impacts.	Force plates (n=5) to assess drop jumps and multi-directional jumping.	Pre- and Post-intervention DXA and bone turnover markers (BAP and CTX).	No differences in bone markers or bone characteristics between groups. Limited report of load data.
Heiniö et al 2015	Female athletes (17 – 40 yrs of age). 19 high impact, 18 odd impact, 17 high magnitude, 17 repetitive impact, 17 repetitive non-impact, 19 CON.	Cross-sectional study on different type of loading athletes.	Force platform to assess CMJ.	DXA scan at single time point.	High impact showed positive correlation between peak force and LS trabecular bone score. High magnitude showed positive correlation between peak force and BMD.

Ahola et al 2009	35 healthy females (35 – 40 yrs of age).	12 month impact intervention (stamping, jumping, running). Supervised session 3 d/wk; 60 min/d. Home session 7 d/wk; 10 min/d.	Daily worn hip accelerometer during all waking hours.	Pre- and Post-intervention DXA and QCT.	6 months high intensity impact positively associated with bone change. Impacts over 3.9 g positively correlated with FN BMD at 12 months. Number of impacts over 1.1 g within 6 months correlated with trochanter BMD.
Rowlands et al 2020	Secondary analysis: 124 males and 96 females (23 yrs of age).	Longitudinal study following bone development in relation to physical fitness and lifestyle. Unsupervised.	Daily worn hip accelerometer, 24 h/d; 5 consecutive days.	DXA scan at single time point.	High intensity physical activity optimises BMC and aBMD and is advantageous for hip aBMD and total body BMC.

Abbreviation: CT – controlled trial. RCT – randomised control trial. INT – intervention group. CON – control group. CMJ – counter movement jump. BUA - broadband ultrasound attenuation. LS – Lumbar spine. HRT – hormone replacement therapy. Tb – trabecular. IKD – isokinetic dynamometer. FN – femoral neck. CSA – cross-sectional area, GRF – ground reaction force, JRF – joint reaction force. BAP – bone-specific alkaline phosphatase.

2.7.1 Accelerometry

Accelerometers, specifically triaxial sensors, activate distinct electromechanical systems that detect changes in acceleration. The capability of collecting wireless data allows the process to be a practical method that other methods (force plates, motion analysis) are incapable of being (Raper et al., 2018). Most commercially available sensors are triaxial, meaning movement can be measured across three anatomical planes; transverse (vertical), sagittal (mediolateral) and frontal (anteroposterior) (Sasaki et al., 2016). As the body moves within three planes it is important to be able to measure across each axes to gain a complete understanding of any movement being performed. Accelerometer data is processed using sampling rate frequencies (Hz), with raw data typically expressed in meters per second or gravitational force (where 9.81 m/s^2 = 1g). The sampling frequency should fulfil the Nyquist principle; the frequency should be at least twice the rate of the highest movement frequency sample (Grenander, 1959). If this criterion is not met, measurements of rapid motions (higher frequency domain) will be distorted as the sampling signal will fold into lower frequencies to be within the bounds of the active sampling rate. The same issues are observed during filtering as bandpass filters adjust frequency signals at different rates. Filtering frequencies are attenuated to facilitate noise artefacts and sensor drifts within the raw signal (Chen & Bassett., 2005). Applying an inappropriate bandwidth cut-off can result in deceptive data as an extensive cut-off will include external noise in the signal, which is not relative to the physiological measurement, whilst a narrow cut-off will extract relevant signals recorded during the movement. Due to the number of sampling rates, frequency rates and accelerometer models, it is rare for research studies to consistently match these three variables (Elvin et al., 2007; Tran et al., 2010).

Accelerometers are available in different formats; skin-mounted accelerometers, IMUs, GPS, pedometers, watches and smartphones. The location of the accelerometer is important as bone is known to respond in a site-specific manner to load (Adami et al., 1999; Winters-Stone & Snow., 2006; Varley et al., 2019). Therefore, back-mounted GPS units and hip-mounted accelerometers cannot provide accurate data on distal bone as accelerations differ between anatomical sites (Nedergaard et al., 2017). Studies have used various anatomical sites when measuring accelerations in the tibial bone; distal (Wee & Voloshin., 2013; Mercer et al., 2003), proximal (García-Pérez et al., 2014) and midpoint

(Chambon et al., 2014). Distal placement is shown to result in greater peak accelerations in comparison to proximal placement, which is likely due to reduced angular motion and gravity interaction within the time domain. Therefore, anatomical placement can contribute to a misestimation of up to ~2 gravitational units (Lafortune & Hennig., 1991). A proximal sensor placement experiences a lower oscillation compared to the distal end meaning the underestimation of a movement is likely. For this reason, precise site-specific placement is important for understanding the magnitude of load at the point of interest when investigating the effects of load on bone. Therefore, site-specific placements of the bone being measured are needed, as using non-site-specific measures to infer distal bone load may not offer a true estimate of the load being produced.

During day-to-day physical activity the intra-variability of accelerometry has been shown to be good (ICC: 0.82) over a week in middle-aged adults (Brady et al., 2023) and good (ICC: >0.79) in year-to-year variability in older adults (Löppönen et al., 2021). During physical activity, the overall, absolute and relative reliability of accelerations in the vertical, anteroposterior and mediolateral axes are also good (>0.75). ICCs are reported to be greater than 0.77 (vertical), 0.88 (anteroposterior) and 0.78 (mediolateral) during linear movements whilst no differences have been shown in inter-examiner (0.86) and intra-examiner (0.87) accelerometry data during walking tasks at various speeds (Kavanagh & Menz., 2008). Furthermore, across two 7-day periods of daily activity (Sirard et al., 2011) sensor reliability is reported to be high (ICC: 0.77 – 0.90). Since accelerometers are shown to be a reliable measure of movement, they may be used to provide information on estimating bone load.

Previous studies observing physical activity and bone characteristics using accelerometers have reported moderate-to-vigorous physical activity and vigorous physical activity are positively associated to BMD, cortical bone mass and bone strength (Haapala et al., 2022; Deere et al., 2012; Gracia-Marco et al., 2011; Sayers et al., 2011). However, these studies apply predetermined cut-off points to categorise exercise intensity that may not reflect the mechanical load required to infer bone accrual. Thus, applying specific loads to understand the influence of physical activity on bone adaptation is necessary. Furthermore, metrics such as daily impacts (Ahola et al., 2009; Vainionpää et al 2009; Jämsä et al., 2006) and acceleration slopes (Heikkinen et al., 2007) are accelerometer-derived metrics that have been previously positively associated with

changes in BMD. These studies have shown impacts exceeding 3.6 g's and acceleration slopes above 1000 m/s^3 induce an osteogenic response in hip BMD and femur BMD, supporting the evidence that high magnitude activity is associated to greater bone adaptation than low magnitude physical activity. It is suggested that peak resultant accelerations may better reflect bone load during sporting activities due to sudden change of direction across multiple planes (Stiles et al., 2013). Since multidirectional, high-impact activity is shown to be osteogenic, assessing peak resultant accelerations and associating them to bone characteristics should be examined. Furthermore, knowledge of accelerometer reproducibility during sport-specific movements and examining associations between accelerations and bone characteristics can encourage others to measure external load as a proxy measure of bone load. One way of measuring movements in applied environments could be with IMUs.

2.7.2 Inertial Measurement Units

IMUs are a variation of accelerometry that measure a body's acceleration, angular rate and orientation. The variability of IMUs is observed to be good when producing resultant accelerations during running (ICC: 0.84 - 0.97) (Sheerin et al., 2016). Higher variability is portrayed in axial accelerations (ICC: 0.73 – 0.95) compared to resultant accelerations, likely due to axial measures relying upon the orientation of the device, whereas resultant measures incorporate all axes (Sheerin et al., 2016). However, both axial and resultant acceleration variability are interpreted as moderate to excellent and have trivial effect sizes (0.01 – 0.17) across all running speeds with most subjects (14/16) showing a mean difference of <5% (Sheerin et al., 2016). During team sports tasks (*e.g.,* change of direction, deceleration, etc) it has also been shown that impact load inter-unit reliability is good (ICC: 0.79 – 0.96) inferring IMUs can be a reliable measurement tool for external load (Armitage et al., 2021).

IMUs are a method purported to estimate bone load (Besier., 2019), but their ability to accurately determine subsequent bone accrual is not established. Current research tends to use a single wearable sensor as a way of capturing bone load data and the development of algorithms to reduce load estimation errors have been progressing (Matijevich et al., 2020) (Figure 2.8). IMU Bone Stimulus is a metric created to estimate the load required to initiate a response in bone to remodel. This was based on the theory that bone is

influenced by load magnitude and the number of cycles (Beaupré et al., 1990). The concept has been tested to predict changes in BMD following exercise (Ahola et al., 2010). The metric is shown to plateau after reaching a certain number of cycles in conjunction with the knowledge that bone cells reduce in mechanosensitivity during repetitive movements (Robling et al., 2001).

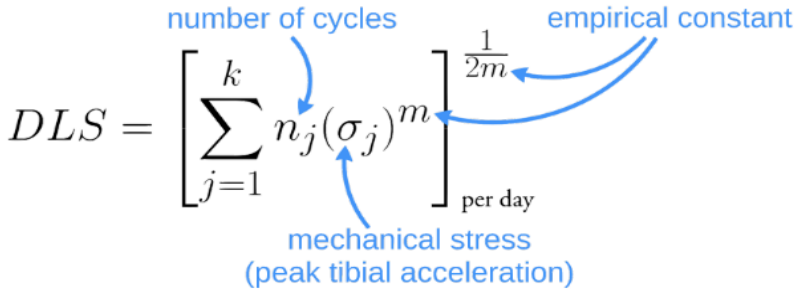


Figure 2.8. Daily Load Stimulus (DLS), or Bone Stimulus metric (Besier. 2019). Integration of number of load cycles (n), peak tibial acceleration (σ) and an empirical constant (m).

Cumulative load is the linear sum of all impacts and as load magnitude influences bone adaptation, cumulative load may present a better association to bone during multidirectional, high impact exercise where monotonous movements are less frequent. Cumulative load is calculated as:

Cumulative Load = (500 x 1g) + (1000 x 4g) + (300 x 10g) = 7500 (7.5k)

The number of impacts is multiplied by the gravitational unit creating a cumulative impact load. This metric may help quantify the external load acting on bone as it allows the user to identify exercise magnitude and frequency which are key determinants for bone adaptation. Although Besier (2019) has suggested theoretical reasoning of using these metrics with bone data, there are no studies known to the author that use the blue trident IMU metrics and measure bone characteristics. Recently, it has been suggested peak axial accelerations can be used as practical indicators of load rate during running supporting the approach of assessing external load with IMUs (Doyle et al., 2024). However, Matijevich et al. (2020) proposed combining IMUs with motion capture and machine learning can improve tibial force estimates up to four-fold compared to current conventional approaches using wearables. Therefore, associations between accelerations

and exercise load may be observed, but they are not able to explain the internal loads experienced by the musculoskeletal system. However, as bone responds to site-specific load (Adami et al., 1999; Winters-Stone & Snow., 2006; Varley et al., 2019), being able to apply IMUs to specific anatomical locations is advantageous. This is useful to both practitioners and researchers as it allows them to study the accelerations at sites relevant to sporting movements. In order to understand an ecologically valid load, studies are required using site-specific applied measurement tools, such as IMUs, to associate external load and bone characteristics. Other than GPS units, external load is scarcely quantified and with the site-specific attachment of IMUs they may be useful for applied research.

2.7.3 Force Plates

Force plates are a means to examine external forces, reporting GRF in three axes (vertical, anteroposterior and mediolateral). Applying Newtons laws of motion (law of acceleration and the law of action/reaction) when a body applies force to the ground, the ground applies a force back of equal and opposite magnitude, which we can measure through GRF. Acquiring data from force plates allows the user to calculate a variety of force metrics dependent on whether the force plates are measuring triaxial or uniaxial (vertical force only). Rate of force development (RFD) refers to the ability of the neuromuscular system to increase force from a low or resting level as quickly as possible (Rodríguez-Rosell et al., 2018), thus RFD is considered an important metric to measure force-time series. Research suggests RFD is the most appropriate metric to measure rapid force production in human movement as it can be measured during static and dynamic conditions (Rodríguez-Rosell et al., 2018). It has been shown to increase with resistance training (Holtermann et al., 2007) and impact training (Jensen & Ebben., 2007) suggesting the force-time series properties of the metric may be favourable over 'force only' variables. Impulse is a metric similar to RFD, derived from force and time, and often referred to as the area underneath the curve (impulse = force x time). Therefore, the greater the peak force or the longer the duration of force, the higher the resultant impulse. Unlike RFD, impulse is not a measurement of instantaneous force production but a measurement of maintaining force. These external load metrics may be useful surrogates of bone load due to their acknowledgement to muscle-derived load.

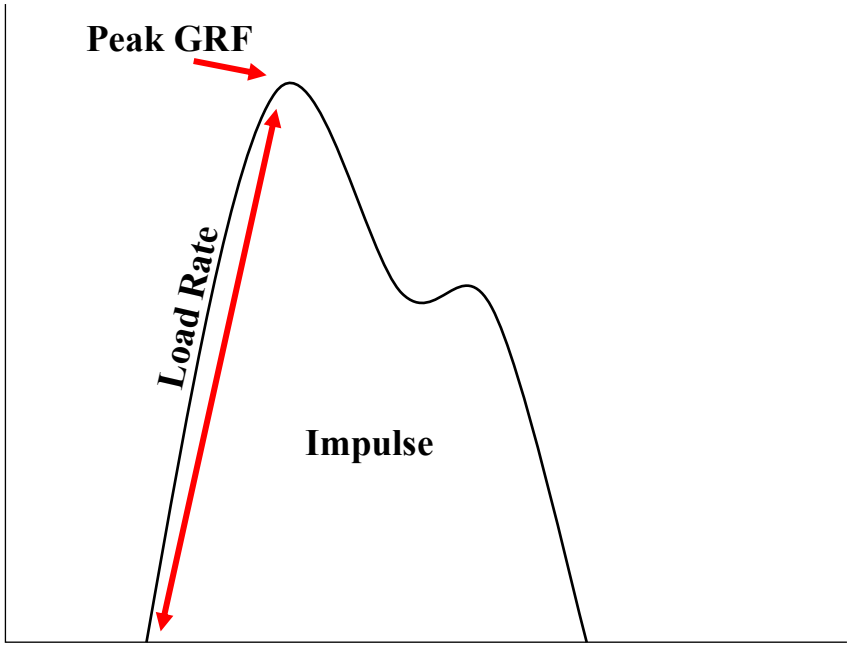


Figure 2.9. Common metrics found in research calculated from GRF trace.

The reproducibility of peak force (within group ICC: 0.93; between group ICC: 0.91 – 0.96) and RFD (within group ICC: 0.83; between group ICC: 0.63 – 0.94) are demonstrated to be moderate to excellent in healthy subjects performing jumps (Hansen et al., 2011; Lombard et al., 2017; Walsh et al., 2006). They are also observed to have low variability during jumps (CV: < 6.05%; Hansen et al., 2011; Lombard et al., 2017), whereas data variability during running are shown to fluctuate as peak and resultant force produce little variation (CV: < 6%). Force-time metrics, such as RFD, produce higher variability (CV: 10 – 21%) in young and older athletes (Korhonen et al., 2010). These studies indicate force plates can be used as reliable measures of external load, but more so during jumping and landing tasks compared to running tasks. There are also differences in reproducibility and variability depending on the metric being calculated. For example, force measurements, such as peak GRF, show higher within and between subject reliability than force-time metrics such as RFD. Therefore, it is important to perform inter- and intra-statistics when testing with force plates to understand the reliability of the metrics being used.

GRF metrics have shown high-impact exercise is associated with an increase in BMD in older women (Kohrt et al., 1997), middle-aged men (Rogers & Hinton., 2010) and gymnasts (Wu et al., 1998). Bailey & Brooke-Wavell (2010) observed an increase in femoral neck BMD and peak GRF of the exercising leg when performing unilateral hops

seven days a week for six months, indicating greater peak GRF may be associated to greater BMD. This was replicated by Allison et al. (2013) showing increases in peak GRF alongside increases in femoral neck BMD and BMC compared to the control leg during hopping exercises over six months. Bilateral jumping using force plates have also shown positive correlations between peak GRF and bone strength (Rantalainen et al., 2010; Rantalainen et al., 2009; Rantalainen et al., 2008). Similarly in high impact athletes, a positive correlation between peak GRF and BMD when performing CMJs has been shown (Heiniö et al., 2015). As GRFs are suggested to impose ~30% of the load on bone (Matijevich et al., 2019) they may be useful to guide the relative intensity of bone load during activity (Bassey et al., 1997). Force production is associated with positive bone health (Hinton et al., 2015), therefore proxy measures may help quantify bone load when investigating bone adaptation. This is beneficial for optimising the measurements of external load, however, the translation from lab to field needs to be developed as force plates are incapable of being used in applied environments.

2.7.4 Motion Capture

Motion capture tracks human movement by producing a skeletal model of the subject using a multi camera system. It produces a biomechanical assessment of locomotion that creates kinetic and kinematic data. The drawback of motion capture is the time-consuming nature meaning, like force plates, it is not an applied method. Furthermore, alternative methods can produce instantaneous feedback (IMU, force plates, GPS), whereas motion capture requires extensive processing and expertise to retrieve data. Historically motion capture is performed with a customised marker system composed of reflective markers attached to anatomical landmarks, however, recently marker less systems are being developed. The importance of developing an effective marker system is highlighted by Stief et al. (2013), where a non-specific model produced higher intertrial variability (8.9%) in hip and knee kinematics than a custom-made lower body model (6.3%). However, when comparing a clustered marker system to an individual marker system during walking trials the test–retest reliability of both methods was found to be good to excellent (ICC: ≥ 0.75; Mentiplay & Clark., 2018). Creating a marker system relevant to the movement being captured is essential for retrieving reliable results, therefore, understanding what is important to the task is required prior to data collection to optimise the reliability and validity of the metrics. Once the marker system is set up

the multicamera system triangulates the subject's 3D position in the field of view, allowing the user to create a skeleton specific to their marker system and produce metrics such as joint moments and joint angles.

Lower limb kinetics and kinematics can be used to observe technique changes during exercise which can result in differences in load. Moments are the measurement of force produced during motion, often calculated at joints. The movement of the joint adjoined with the moment is a metric that can offer insight into the load experienced during dynamic tasks. External joint moments of the hip have been shown to increase the predictability of proximal femoral BMD and BMC compared to height and body mass during walking and jogging in middle aged healthy adults (Moisio et al., 2004). Studies in postmenopausal women performing habitual walking have shown positive correlations between motion capture derived hip power and BMD (Choi et al., 2021; El Deeb & Khodair, 2014). It would be hypothesised that joint moments and power during high load movements, such as jumping, would be associated to higher gains in BMD and BMC. A non-invasive musculoskeletal model assessing tibial strains during multiple drop jump heights has shown drop jumping from 52cm was associated with more peak strain and maximum shear strain in comparison to 26cm and 39cm drop jumps (Wang & Dueball, 2018). The model was developed as a non-invasive alternative of using strain gauges to measure bone loads, and although this provides insight into the effects of load during high-impact activity, there was no attempt to assess the effects this may have on bone accrual (Wang & Dueball, 2018). This highlights the disconnect in bone load studies that concentrate on measuring load without assessing the associations to bone adaptation. Therefore, motion capture may be considered in a lab-based setting as it can offer valuable insight into joint kinetics and kinematics associated to changes in BMD (Choi et al., 2021; El Deeb & Khodair, 2014; Moisio et al., 2004). Although motion capture is not a feasible approach to measure bone load in an applied environment, lab-based research can help progress the understanding of mechanical load and bone adaptation.

2.7.5 Other quantification measures

GPS units utilise trigonometry from multiple satellites to determine the position of the GPS tracker and integrate micro inertial sensors in the forms of triaxial accelerometers, magnetometers, and gyroscopes to track further information on movement within three

axes (Malone et al., 2017). The popularity of the devices in sport and its practicality suggest it may be a useful method to measure external load during human movement. The integration of accelerometry allows the user to detect force measurements during movements useful for impact exercise. There are studies assessing GPS-derived load metrics and their association with bone adaptation (Varley et al., 2023; Varley et al., 2022). Varley et al. (2023) demonstrated a positive correlation between tibial strength, BMC, and GPS metrics (acceleration, deceleration, total distance) in professional male footballers over a season. Dynamic, high-load movements are important for creating an osteogenic response within bone, therefore monitoring movement with GPS may be a useful method. Monitoring impact load would allow an evaluation and quantification of external load during impact-based, multi directional exercise. However, it is understood that GPS may not be the optimal method for developing bone load metrics, as they do not measure site-specific external load. Therefore, to increase the validity of measuring load at distal bone it requires site-specific measurements to be taken at the area of interest *e.g.,* tibial placement when examining tibial adaptation. A common trait of the previously mentioned measurement tools is the inability of them to measure muscle-derived load which is also imposed on bone. A way of measuring muscular activity without being invasive is through electromyography.

Surface electromyography (sEMG) records the electrical activity of muscle and is a superficial measurement of muscular force (Kleissen et al., 1998), therefore, it may be used to interpret the muscle-bone relationship. The technique and experimental conditions require expertise to perform and interpret the method accurately with the sensor placement and testing environment influencing the output. A location away from the innervation zone of the muscle (the region where nerve fibres attach to skeletal muscle fibres) is recommended as the most optimal location to reduce the variability and likelihood of invalid estimates produced by muscular crosstalk in the sEMG signal (Hermens et al., 2000). High pass filtering can be used to suppress movement artifacts that may occur from the surrounding environment, however, performing 3D motion capture or video is better for the experimenter to understand the muscular activity during the movement (Kleissen et al., 1998). As muscle imposes load onto bone it may be assumed sEMG can inform on muscular contractions relevant to bone load. This has been observed using multiple jumping methods in early postmenopausal women, where greater forces were recorded in the semitendinosus and tibialis anterior during CMJ's and drop

jumps compared to heel drops (Montgomery et al., 2019). However, bone characteristics were not assessed meaning associations between muscular activity and bone characteristics could not be drawn. This highlights there may be potential for sEMG to relate muscle activity with bone load, but there is currently insufficient evidence to suggest the method can be used for this purpose.

Finite element models are a mathematical representation of bone used to assess how bone geometry, characteristics and mechanical loading can influence the tissue. They are widely used to explore mechanoadaptation in response to specific loading conditions that are beyond the capabilities of human interventions. The experimental method allows the exploration of bone formation to inform future study design (Meslier & Shefelbine., 2023). The design and inform process is observed in radial bone adaptation (Troy et al., 2020), femoral adaptation (Kersh et al., 2018) and osteoporosis exercise prescription (Martelli et al., 2020). The extensive analysis this method allows is insightful for bone physiology and has a place to progress our understanding in bone load. The drawbacks of the technique, however, are that they are time-consuming, lack practicality and require expertise to develop models. It should continue to be used alongside applied research but not as an alternative method to study bone load.

2.8 Summary

Impact based mechanical loading is important for bone health and can be used to promote positive bone adaptations, with load magnitude, frequency, and duration being important determinants for bone accrual. The use of rest periods between load bouts is thought to be a method of increasing bone formation, but it is not clear to what extent they can be used when load magnitude and duration are matched. Research performing impact-based exercise have measured external load to understand the effects mechanical loading has on bone adaptation. The association between external load and bone characteristics in applied settings, however, is not well established and it is not known what methods are currently used in applied practice. The scarcity of studies using bone characteristics and quantitative load data contributes to the ambiguity surrounding bones adaptation to exercise. Therefore, impact-based intervention studies measuring load and bone are desirable. This thesis aims to contribute to the advancement of knowledge in the field of bone adaptation to mechanical loading.

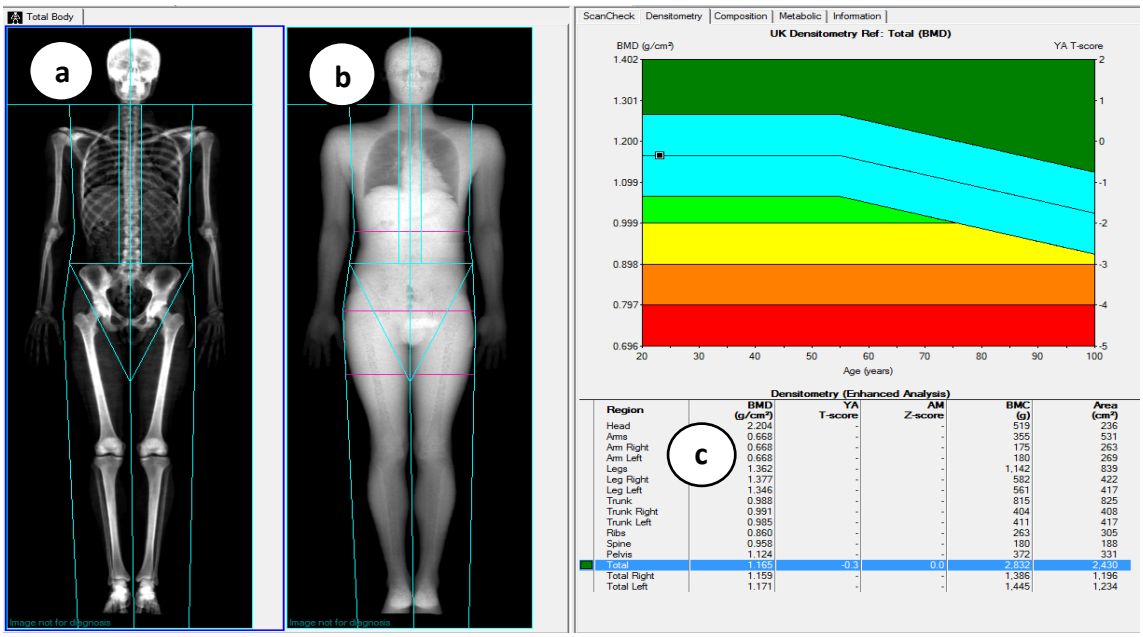
3 General Methodology

The methodologies described within this section have been used within the experimental chapters. To see details on protocols and measures taken during an individual experimental study please refer to the methods section within the individual chapters. All studies had approval from the Nottingham Trent University Ethical Advisory Committee. Chapter 6, 7 and 8 had dual approval from the National Health Service Research Ethics Committee and the Nottingham Trent University Ethical Advisory Committee.

3.1.1 *DXA*

Dual-energy x-ray absorptiometry (DXA, GE Healthcare, UK) was used as a method to assess bone characteristics and body composition in chapter 6, 7 and 8 (Figure 3.1). The underpinning theoretical basis of DXA states that across the photon energy range, the x-ray transmission through a physical object can be disintegrated into areal densities of any two chosen reference materials: bone and soft tissue (Lehmann et al., 1981). The radiation energies (low and high) emitted by the scan are variably attenuated based on the anatomical density and thickness, and the intensity of the emitted energy (Messina et al., 2020). The x-ray relies on the principle of higher photon energy equalling lower attenuation, meaning the denser the tissue, the more beams are attenuated *e.g.,* bone (Bazzochi et al., 2016). Bone and soft tissue can therefore be distinguished due to the higher atomic number of calcium and phosphorous in bone compared with the carbon, nitrogen and oxygen atoms in soft tissue (Blake & Fogelman, 2010). Upon analysis, DXA-derived BMD is a pixel-by-pixel map of BMD over the scanning field or a derivation from a specific location (*e.g.,* left leg), whereas BMC is calculated by multiplying average BMD by the area (Blake & Fogelman, 1997). DXA is an advantageous, gold standard method to assess bone and body composition for monitoring treatment response due to its high precision (CV: 1.12 – 2.21%) and ease of use (Blake & Fogelman, 2010; Patel et al., 2000). The reliability of bone measurements during DXA, are also shown to be excellent (ICC: 0.99; Lodder et al., 2004). However, the limitations of this method are mainly subject, or operator induced. For instance, internal artifacts (*e.g.,* implants or orthopaedic hardware) and external artifacts (*e.g.,* clothing or jewellery) can affect BMD accuracy, therefore if it is not possible to remove the artifacts, it is recommended that the region is omitted from the image for a truer reading (Morgan & Prater, 2017). Participants who underwent DXA scans as part of the studies presented in the present thesis did not produce any artifacts, therefore BMD measurements were not

affected. Body hydration and exercise can influence body composition results, particularly lean mass and total mass of body segments. To combat this issue and keep the biological variability as low as possible, measurement standardisation was performed by keeping the time of day consistent between measures for participants, limiting activity prior to a scan and limiting food and drink intake prior to a scan. When possible scans were performed in the morning as an overnight fast, rest and euhydration allow for the best conditions for reproducible measurements (Bazzochi et al., 2016). However, this was not always possible due to other commitments participants had to attend to (i.e., work), therefore in this case participants would be informed to restrict exercise and food and drink intake before scanning. Furthermore, the most prominent source of error for this methodology is operator related, as incorrect patient input and positioning lead to wrongful data output (Morgan & Prater, 2017). To minimise the possibility of these occurring during chapter 6, 7 and 8, the scans were conducted by the same trained operator to reduce any inter-operator variability.



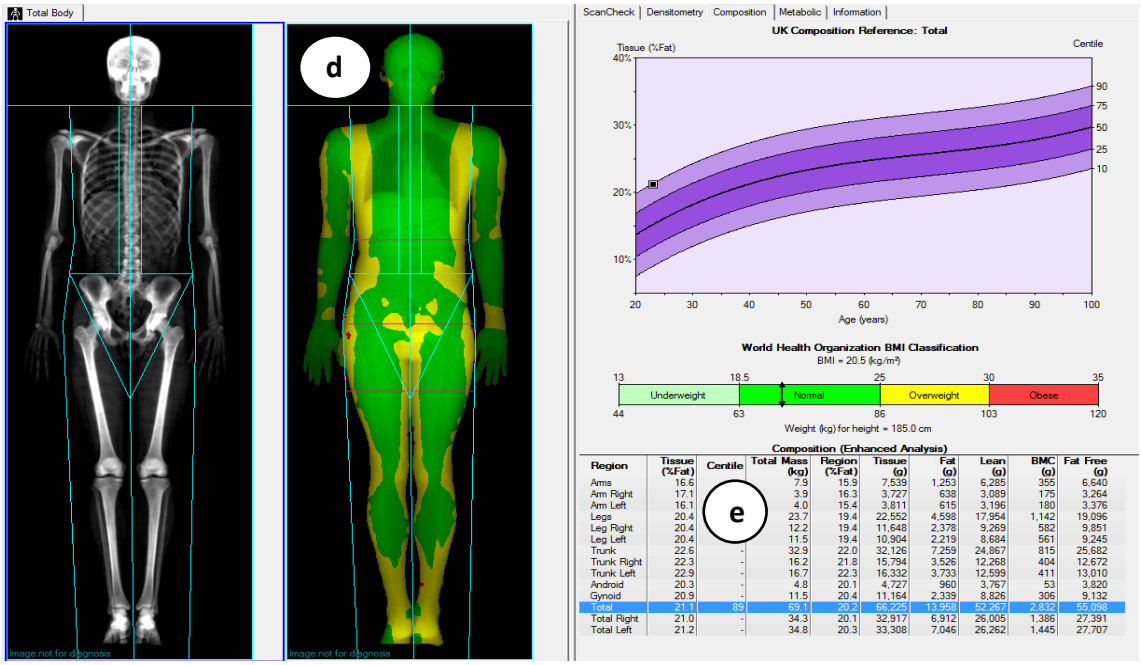


Figure 3.1. Data retrieved from a whole body DXA scan. a) X-ray image of skeleton, b) X-ray image with soft tissue, c) bone densitometry results, d) X-ray image with fat % level: green – low, yellow – medium, red – high, e) body composition results.

Before each scanning session, the DXA was calibrated using a phantom of a known density to ensure the system's standard of quality was precise and repeatable (Morgan & Prater, 2017). Participants removed any jewellery or metal they may have had on their body and were then positioned supine on the scanner bed, with their ankles and knees strapped using manufacturer issued Velcro straps to restrict any involuntary movement. The participants were instructed to lay motionless for the duration of the scan with their arms by their sides (Figure 3.2). If any movement artifacts were present, the image was classified as invalid, and the scan was repeated. Based on the purpose of this thesis, the following metrics were extracted: BMD (g/cm^2), BMC (g), bone area (cm^2), lean mass (g) and fat mass (g).

Figure 3.2. DXA scanner used within chapter 6, 7 and 8.

3.1.2 *PQCT*

Peripheral quantitative computed tomography (XCT2000L, Stratec Medizintechnik) was used to assess tibial bone mass, density and geometry in chapter 6 and 7 (Figure 3.3). pQCT is a low-cost and low-dose application of QCT used to assess bone architecture and strength in distal bone (Cervinka et al., 2010). Similar to DXA, pQCT utilises x-rays and provides an image based on beam attenuation of the tissues, however, unlike DXA it uses three-dimensional imaging to provide insight into the structure of cortical and trabecular bone (Lalayiannis et al., 2021). The product of pQCT is a process of scan data acquisition and tomographic reconstruction via mathematical calculations based on the images (Adams, 2009). The images and results are created by splitting the region of interest into predetermined sized slices by the operator. Before participant scans are performed quality assurance calibration is required using a phantom. Within the present body of work, a cone phantom was used on the day of each scan to ensure the pQCT was measuring accurately. The advantages of pQCT are the smaller size, higher mobility, lower radiation dose and site-specific applicability compared with whole body QCT scanners. Due to its application at perpendicular sites where x-rays are not largely attenuated by surrounding soft tissue, pQCT can determine cortical thickness in the lower limbs more accurately than QCT can in the lumbar spine (Cervinka, 2014). Furthermore, geometry-based parameters (*e.g.,* SSIX, SSIPOL) improve the prediction of bone strength

and bone failure loads at the tibial epiphysis and diaphysis (Cervinka, 2014) and are associated with whole bone strength (Cointry et al., 2014; Kontulainen et al., 2008; Siu et al., 2003). Its data acquisition and reconstruction imaging methods provide similar information as QCT on macro-structural traits in the distal tibia (Sievänen et al., 1998), but also inherit the resolution-related benefits and limitations of QCT-based densitometry. Limitations unique to pQCT scanners are the positioning and selection of reference points on long bones (Adams, 2009). The reference line requires standardisation and is selected to produce comparable data within research that can be difficult to replicate between studies, hence the limited comparison due to scanning variability (Adams, 2009). The current thesis used the distal tibia end plate for all scans as the reference line to ensure reproducible sites were measured between legs and participants.

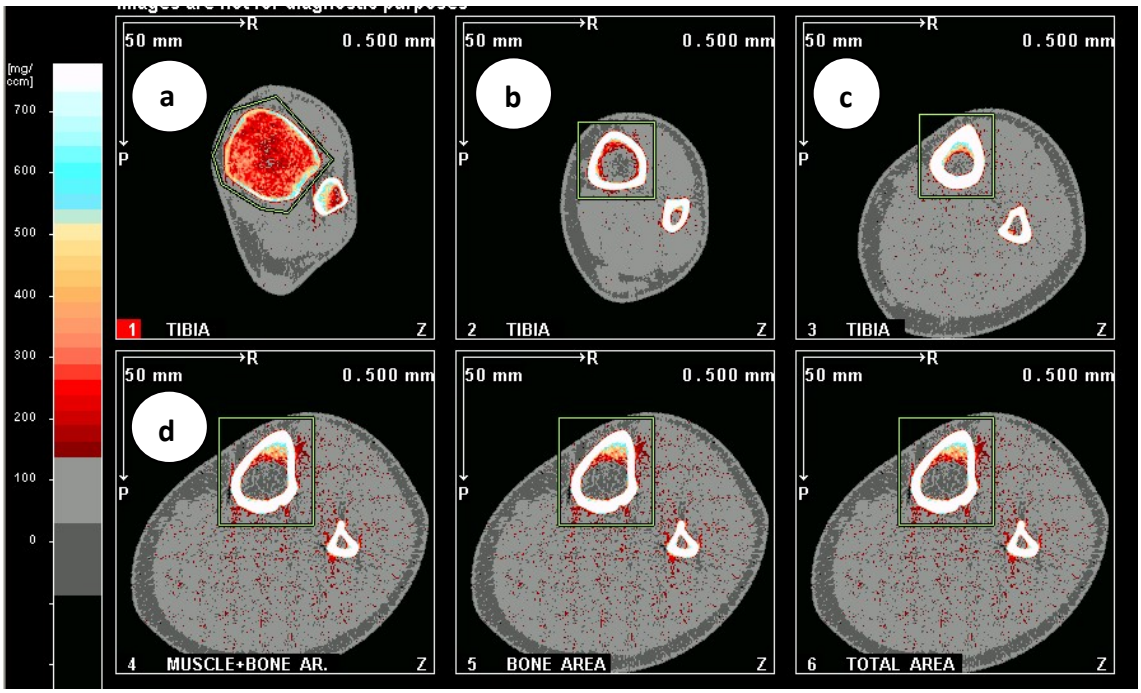


Figure 3.3. Data retrieved from tibial pQCT scan. a) 4% sectional image, b) 14% sectional image, c) 38% sectional image, d) 66% sectional image.

Tibial length was determined as the medial aspect of the tibial plateau to the medial malleolus. Participants were asked to remove their shoes and socks and expose their lower leg. The participant's leg was then placed in the scanner with their foot secured in a purpose-built integral attachment. The leg was aligned with an integral laser and clamped at the knee to restrict movement whilst the participant was directed to remain as still as possible during the scan (Figure 3.4). A scout scan was performed to confirm the location

of the distal end plate. In line with previous literature sectional images were obtained at the distal sites (4%, 14%) and the diaphysis of the tibia (38%, 66%) from the positioning line (Lalayiannis et al., 2021). The 4% site was used to obtain trabecular values at the metaphyseal bone, 14% and 38% were measuring diaphyseal sites for cortical values and the 66% site was used to obtain fat and muscle estimations (Lalayiannis et al., 2021). A voxel size of 0.5mm and slice thickness of 2.5mm was used for all measurements. A contour mode, with a threshold of 180mg·cm3, was used to separate soft tissue and bone. If any movement artifacts (inaccuracies in the measurement caused by motion) were present following the scan, the image was classed as invalid, and a repeat scan was performed. Based on the aims of this thesis, the following metrics were assessed: trabecular density (g/cm^2), cortical density (g/cm^2), cortical thickness (mm), periosteal circumference (mm), anteroposterior axial bone strength (SSIX), mediolateral axial bone strength (SSIY) and torsional polar bone strength (SSIPOL). As both DXA and pQCT scans emit radiation it was not possible to perform repeated scans on participants to determine reliability measures, as stated by ethics. Although the radiation exposure is minimal during both techniques, unnecessary exposure was avoided as it is the responsibility of the investigator to ensure the radiation exposure is kept as low as possible.

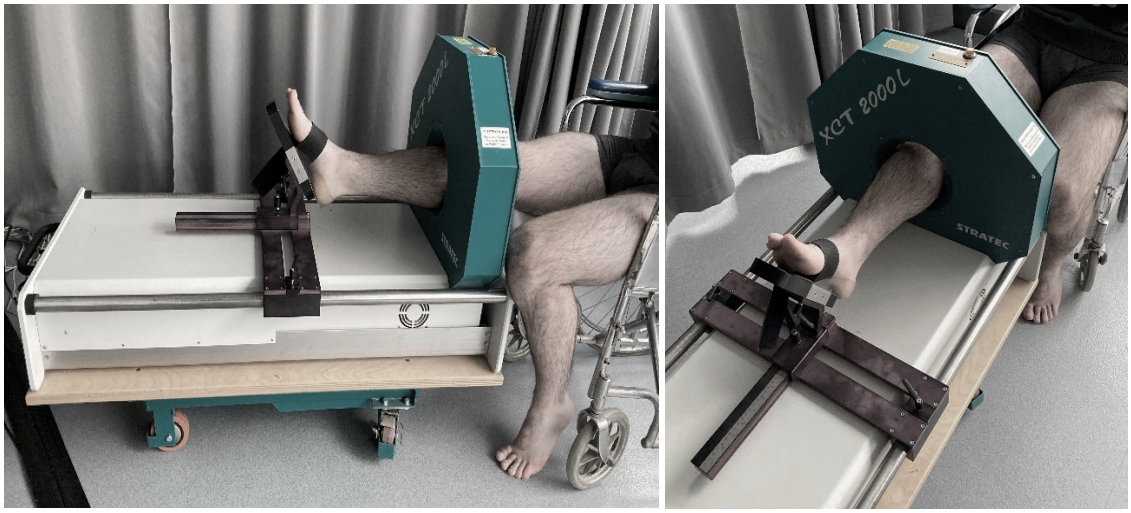


Figure 3.4. pQCT scanner used within studies 4 and 5.

3.1.3 *IMU*

The assessment of external load in an applied setting is performed consistently in the current body of work. This is shown throughout chapter 7 and 8. Therefore, based on previous literature and the practicality of the method (Epifano et al 2022; Besier., 2019;

Moore & Willy, 2019), IMUs were used to assess site-specific external load at the 14% site of the tibia. IMUs are small electronic devices that combine multiple sensors such as accelerometers, gyroscopes and magnetometers. The mechanical accelerometers consist of a mass suspended by springs in which the displacement of the mass is measured, giving a signal proportional to the force acting upon it and coinciding with Newton's second law (Ribeiro & Santos, 2017). A gyroscope is a spinning disc in which the axis of rotation is free to assume any orientation unaffected by tilting or rotation of its attachment. They are applied as angular velocity sensors and are useful for the measurement of motion and posture of the segment they are fixated on (Ribeiro & Santos, 2017). If equipped with magnetometers the IMU device has a total of 9 degrees of freedom meaning it can measure in the x, y and z axes of each sensor. Magnetometers measure the bearing magnetic direction and can increase the accuracy and performance of IMUs by improving the drift error, determined as an accumulation of small errors of measurement in accelerometer and gyro measurements (Ahmad et al., 2013). Within the present thesis, IMUs (dimensions 42 x 27 x 11 mm, mass 9.5 grams, operating range 200g; Blue Trident, Vicon Motion Systems Ltd, Oxford, UK), recording at 1600 Hz were secured with a self-adhesive overwrap (Lightpast Pro, Vivomed) at the 14% distal site of each tibia to match the 14% site of the pQCT scan. Tibial length was measured between the medial aspect of the tibial plateau and the medial malleolus. IMU data processing and analysis differed within chapter 7 and 8, therefore please refer to each chapter for a detailed methodology. Intra-rater reliability measures were performed in both chapters 7 and 8 for IMU data.

3.1.4 *Development of in vitro method*

The effects of rest periods on bone formation were assessed using a Flexcell bioreactor (Flexcell Int. USA). Before the study commenced, a suitable and reliable *in vitro* model was developed. This section describes the development process of the model (Figure 3.5).

Initially, a growth medium (GM) was created using Minimum Essential medium α (MEMα, Gibco) supplemented with 10% foetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin solution (Invitrogen). The solution was inverted 5 times to ensure it was mixed. 25 ml of GM was transferred to a T75 flask and incubated for 10 minutes whilst mouse pre-osteoblast cells (MC3T3, ATCC) were placed into a water bath to thaw. The GM was removed from incubation and 1 ml of the cell line was transferred to the

solution. To ensure the cells were dispersed within the GM they were aspirated and released ten times. The flask was placed in the incubator at ~37^{o}C and GM was replaced every two days during cell culturing until 80% confluency (concentration of cells) was reached. To prevent disruption amongst the osteoblasts when changing medium the new GM was placed in a water bath for 10 minutes at ~37^{o}C to replicate the incubation environment. The aspirated GM was discarded.

To culture the cells, GM was removed leaving the cell line attached to the flask. 25 ml of phosphate-buffered saline (PBS, Sigma) was added to and aspirated from the flask to wash the surface area. This process was repeated twice. 5 ml of trypsin was then added to the flask to cause cell detachment which was observed with a microscope to check for cell movement. Once detachment was confirmed 5 ml of GM was added to create a ratio of GM and trypsin of 1:1. The solution was aspirated and transferred to a falcon tube and centrifuged at 1000 rpm for five minutes. White pellets at the bottom of the tube were slightly visible depicting the presence of cells. Upon completion, 24 ml of GM was added, and the solution was aspirated ten times from the bottom of the tube and released at the top to homogenise the solution. Once homogenised 4 ml of the solution containing the cell line was added to 6 ml of GM (2 flasks contained 10 ml) and placed into incubation. The excess solution was aliquoted alongside 1ml solution (90% GM and 10% dimethyl sulfoxide (DMSO)) and placed in an Eppendorf tube stored at -80^{o}C. To create independent experiments this process was performed using 3 individual cell passages therefore a T75 flask containing 10 ml of solution equated to one experiment. This was repeated three times meaning a total of 3 flasks were used to culture cells. This was repeated for each of the loading protocols (n = 3).

Daily checks were performed to monitor the progress of cell confluency within each flask. Once 80% cell confluency was observed they were seeded into 6 well plates. When observed on occasion 80% confluency was exceeded meaning the cells were discarded and the process of subculturing of the cells was restarted. The first part of cell seeding was the same as subculturing the cells as mentioned in the previous paragraph using PBS and trypsin to detach the cells from the flask. Once detached and transferred to a falcon tube 10 µl of the solution was placed into a hemocytometer (C-chip, Cambridge Bioscience, UK) and placed under a microscope for cell counting. The cells counted within the 10 µl solution were a representative for the total cells within the flask thus the

cells present was determined as the number of cells counted multiplied by 10^4. The cell density required for this experiment was 15,000 cells/cm^2 therefore as each well was 9.6 cm^3 and there were 6 wells per plate this meant the total number of cells needed from each flask was 864000 (15000 x 9.6 x 6). The number of cells needed was divided by the number of cells in the solution and multiplied by 100 to obtain a percentage of the total volume of cells needed (a ratio of GM to cells). The wells were seeded with 1.5 ml of solution. This ratio was subject to change dependant on the sub cultured flask being used as the number of cells generated from each experiment could vary.

Differentiation medium (DM) was created to induce osteogenic differentiation where osteoblasts secrete a mineralised extracellular matrix which contributes to bone formation. Firstly, tin foil was wrapped around a beaker as the compounds were light sensitive and then 0.0017612g of ascorbic acid (Sigma, A4403-100MG) and 0.0432079g β-glycerophosphate (Sigma, G9422-10G) was added followed by 20 ml of GM. The solution was mixed using a vibration plate on a low setting for 2 minutes. Falcon tubes were prepared in tin foil for 10 ml of the solution to be distributed using a sterilised syringe attached to a 0.2 µm filter and then 40 ml of GM was added to each tube. This was now complete DM to be used or stored in a refrigerator. DM was used within the well plates after the first 24 hrs and then replaced every other day whilst the cells were at rest (not receiving load). To change the DM within the wells the new DM was placed in a water bath (~37^{o}C) to replicate incubation. The medium within the wells was aspirated and disposed of on all days other than the time point being analysed. Therefore, on days 1, 3 and 12 the medium taken from the wells was placed into a falcon tube and stored at -20^{o}C for further analysis. After the medium was aspirated, the wells were washed with 1ml PBS and then 1.5 ml of fresh DM was placed into each well.

To induce load a Flexcell bioreactor (Flexcell Int. USA) was used on an *in vitro* model. Prior to placing the well plates into the bioreactor, the apparatus was sterilised using 0.1% bleach solution on the plate and base. The loading posts where the load is imposed were lubricated pre experiment to ensure the collagen-coated flexible bottom 6-well plates (Biopress, Flexcell) would not be damaged. The plates and cover were cleaned before they were placed into the Flexcell and incubator. Once the well plates were put onto the Flexcell base they were placed within the incubator and the bioreactor valves were clipped into the base. Once secured a sterilised glass cover was placed on top of the apparatus to

secure the well plates during loading. The Flexcell software was set up to apply 5 hours of 5000 μS (0.5% elongation), at a frequency of 1 Hz based on a review by Baudequin et al. (2019). To examine the effects of intermittent load in comparison to continuous load on bone formation the following conditions were applied:

- Intermittent load – 1 hour of load was followed by 3 hours 48 minutes of unloading in each 24 hour period.
- Continuous load – 5 hours of load was followed by 19 hours of unloading in each 24 hour period.
- Control – unloaded condition that received no load.

Data analysis was performed on day 1, 3 and 12. When removing a 6-well plate at its time point it had to be replaced with an empty well to ensure the load was applied consistently on the remaining experiments. Upon completion each condition was qualitatively analysed with alizarin red staining (ARS) using the well plate whilst the DM was aspirated and stored for quantitative analysis using ARS, ALP and PINP on a future date. Whilst developing the methodology for the protocol it was initially found that the Flexcell was applying a load above the level required (5601 μS – 5763 μS) due to an issue with the pressure valve supplying the bioreactor load. To amend the issue the pressure tubes supplying the load were washed out and dried to remove any water that was being built up in the lines which occurred from a loose attachment to the Flexcell. After securing the lines, the load was amended to range between 4954 μS - 5028 μS, therefore reducing the variability of the strain applying the intended load.

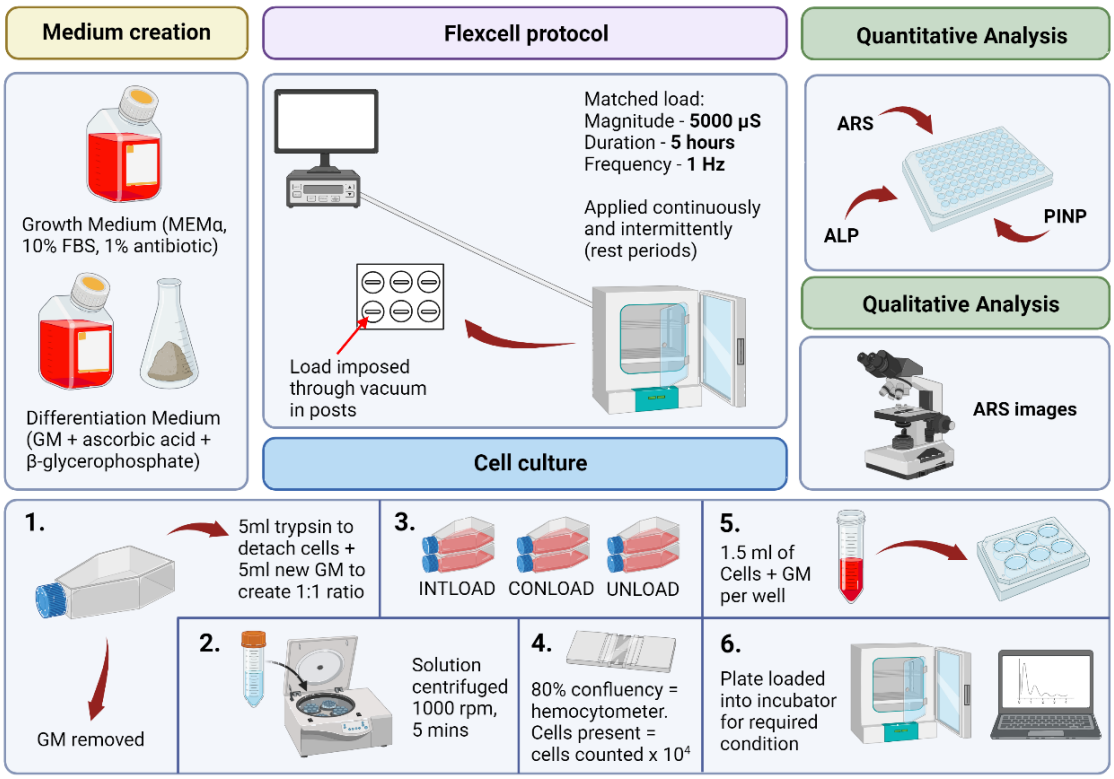


Figure 3.5. Schematic of methodological processes required during *in vitro* study (created with Biorender.com).

Alizarin Red S staining (Sigma-Aldrich) was performed to determine the presence of extracellular matrix mineralization on day 1, 3 and 12. After the medium within each well was removed the cells were washed with 1 ml PBS. Cells were fixed to the well with 1 ml of 10 % formalin to cover the cellular monolayer. After ten minutes formalin was carefully aspirated, and the wells were washed with 1 ml DH_2O. ARS staining solution was added to cover the monolayer, and the plate was covered in tin foil and incubated in the dark at room temperature. After 45 minutes of incubation the solution was aspirated, and the monolayer was washed four times with 1 ml DH_2O. Post wash 1 ml of PBS was added to each well and the plate was analysed under a microscope for qualitative imaging (see chapter 4 for images). To quantify ARS staining PBS was aspirated and 1 ml of 10% acetic acid was added to each well post qualitative imaging. Using a cell scraper, the cells were removed from each well and placed in a microcentrifuge tube to be incubated for 10 minutes in a 80°C water bath. Following incubation, the tubes were chilled on ice for 5 minutes and then centrifuged for 15 minutes at 17,000 g. 500 µl of the supernatant was transferred to a new Eppendorf tube and 200 µl 10% ammonium hydroxide was added to

neutralise the acetic acid. The solution was aliquoted with 150 µl into a 96 well microplate and optical density was read using a plate reader at a wavelength of 405 nm.

ALP was analysed using a colorimetric assay kit (Biovision, Abcam) on cell medium. ALP buffer, ALP enzyme, pNPP solution and stop solution were placed on a vibration plate for one minute at a low level prior to being opened. A 1mM pNPP standard was prepared by diluting 40 µl pNPP 5mM standard in 160 µl of assay buffer. Using 1mM standard, a standard curve (Appendix III) was created using the following dilutions:

Standard	pNPP 1 mM Standard (µl)	Assay buffer (µl)	Final volume standard in well (µl)	End amount of pNPP in well (nmol/well)
1	0	300	120	0
2	10	290	120	4
3	20	280	120	8
4	30	270	120	12
5	40	260	120	16
6	50	250	120	20

For sample wells, 80 µl of medium was added. 20 µl of stop solution was added to each background sample control to terminate ALP activity and mixed well by aspirating and releasing three times. 50 µl of 5 mM pNPP solution was added to each well containing the samples and control samples but not the pNPP standard well. The 96 microplate was incubated at 25°C for 60 minutes protected from light and then 20 µl of stop solution was added to stop the reactions. The plate was transferred to a shake plate to gently agitate the solution and then measured on a plate reader at an optical density of 405 nm.

A PINP assay (IDS, Immunodiagnostic Systems) was used for the quantitative determination of N-terminal propeptide of type I procollagen using cell medium. All standards (0 - 5) and controls (1 - 2) were prepared by adding 0.5 ml DH_2O to each solution and inverting them several times to ensure complete reconstitution. All reagents were brought to room temperature prior to the protocol. Firstly, 50 µl of each standard (0 – 5) and control (1 – 2) was pipetted into the appropriate wells of the 96 well microplate.

5 µl of sample with 45 µl sample diluent (PBS buffer containing PINP) was also added to their appropriate wells. 50 µl of biotinylated PINP was added to each well and sealed with an adhesive plate seal. The microplate was incubated at room temperature for 60 minutes on a plate mixer. After incubation each well was washed three times with 250 µl wash solution (PBS and Tween) and excess solution was removed by tapping the microplate firmly on absorbent tissue. 150 µl of enzyme conjugate (peroxidase conjugated to avidin) was pipetted into each well. The plate was then covered with adhesive plate seal again and incubated at room temperature for 30 minutes. Post incubation the wells were washed as previously described. 150 µl of TMB substrate (tetramethylbenzidine and hydrogen peroxide) was applied to each well and the microplate was sealed for a 30-minute incubation in the dark. Finally, 50 µl of stop solution was added to each well. The absorbance of the completed reaction was assessed from a microtiter plate reader at an absorbance rate of 450 nm and a reference of 650 nm within 30 minutes of completing the reaction. The intensity of the developed colour was inversely proportional to the concentration of PINP in the original sample.

3.1.5 *Statistical analysis*

The statistics used are reported in the methodological section of each chapter. All statistical analyses were performed using SPSS (IBM, SPSS Statistics, v.29). *P* values of <0.05 were deemed significant.

4 Intermittent tensile strain induces an increased response in bone formation markers compared to continuous load in mouse pre-osteoblasts when loading magnitude is matched.

4.1 Introduction

Bone can experience a variety of loads during exercise such as tension, compression, or torsion (Vashishth et al., 2001). Controlling the magnitude, frequency, duration, and type of load with a bioreactor allows conditions to be manipulated on cellular models. *In vitro*, bioreactors have been used to impose different types of load on cellular models (Zhong et al., 2013; Plunkett et al., 2010; Murray & Rushton., 1990). For example, the application of tensile load promotes osteoblast quantity and functionality important for bone formation, whereas compressional load has been observed to decrease the OPG/RANKL ratio and promote osteoclastogenesis (Zhong et al., 2013). Whilst compression and fluid shear stress are commonly experienced *in vivo*, tensile stress remains a common and easy method to trigger the cell response *in vitro* (Baudequin et al., 2019). The magnitude of mechanical load has also been shown to influence cellular bone formation, with higher loads causing increased osteoblastic activity (Jaasma et al., 2007). *In vitro* studies remove the systemic response that is present in human studies and help to understand the effects of load, which can optimise *in vivo* bone formation protocols. At present, the optimal loading regimen to promote osteogenesis, in terms of load magnitude, frequency, duration and type, remains unclear.

In vitro studies provide good insight into the mechanobiological response of bone cells and bone formation markers ALP and PINP are commonly used to assess osteoblast activity. ALP facilitates mineralisation by increasing local inorganic phosphate rates and reducing extracellular pyrophosphate concentrations, which act as an inhibitor of bone formation (Vimalraj., 2020). High magnitude cyclic load has been shown to cause greater osteoblast proliferation and ALP expression than low magnitude cyclic load (Yu et al., 2014; Jagodzinski et al., 2004). A bout of dynamic load lasting 2 hours resulted in the upregulation of ALP activity in human bone marrow mononuclear cells (Sittichokechaiwut et al., 2010). PINP is a marker of osteoblast activity and is produced during the extracellular process of type I collagen, the most abundant organic component of bone matrix. It has also been correlated with parameters of formation, such as osteoid thickness and volume (Chavassieux et al., 2015). PINP is shown to increase protein content in mechanically loaded osteocytes and osteoblasts after 1 hour (Wu et al., 2016) and 5 days (Vazquez et al., 2014). Osteoblasts are shown to respond acutely to load by releasing signalling factors, however, it takes days or weeks to initiate mineralisation. For

example, MC3T3 cells require at least 3 to 12 days before mineralisation begins (Addison et al., 2015; Kato et al., 2001). Rest periods inserted between high-frequency loading are shown to augment osteogenesis (LaMothe & Zernicke., 2004), but it is not clear what load regime optimises bone response. Incorporating rest periods during loading regimes can suppress potential deleterious effects of overloading and may improve osteoblast development.

Animal models have demonstrated that periods of rest incorporated into cyclic loading cycles can enhance the relative bone formation rate ~2-fold compared to continuously applied cyclic loads (Burr et al., 2002). Furthermore, periosteal bone formation, areal BMD and BMC have been shown to increase in rodents when implementing rest periods between loading cycles (Srinivasan et al., 2002; Robling et al., 2001). The relative mineralising surface, defined as the size of the interface between quiescent and newly formed bone, has been shown to increase after 14 seconds of rest incorporated between loading bouts in comparison to 0.5, 3.5 and 7 seconds (Burr et al., 2002), whilst mechanosensitivity fully restores after 8 hours of rest (Robling et al., 2001). Interestingly, George et al. (2022) compared continuous and intermittent loading in experimental and theoretical models. They observed intermittent load, in rats, increased cortical thickness, whereas continuous load decreased cortical thickness. A similar response was shown in their theoretical model where intermittent load favoured bone formation whilst continuous load promoted bone resorption (George et al., 2022). Rest periods are thought to resensitise bone to the effective mechanical load, which if implemented over time would likely translate into a greater osteogenic stimulus and promotion of bone formation. A major limitation of existing models is that these approaches do not match absolute load between conditions. Failure to do this exposes cells to different accumulated loads, even when the magnitude and frequency of load are matched, meaning the model is experiencing a different total load (LaMothe & Zernicke., 2004).

Exercise bouts consisting of intermittent load may have a role in optimising bone mechanosensitivity. It is not clear how the method of loading affects osteoblast activity when loading magnitude and duration are matched. Therefore, this study aimed to assess the pre-osteoblast response to cyclic intermittent and continuous loading patterns. It was hypothesised that intermittent load will induce a heightened response in bone formation markers (ALP and PINP) compared to continuous load.

4.2 Methods

4.2.1 Cell culture

Mouse pre-osteoblast cells (MC3T3, ATCC) were cultured in complete growth medium (GM) composed of Minimum Essential medium α (MEMα, Gibco) supplemented with 10% foetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin solution (Invitrogen). Cells were seeded on a 6-well plate at a density of 15,000 viable cells/cm^2 and incubated in a humidified 5% CO_2 atmosphere at 37°C. Once the cells reached 80% confluence, the GM was discarded, and the cells were washed with phosphate-buffered saline (PBS, Sigma). Differentiation medium (DM) containing αMEM, 10% FBS, 1% penicillin-streptomycin solution, ascorbic acid and ß-glycerophosphate was then added. DM was changed every 2 days. The total culture period was 24 hours in GM followed by 12 days in DM. Cells were collected for analysis on days 1, 3 and 12.

4.2.2 Mechanical loading

The computer-controlled bioreactor (Flexcell Int. USA) was employed to deliver mechanical loading. After switching to DM, MC3T3 cells on a collagen-coated flexible bottom 6-well plate (Biopress, Flexcell) were transferred to the bioreactor to undergo cyclic loading conditions under continuous tensile strain (n=3) or intermittent tensile strain (n=3) (Figure 4.1). Loading conditions were matched for the duration under strain (5 hrs), magnitude of strain (5000 μS = 0.5% elongation) and frequency (1 Hz). The magnitude of the strain was selected based on previous studies that state 5000 μS (0.5% elongation) is the upper end of physiological strain that allows for a window of optimal bone formation (Baudequin et al., 2019). Intermittent loading consisted of 1 hour of loading followed by 3 hours and 48 minutes of rest every 24 hours. During continuous conditions, 5 hours of strain were followed by 19 hours of rest. The unloaded condition was used as a control. Cells were collected on days 1, 3 and 12 to perform ARS, ALP and PINP analysis.

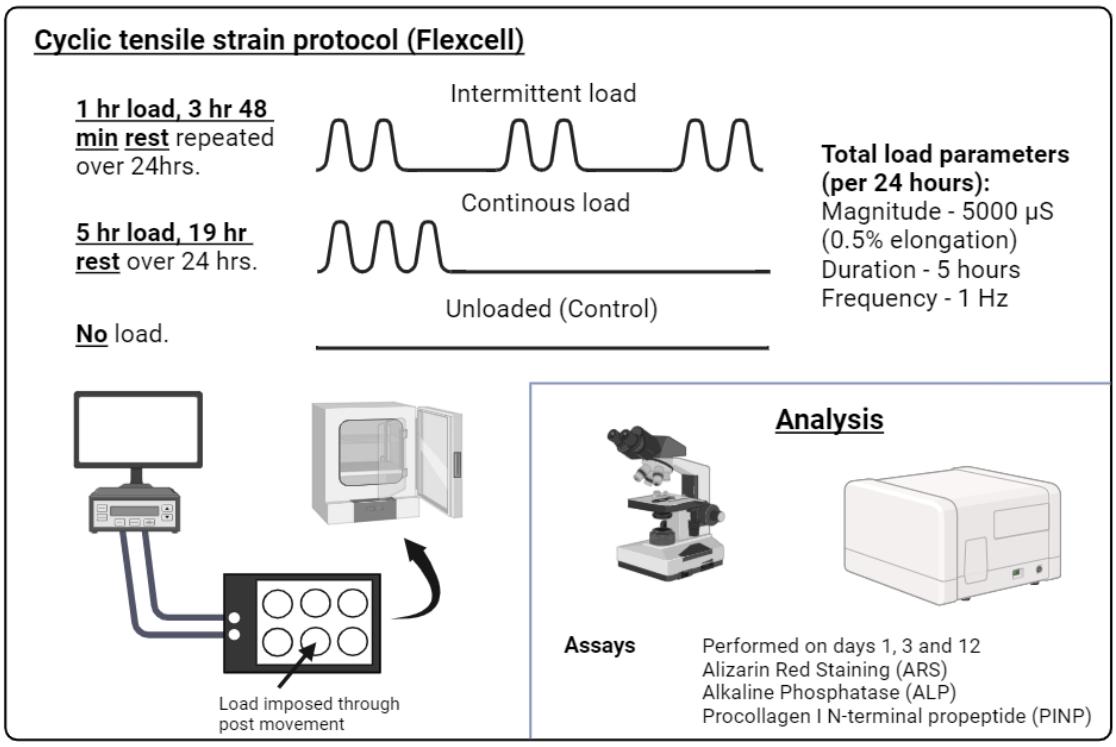


Figure 4.1. Schematic of methodological processes required during *in vitro* study (created with Biorender.com).

4.2.3 ALP activity

The assay (interassay CV, 4-7%; Alkaline Phosphatase Assay kit (Colorimetric), Biovision, Abcam) was performed using multiple standards prepared of assay buffer and pNPP diluted between 0 and 50 µL. Enzyme solution was added to each well before being incubated at 25°C for 60 minutes protected from light. Stop solution was added to each well after the incubation period to conclude the reaction. Post incubation the plate was gently agitated on a plate shaker and measured at an optical density of 405 nm on a plate reader. ALP activity was calculated as:

$$ALP\ Activity = \left(\frac{B}{\therefore T * V}\right) * D$$

Where:

B = amount of *p*NP in a well calculated from standard curve (µmol). ΔT = reaction time (minutes). V = original sample volume added into the reaction well (mL). D = sample dilution factor.

4.2.4 PINP assay

The assay (interassay CV, 9-15%; IDS, Immunodiagnostic Systems) was performed using a competitive enzyme-linked immunosorbent where 50 ml of each calibrator, control and diluted sample were incubated with a biotinylated PINP reagent in microtiter wells. Rinsing with DH_2O and wash buffer was followed by the addition of enzyme avidin to the wells before the rinse was repeated. Colour was developed using a chromogenic substrate (TMB). The absorbance of the completed reaction was assessed from a microtiter plate reader at an absorbance rate of 450 nm and a reference of 650 nm within 30 minutes of completing the reaction. The intensity of the developed colour was inversely proportional to the concentration of PINP in the original sample.

4.2.5 ARS staining

Alizarin red staining (ARS; Sigma-Aldrich) was performed to determine the presence of extracellular matrix mineralization. Prior to the fixation of the cells, the DM was aspirated and transferred into a falcon tube and stored at -20°C for ALP and PINP analysis. On days 1, 3 and 12 cells were fixed in 10% formalin and stained with alizarin red staining solution (pH 4.0) at room temperature and protected from light for 45 minutes. 10% acetic acid was used to collect cells followed by 10 minutes of incubation in an 80°C water bath. After this the solution was centrifuged at 17,000g for 15 minutes within microcentrifuge tubes. To neutralise the acetic acid, 10% ammonium hydroxide was added to the supernatant. The liquid was then aliquoted to a 96-well plate and optical density was read on a spectrophotometer at a wavelength of 405 nm. Three independent experiments were performed with duplicates for each of the 6 wells (Figure 4.2).

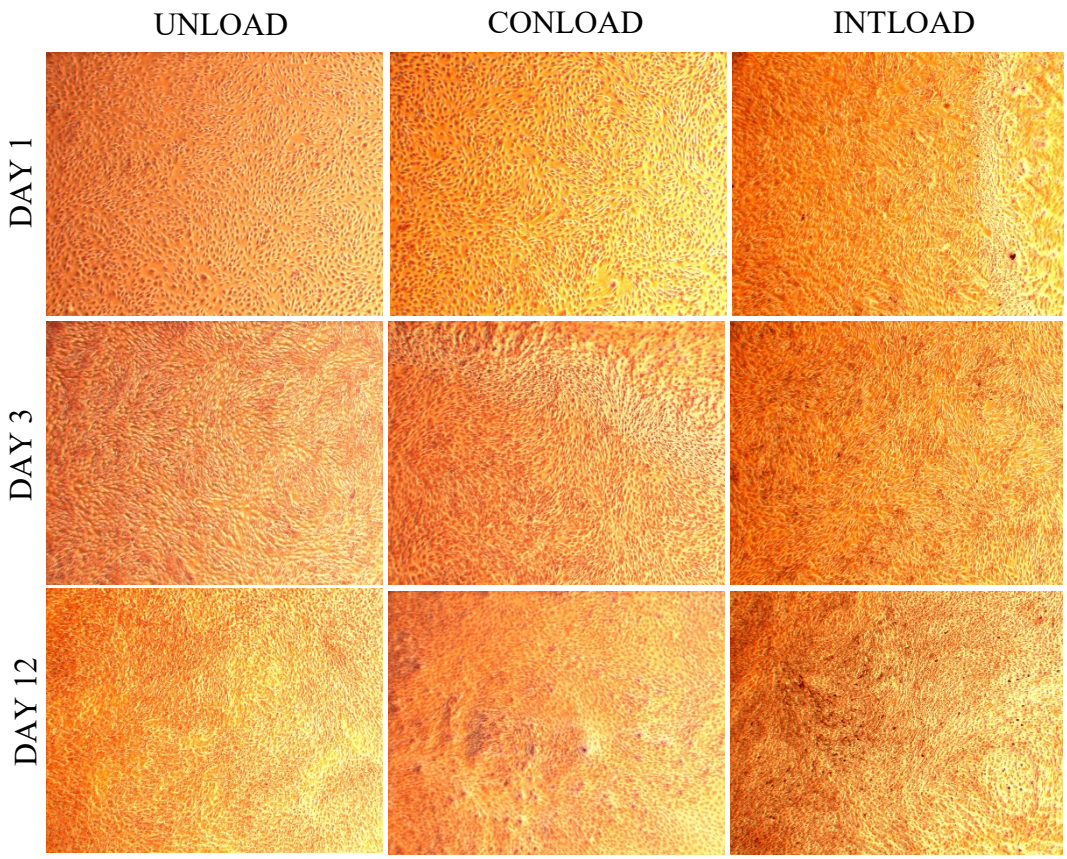


Figure 4.2. Raw images of osteoblasts (x4). Qualitative alizarin red staining at days 1, 3 and 12 for each loading condition. Darker red areas correspond to calcium-rich deposits (mineralisation). Unload = control, Conload = continuous load, Intload = intermittent load.

4.2.6 Statistical analysis

Data were checked for normality of distribution with Shapiro-Wilks tests (IBM, SPSS Statistics, v.29). A two-way repeated measures ANOVA compared group differences between loading conditions and time. To compare within-group differences a one-way repeated measures ANOVA was performed on loading conditions for ALP activity, PINP and ARS at each time point and Tukey's posthoc analysis was applied. Kruskal-Wallis tests were used if data were non-parametric. Statistical significance was accepted at the 95% confidence level ($P<0.05$). Means are expressed as M.

4.3 Results

4.3.1 Alkaline Phosphatase activity

There was a significant difference in timepoint *(P*<.001) and loading condition *(P*=.004) between groups in ALP activity. ALP activity was greater in the intermittent load condition compared to the continuous load condition on day 1 (M Intload .390 Conload .299, +30%, 95% CI: .007-.174, *P*=.035), day 3 (M Intload .404 Conload .253, +59%, 95% CI: .123-.178, *P*<.001) and day 12 (M Intload .440 Conload .313, +40%, 95% CI: .056-.199, *P*=.004; Figure 4.3a). ALP concentrations were also greater in the intermittent load condition compared to the unloaded condition on day 1 (M Unload .205, +90%, 95% CI: .101-.268, *P*=.001), day 3 (M Unload .221, +82%, 95% CI: .155-.210, *P*<.001) and day 12 (M Unload .258, +70%, 95% CI: .111-.254, *P*<.001; Figure 4.3a).

4.3.2 PINP Assay

There was a significant difference in timepoint between groups in PINP concentrations (*P*<.001). PINP concentrations were greater in the continuous load condition compared to intermittent load on day 3 (M Conload 66 Intload 31, +112%, 95% CI: 12-56, *P*=.007) (Figure 4.3b).

4.3.3 Alizarin red staining

There was a significant difference in timepoint between groups in ARS (*P*=.006). Within the unloaded group there was a significant difference in ARS between days 1 and 12 (M Day1 0.09 Day12 0.14, +56%, 95% CI: -.07 to -.03, *P*<.001) and days 3 and 12 (M Day3 0.11 Day12 0.14, +27%, 95% CI: -.05 to -.01, *P*=.004). No significant differences were shown between loading conditions (Figure 4.3c).

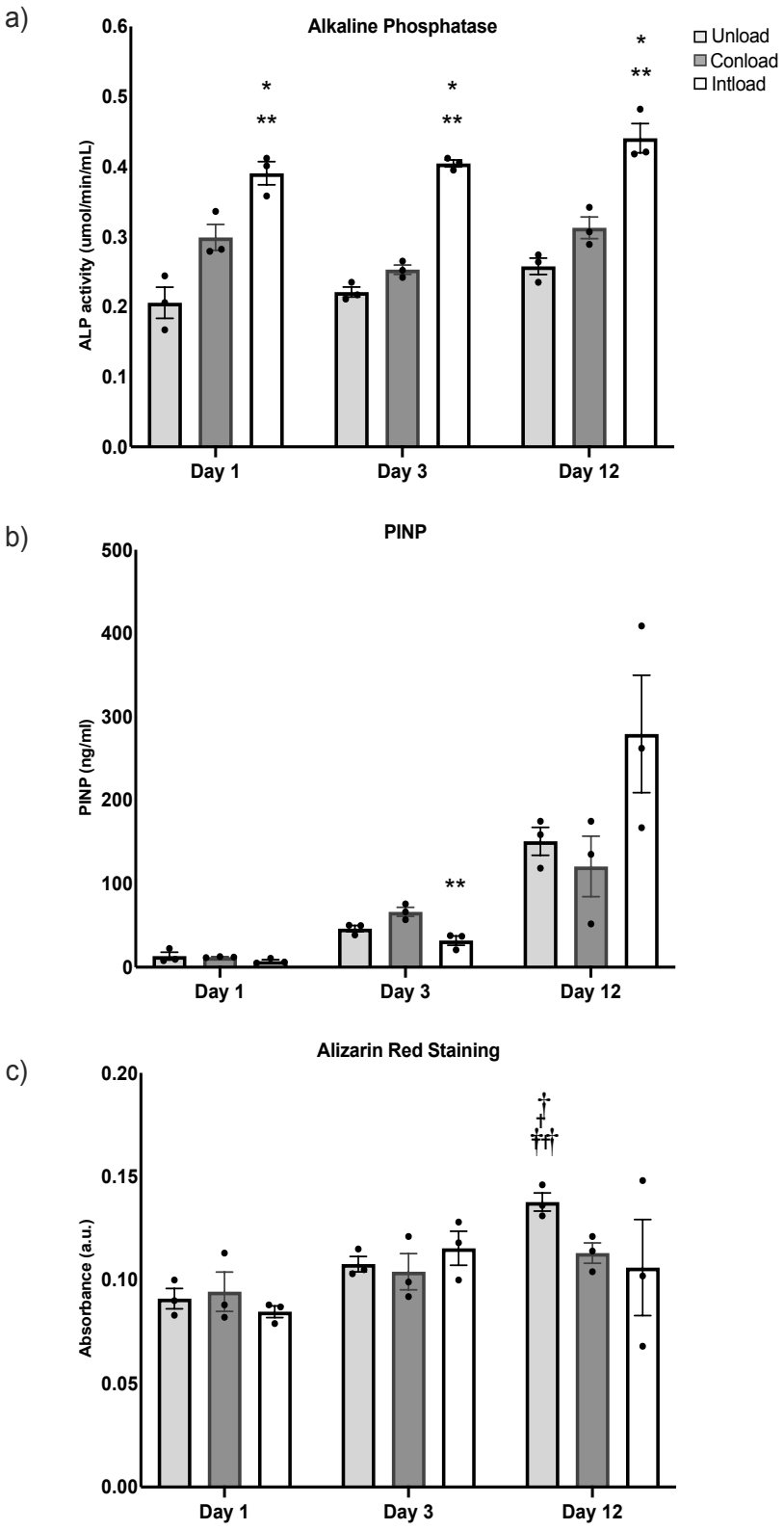


Figure 4.3. a) ALP activity b) PINP activity and c) ARS absorbance across loading conditions. *P<0.05 compared to unloaded. **P<0.05 compared to continuous loading. $^{\dagger}P$<0.05 compared to day 1. $^{\dagger\dagger}P$<0.05 compared to day 3. Error bars represent standard error means.

4.4 Discussion

Bone formation marker ALP was 30% - 90% higher in the intermittent load condition compared to continuous load when loading magnitude was matched. Our data support the hypothesis that the osteogenic effects of implementing rest periods between loading cycles facilitate an increased response in bone formation when observing ALP (Burr et al., 2002; Robling et al., 2001). It is speculated that the rest periods allow mechanosensitivity to be restored and lead to heightened osteoblast activity.

This is the first study to apply the same loading parameters (magnitude, duration, frequency) across intermittent and continuous loading *in vitro,* and report the osteogenic potential of intermittent load on osteoblast activity. The osteogenic effect of intermittent loading shows the extent to which mechanosensitivity can be restored to enable the optimal stimulation of osteoblasts. The present study supports previous findings in rodents where the utilisation of 10 second rest periods between low magnitude loading cycles caused an increase in bone formation in comparison to continuous load (Srinivasan et al., 2003; LaMothe & Zernicke 2004). One critical difference between previous studies and the present study is the difference in load magnitude between conditions. For example, George et al. (2022) exposed rats to continuous (45 min/day at 70% maximal aerobic speed) and intermittent (42 min/day at 50-100% maximal aerobic speed) running for 8 weeks. Although similar protocols, the continuous and intermittent conditions are likely to have inflicted different cumulative loads, which may have been the cause for the difference in bone response rather than the addition of rest periods. Similarly, LaMothe & Zernicke (2004) did not match loading conditions, as the intermittent group were exposed to 10 fewer loading cycles compared to the continuous loading group. The present study subjected osteoblasts to the same load (5000 μS = 0.5% elongation), which is previously noted as a physiological strain that allows for optimal formation (Baudequin et al., 2019), as well as the same frequency (1 Hz) and duration (5 hours). Intermittent loading was found to produce higher levels of ALP activity compared to the continuously loaded condition. As the loading magnitude was matched between conditions in the present study, it is therefore viable that the loading application contributes to the bone marker response. Mechanical loading protocols designed to induce osteogenic effects are an attractive means to combat osteoporosis. The present data suggests high-frequency loading used intermittently may augment osteogenesis by increasing osteoblast activity.

In practical terms, this suggests the monotonous nature of activity such as running is suboptimal for bone accrual. It is therefore suggested that exercise programmes designed to improve bone health should incorporate rest periods.

The current study explored the effects of long-term rest periods compared to short-term rest periods *in vitro*. Previous research has shown longer rest periods (7 hours) allow osteoblasts to restore their mechanosensitivity between loading bouts by increasing the expression of bone formation marker cyclooxygenase-2 (COX-2) present in early-stage formation (Jaasma & O'Brien, 2008). Rest periods of 30 minutes are also shown to enhance calcium response (Godin et al., 2007). Short-term rest periods (5-15 seconds) incorporated within loading bouts are also shown to promote osteoblast activity by increasing osteopontin compared to continuous load (Batra et al., 2005). Similarly, oscillatory flow with short-term rest insertion is shown to increase the frequency and size of calcium transients and upregulate intercellular calcium (Donahue et al., 2003). The present study supports previous evidence of stimulating osteoblast activity by showing increases in ALP during intermittent load suggesting longer resting periods may provide osteogenic stimulation for osteoblasts *in vitro*.

Bone formation marker PINP was lower in the intermittent loading condition compared to continuous loading on day three (Figure 3b), however no differences were observed on day 12. This suggests osteoblasts may not respond positively to loading in the acute period. The acute variability in PINP response may be due to it being an indicator of matrix deposition and therefore unlikely that its activity levels will peak in the hours following an intervention (Dolan et al., 2022). This premise is supported by studies showing no difference in the PINP response to acute exercise after 24 hours (Evans et al., 2020; Dror et al., 2022; Kouvelioti et al., 2018) and 72 hours (Scott et al., 2011). However, a local effect of loading on bone formation undetected by the marker cannot be ignored, as findings from humans (Vainionpää et al., 2009) and animals (Zhang et al., 2011) imply loading may promote osteogenesis without detecting changes in PINP. Due to practical reasons, the *in vivo* assessment of PINP greater than 72 hours post-intervention is not commonly conducted and therefore the present findings cannot be compared to human studies. The present findings suggest that loading does not have any effect on PINP concentration. Alizarin red staining was also measured during the current study. ARS assay identifies calcium deposits that signify mineralisation. No differences

were observed during either of the loading conditions yet, there was a significant difference over time in the unloaded condition. However, this method demonstrates moderate sensitivity meaning early differentiation or slight differences in mineralisation are difficult to detect (Serguienko et al., 2018). As the current study was conducted over 12 days it may be premature to identify significant mineralisation from the osteoblasts as it is proposed mineralisation is not detected until after 16 days in MC3T3 cells (Quarles et al., 1992). It is possible the differences in ARS observed in the unloaded condition may be a false positive result as ARS has been suggested to produce results in the presence of calcium-binding proteins and proteoglycans (Bonewald et al., 2003). Furthermore, the elevation in the control group compared to the loaded conditions may be a result of heightened activity in osteoblasts during loading. Osteoblasts under load may prioritise remodeling or structural protein production (*e.g.,* collagen) over calcium deposition, whilst the control condition is not influenced by mechanical stimuli (Klein-Nulend et al., 2012).

4.4.1 Limitations

The data of the present study were limited to acute bouts of loading of up to 12 days, therefore, the bone response following this period is not known. This study suggests bone formation markers offer insight into osteoblast activity *in vitro*, however, human studies need to be examined to confirm if a similar response to intermittent load occurs *in vivo*. Further research is warranted to investigate the effects of intermittent exercise on bone adaptation in humans.

4.4.2 Conclusions

In summary, the present study showed intermittent load increases bone formation marker ALP compared to continuous load when load magnitude, frequency and duration are matched. It can be hypothesised that the intermittent nature of load allowed the osteoblasts to resensitise and restore the mechanosensitivity (George et al., 2022), resulting in a heightened osteogenic response. The findings may be of interest to researchers and practitioners exploring exercise programmes for optimising bone accrual in human participants.

5 Perspectives from research and practice: A survey on external load monitoring and bone in sport.

5.1 Introduction

Mechanical load can be separated into two categories; internal load and external load (Impellizzeri et al., 2019). Internal load is the biological stress imposed upon an individual, such as heart rate or blood lactate (Bourdon et al., 2017), whereas external load can be described as the work completed (*e.g.,* acceleration or force) independent of the internal characteristics (Halson., 2014). External load has an important relationship with the mechanical stresses imposed on the musculoskeletal system (Vanrenterghem et al., 2017). Monitoring external load is important in sport as it provides objective data on physical attributes in response to prescribed training (Newton et al., 2019) and can be used to optimise performance (Heishman et al., 2018; Mooney et al., 2011). High intensity external load has been associated with an increase in injury risk of up to 270% in rugby league (Gabbett & Ullah, 2012) and football (Bacon & Mauger., 2017) and, as such, monitoring external load in applied settings has increased in popularity to try to mitigate against injury (Burgess, 2017; West et al., 2020). Methods measuring external load in relation to bone allows support staff to understand how external load is associated to bone characteristics and its applicability within an applied setting. Subjective methods (*e.g.,* questionnaires or rating of perceived exertion) are often used to monitor athlete load, but these metrics lack reliability and validity in comparison to quantitative data as they depend on the athlete's perception (Borresen & Lambert., 2009). Although there are methods studying bone response that are insightful (*e.g.,* strain gauges, BTMs), they are restricted due to their invasiveness (Szulc et al., 2017; Yang et al., 2011). Metrics derived from applied technologies (*e.g.,* GPS, IMU, force plates, motion capture), however, have the potential to be associated with bone characteristics.

Associations between bone characteristics and physical performance (*e.g.,* high-speed distance associated with bone mass, trabecular and cortical density, and peak speed associated with bone mass, cortical density and thickness) indicate bone is influenced by exercise intensity. Studies have attempted to understand the relationship between physical activity and bone, although these have been performed in non-athletic populations (Heikkinen et al 2007; Jämsä et al., 2006; Marin-Puyalto et al., 2019) or associated to injury (Milner et al., 2006) rather than bone structural characteristics. Higher GRFs are shown to increase osteogenic loading (Kohrt et al., 1997), with GRF intensity thought to be a better predictor of BMD and skeletal adaptation than load volume (Rogers & Hinton,

2010). However, force plates are not capable of monitoring load in the applied environment, therefore practitioners often use measurements tools, such as GPS, that do not restrict the athletes. Accelerometery-derived data has observed vigorous physical activity to be associated to higher BMD and BMC (Marin-Puyalto et al., 2019). IMUs are a novel approach to monitor bone stimulus in the applied field, utilising site-specific segmental acceleration as opposed to whole-body load that GPS measure (Armitage et al., 2021; Pino-Ortega et al., 2019; Wiig et al., 2019). IMeasureU (Auckland, New Zealand) offer a bone stimulus metric that combines the number of loads and magnitude of loads to predict the stimulus response of bone (Armitage et al., 2021), but these claims are unsubstantiated. GPS is a popular method to monitor external load in the field and GPS-derived metrics have been associated to bone adaptation (Varley et al., 2023; Varley et al., 2022). For example, acceleration and total distance derived from GPS were positively correlated with BMC and tibial strength in footballers (Varley et al., 2023). Acceleration-derived metrics from IMUs and GPS are often used to quantify external load (Gabbett, 2016), but research is limited on whether these methods can be associated to bone adaptation. Therefore, the efficacy of monitoring external load as a proxy of bone load in an applied environment is not well known.

Bone stress injuries (BSI) are often associated with alterations in training programmes (Fredericson et al., 2006). As such, the ability to monitor external load accurately and reliably as a proxy for bone load, offers the potential to reduce BSI risk and help to ensure that athletes are not exposed to sudden excessive load cycles. It is argued that external load monitoring can be used to manage bone load and reduce the incidence of BSI, as prompt increases in load are prominent in their pathophysiology (Warden et al., 2021). Whilst this might be the case, it remains unknown to what extent athlete support staff estimate bone load, and, if they do, what methods and metrics they use to do so, given that there is no consensus on the optimal method for monitoring bone adaptation. Therefore, the aims of this study were two-fold; (1) to identify the methods used to monitor external load and ascertain if these methods are used to estimate bone load by surveying support staff, and (2) to assess the measurement tools used to estimate bone load through a narrative review.

5.2 Materials and Methods

5.2.1 Participants

Support staff (n=71) from sports clubs and national governing bodies (Figure 5.1) were recruited worldwide (UK n=48, 67%; Rest of Europe n=7, 10%; North America n=7, 10%; Australia n=5, 7%; Africa n=2, 3%; Asia n=1, 1%; South America n=1, 1%) via email or word of mouth. The role occupied by those surveyed included: Strength and Conditioning Coach (n=29), Sports Scientist (n=22), Physiotherapist (n=13), Coach (n=2), Physiologist (n=1), Sports Therapist (n=1), Athletic Trainer (n=1), Researcher (n=1) and Nutritionist (n=1). The majority of support staff worked with National (n=36) or International athletes (n=24), whereas others worked with Regional (n=6) or University/Collegiate athletes (n=5).

5.2.2 Procedures

Participants were asked to provide informed consent and complete a survey related to external load monitoring and bone in sport between July 2020 and August 2020. The internet-based survey platform (Jisc, Bristol, UK) was used, with the survey being completed anonymously. It comprised of 19 multiple choice questions relating to external load monitoring in sport. Respondents were able to elaborate on their answer with the 'Other' option if they wished to do so.

Participants met the inclusion criteria if their role involved working in a support staff capacity in an applied sporting environment. Prior to taking part in the study, each participant provided informed consent. Ethical approval was granted by the Non-Invasive Human Ethics Committee from Nottingham Trent university (126V2).

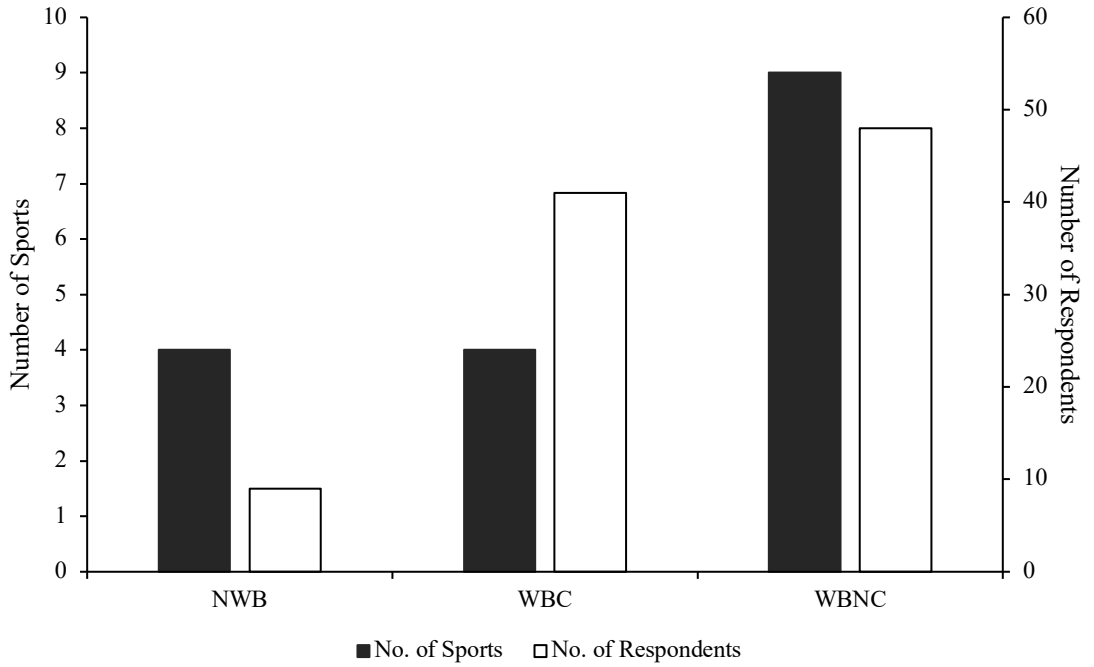


Figure 5.1. Number of survey respondents alongside number of sports classified into sporting conditions. Sporting conditions are classified as; non weight-bearing sports (**NWB**) including Cycling, Swimming, Canoeing and Rowing; weight-bearing contact sport (**WBC**) including Football/Soccer, Rugby, Judo and American football; weight-bearing non-contact sport (**WBNC**) including Cricket, Athletics, Basketball, Volleyball, Field Hockey, Baseball, Triathlon, Dance and Squash.

The survey (Appendix IV) divided the topic of 'external load monitoring' into two sections; a) If/How external load is monitored and, b) What methods/metrics are used to estimate bone load. Multiple choice and free text options were provided on the common methods identified within research for sports performance and external load quantification. Frequency based descriptives were produced on fully completed surveys.

Alongside the survey, a narrative review was performed using PubMed as a database to assess how external load is associated to bone. Google Scholar was used as a complimentary database. The search strategy used the keywords "bone load", "external load", "non-invasive bone load" and "bone and exercise". Articles were included if they met the following:

- The methodology presented in the study was non-invasive (meaning the research was performed in an applied environment and not intrusive for participants)

- Human trials only
- The loading metric used had bone health, load or injury as outcome variables.
- Fully published, peer reviewed articles

Various metrics were reported within the studies, although the methodologies adopted were consistent between each study.

5.3 Results

5.3.1 Current use of external load monitoring in sport

Most support staff reported monitoring external load with their athletes (92%). For the 8% that did not monitor external load, this was primarily due to a lack of equipment (67%). The most common methods used by support staff were GPS, force plates, IMU and motion capture. Only 28% of support staff, however, used the methods to estimate bone load with 40% stating the main barrier was a lack of knowledge (Table 5.1).

Table 5.1. Support staff response for monitoring external load in sport.

	Yes, n (%)	No, n (%)	Unsure, n (%)		
Does your club / organisation monitor external load in your athletes? (n = 71)	65 (92)	6 (8)	0 (0)		
What is the primary reason you don't monitor external load? (n=6)	Lack of Time: 0 (0)	Lack of equipment: 4 (67)	Lack of knowledge: 0 (0)	Don't feel it is needed: 1 (17)	Other: 1 (17)
What systems do you use to monitor external load? (n = 65)	GPS: 55 (85)	IMU: 11 (17)	Force Plates: 31 (48)	Motion capture: 10 (15)	Other: 16 (25)
Do you use any of the external load metrics attained to estimate load on bone? (n = 65)	18 (28)	43 (66)	4 (6)		
What is the primary reason you don't relate external load to bone? (n=43)	Lack of Time: 6 (14)	Lack of equipment: 7 (16)	Lack of knowledge: 17 (40)	Don't feel it is needed: 7 (16)	Other: 6 (14)

5.3.2 Methods to estimate bone load

GPS was the most common method for monitoring external load (n=55, 85%) and most commonly used to estimate bone load (n=11, 50%). The use of GPS to inform on bone related outcomes (21-38%) was not as prevalent as using IMUs (50-100%) or motion

capture (40-100%) (Table 5.2). Force plates were well utilised to monitor external load, but the least prevalent in relation to bone related outcomes (12%) (Table 5.2).

Table 5.2. Support staff responses to the metrics used when measuring external load and the metrics used in relation to bone.

Method Metric	Respondents who measure external load, n (%)	Respondents who measure external load to estimate bone load, n (%)	Prevalence of use to estimate bone load
GPS			
PlayerLoad	29 (45)	11 (50)	38%
Total distance	48 (74)	10 (46)	21%
High speed distance	47 (72)	10 (46)	21%
IMU			
Impact load	9 (14)	5 (23)	56%
Step count	3 (5)	3 (14)	100%
PPA	6 (9)	3 (14)	50%
Motion capture			
Torque	3 (5)	2 (9)	67%
Moment	5 (8)	2 (9)	40%
Stiffness	3 (5)	3 (14)	100%
Force Plates			
Peak ground reaction force	25 (39)	3 (14)	12%
RFD	26 (40)	3 (14)	12%
Impulse	17 (26)	2 (9)	12%
Other	19 (29)	7 (32)	37%

A total of 16 articles were included in the narrative review (GPS n=1; Force plates n=10; IMU n=1; Motion capture n=4) (Table 5.3).

Table 5.3. Articles included in narrative review (n=16)

Measurement tool	Study (year)	Experimental model	Main results
GPS (n=1)	Varley et al. (2022)	GPS training load across a football season x 3 time points. DXA and pQCT scans x 4 time points.	Correlations between training load variables and BMC and tibial strength.
Force plates (n=10)	Jämsä et al. (2006)	Postmenopausal women. 12 month high impact exercise intervention. 3 x a week. GRF and accelerometer. DXA scan.	BMD change at proximal femur correlated with accelerations exceeding 3.6 g.
	Kohrt et al. (1997)	Healthy older women. 9 month intervention, GRF and JRF exercise group. DXA scan.	Increase in whole body BMD for both GRF and JRF groups. Femoral neck BMD increase in GRF group.
	Rogers & Hinton. (2010)	Physically active middle aged men. Bone loading scores based off GRF exercise. DXA scan.	Bone loading during young adulthood was a predictor of BMD. GRF good predictor for increased BMD.
	Bailey & Brooke-Wavell. (2010)	Premenopausal women. 50x hops 2x, 4x or 7x a week intervention for 6 months. GRF measures. DXA scan.	Femoral neck BMD significantly higher in 7 days a week group. BMC increased at femoral neck in 7 day group.
	Matijevich et al. (2019)	Young healthy subjects. Treadmill run on range of slopes (-9 - 9 degrees) and speeds (2.6 – 4.0 m/s). Vicon motion capture and GRF. Lower extremity marker system. Model of tibial load.	Ankle force indicative of tibial bone load. GRF metrics not strongly correlated with increases in tibial bone load.
	Allison et al. (2013)	Older men. 50x hops, 7 days a week intervention for 12 months. GRF measures. DXA scan.	BMD and BMC increased in the exercise leg and decreased in the control leg. Cross-sectional moment of inertia increased in exercise leg.
	Rantalainen et al. (2008)	Healthy young men. Max GRF measured during bilateral jumping. Muscle torque measured with dynamometer. pQCT tibial scan.	GRF and eccentric torque positively correlated with tibial bone strength.

	Rantalainen et al. (2010)	Premenopausal and postmenopausal women. CMJ performed on force plates. pQCT tibial scan.	Premenopausal group had higher bending and compressive bone strength. Higher peak GRF and impulse in premenopausal group.
	Wu et al. (1998)	Rhythmic gymnasts. Muscle strength measured from IKD. GRF measured. DXA scan.	BMD higher in take-off leg and landing leg. Force significantly higher in take-off than landing leg.
	Rantalainen et al. (2009)	Young male students. Bilateral jumping until exhaustion. GRF measured. Blood biomarkers measured.	Maximal GRF and P1NP marker were positively associated. Negative correlation between maximal GRF and CTX form pre and 2 days post intervention.
IMU (n=1)	Besier. (2019)	Bone stimulus metric created from number of cycles and peak strain.	No experimental data.
Motion capture (n=4)	Milner et al. (2006)	Habitual runners. Treadmill run at 3.7 m/s. Vicon motion capture. Lower extremity marker system. Tibial x-ray.	Greater vertical loading rate, impact peak, peak tibial shock and knee joint stiffness in tibial stress fracture group compared to controls.
	Laughton et al. (2003)	Rear foot and forefoot strike runners. Running with and w/o orthotic devices. Accelerometer, GRF and motion capture. Model of lower limbs.	Positive correlations between peak positive tibial acceleration and anteroposterior GRF load rate. Forefoot strikers experience greater tibial shock.
	Choi et al. (2021)	Older population. Barefoot walking over 9m. Motion analysis and GRF. DXA of femoral neck.	Maximum hip power and BMD positive correlation in trochanter. Hip power-time integral positive correlation with femur.
	El Deeb et al. (2014)	Postmenopausal women. 10m walking gait trails. Qualisys motion system and GRF. Whole body marker system. DXA scan at femoral neck.	Low BMD associated with hip and trunk moments. Less power generated in hip with low BMD.

1

5.4 Discussion

5.4.1 Main survey findings

The current study aimed to identify external load is monitored in an applied environment and ascertain if these methods were used to estimate bone load. The secondary aim of this study was to perform a narrative review to assess if the practitioner view reflected bone load research. The key findings of the study show external load is widely monitored by support staff, primarily using GPS, force plates, IMU and motion capture (Table 5.1), but fewer use external load to provide an insight on bone response to exercise. Although these methods have been validated and shown to be reliable measures of performance related variables (Barrett et al., 2014; Coutts & Duffield., 2010), the validity of associating them with changes in bone are not well established (Matijevich et al., 2019).

5.4.2 GPS and bone

GPS was the most common method used by support staff to monitor external load (Table 5.2), likely due to its capacity for real time data interpretation (Theodoropoulos et al., 2020). As GPS technology has developed, micro inertial sensors (triaxial accelerometers, magnetometers, gyroscopes) have been integrated into the devices, providing support staff with a wide range of metrics to indicate external load and undertake activity profiling (Malone et al., 2017). The use of GPS has been shown to offer an accurate and reliable method to quantify the habitual movement of athletes (Vanrenterghem et al., 2017). Support staff, however, should be cautious when measuring maximal accelerations as the sampling rate of commercial devices in research (~1Hz; GPSports, Catapult innovations) may result in missing data over a short period of time (Coutts & Duffield., 2010). Furthermore, comparing high intensity running between GPS devices may be unreliable, as differences have been shown between manufacturers (Coutts & Duffield., 2010). Therefore, high intensity metrics (i.e., high intensity running) may not be reliable between devices as a result of high coefficient of variation (32.4%) when analysing high intensity movements.

Although GPS is used to monitor physiological markers relative to performance (Malone et al 2017), less is known about how the metrics can monitor bone load. This is despite our findings showing that total distance and high-speed distance are commonly used to

estimate the load placed upon bone by support staff (46%; Table 5.2), as they may assume that greater distances and higher peak speeds result in greater bone load. Bone, however, desensitises to repetitive, unidirectional loading (Burr et al., 2002; Warden et al., 2021), meaning total distance might be an informative metric when it comes to determining the bone stimulus. This has been reported in rats where excessive high-magnitude load (14,000 load cycles on each limb per day) did not have any effect on bone (Yingling et al., 2001). In humans, tibial strength and BMC have been shown to positively correlate with GPS training load metrics, such as acceleration, deceleration and total distance, in professional male footballers over a season (Varley et al., 2023). This suggests dynamic, high-load movements are important for creating an osteogenic response within bone and may be monitored using GPS. The effect sizes in this study, however, were moderate to low (Varley et al., 2023), suggesting the practical use of GPS to estimate bone load is yet to be established.

Data is available on GPS-derived PlayerLoad (commercially used metric to estimate workload completed in a given period) and distance covered in relation to sports performance and fatigue (Halson, 2014), yet there is no robust evidence to suggest that these metrics can be used to estimate bone load. Despite this, our findings show that PlayerLoad is the most prevalent metric (50%; n=11 of support staff surveyed) used to estimate bone load. Comparing PlayerLoad between athletes may not be reliable due to the variability of the measurement suggesting it may not reflect differences of internal load. Another issue with the reproducibility of PlayerLoad results from the ambiguity surrounding the measurement (Bredt et al., 2020). This ambiguity stems from the inconsistent definitions within literature surrounding the metric, resulting in a lack of clarity between studies (Castillo et al., 2017; Schelling & Torres, 2016). Some research defines the variable as a ‘vector magnitude representing the sum of accelerations from each direction’ (Castillo et al., 2017), whereas others define it as the ‘instantaneous change in rates of resultant accelerations over time’ representing the acceleration load for an activity (Schelling & Torres, 2016). This limits the application of PlayerLoad as a tool for monitoring external load, as well as using this as a metric to estimate bone load. Research needs to present a clear and consistent use of how PlayerLoad is calculated in order to offer a standardised and reproducible metric for support staff to understand.

A limitation of GPS is the sensor location, which is commonly positioned between the shoulder blades. The reliability of positioning a GPS at the scapulae (ICC .60-.93, CV 4.6-18.2%) compared to the centre of mass (ICC .65-.97, CV 3.6-14.7%) shows a moderate to high test-retest reliability for both locations (Barrett et al., 2014). However, wearing the device at the scapulae can underestimate metrics (i.e., PlayerLoad) due to the lack of sensitivity to subtle movements during high-speed running (Barrett et al., 2014). As stress related bone injuries occur predominantly in the lower limbs measurements at the scapulae may not be a valid method to understand the load experienced at distal limbs (Fredericson et al., 2006). As associations between GPS-derived metrics and bone characteristics are not well established there is no clear evidence to show the metrics can be used in relation to bone load. The 21 - 38% of support staff that relate GPS metrics to bone should therefore do so with caution. This dearth of robust scientific data should be addressed by researchers.

5.4.3 Force Plates and bone

Force plates were reported as the second most utilised method for measuring external load by support staff within the present study (n=31, 48%). Force plates are mechanical sensing systems that measure GRF's when contact is made with an external force (i.e., an athlete), and can be used to gather kinetic data. Support staff often use the device within a gym environment to measure the effects of training programmes. Despite only ~14% of support staff using GRF in relation to bone, researchers have shown associations between GRF and bone adaptation (Kohrt et al., 1997; Rogers & Hinton, 2010).

Femoral neck, trochanter and ward's triangle BMD have been shown to increase when frequently performing daily impacts and accelerations over a 12-month exercise intervention (Jämsä et al., 2006). Greater increases in BMD at the femoral neck have also resulted from daily hopping compared to hopping for 2- or 4 days, with load frequency being the only difference between groups (Bailey & Brooke-Wavell, 2010). This could be important for offering guidelines on exercise-induced bone load without increasing exposure to injury. Internal forces acting upon bone are higher than surrogate measures, such as GRF, but the accessibility of GRF's makes them a surrogate of internal bone load intensity during impact exercises (Allison et al., 2013). This is proposed by Rogers & Hinton (2010) whereby classifying bone load into categories: 0 (GRF 1 x bodyweight); 1

(GRF between 1 and 2 x bodyweight); 2 (GRF between 2 and 4 x bodyweight) and 3 (GRF > 4 x bodyweight) showed greater bone load (category 3) has a positive linear relationship with whole-body BMD and a positive effect on skeletal health in later life (Rogers & Hinton, 2010). The applicability of this scoring system in relation to bone load is questioned due to two points; (1) the lack of validity surrounding the biomechanical GRF (Groothausen et al., 1997) and (2) the retrospective measure of physical activity across the lifespan. As far as the current author is aware there is no validation for the GRF scores relevant to bone load and, therefore, although it might be a good theory, it cannot be truly classified as being a bone load metric. Furthermore, as is highlighted in the study by Rogers & Hinton (2010), the recall bias of physical activity across the lifespan from a middle-aged population is likely to have significant error, which will have affected the bone load metric. Using GRF in this format has potential to inform on intervention strategies in an applicable and simple technique but validation of GRF in relation to bone load needs to be examined for it to be used as a predictor of skeletal adaptation.

The ability to create a high force rapidly during muscular contractions may be a relevant measurement to inform on bone adaptation. This is due to the functional link between muscle and bone, in which both biological structures directly influence one another (Ashe et al., 2008; Schoenau, 2005). Rate of Force Development (RFD) is reportedly used by ~14% of staff (n=3) who use force plates to estimate bone load. The incorporation of time-based analysis makes the measure more indicative of neuromuscular performance, as opposed to peak force which may be more indicative of movement strategies (Lombard et al., 2020). Neuromuscular performance has been associated with bone strength (Rantalainen et al 2008) as tibial strength was higher in those that produce greater eccentric torque and predicted bone strength in pre- and postmenopausal women (Rantalainen et al., 2010). Muscular forces impose a large load on bone demonstrated by the associations shown between bone mass and muscle mass (Macdonald et al., 2006; Ruff, 2003). Therefore, using RFD alongside other GRF-derived metrics may be advantageous to assess adaptations in bone (Mosti et al., 2014) since muscular forces influence bone characteristics. Similarly, impulse is a product of a resultant force and the duration of this force. Impulse was used by ~9% (n=2) of support staff who use external load monitoring in relation to bone. Impulse incorporates neuromuscular performance and body mass and has been shown to have a strong linear association with maximal power. Neuromuscular performance, represented by impulse, has been related to skeletal

robusticity (skeletal strength relative to body size) through a regression model (Rantalainen et al., 2010), with a 1% improvement in impulse associating to a 0.5% increase in skeletal robusticity. This is proposed as an alternative to using body mass as a predictor of skeletal robusticity, and using longitudinal measurements (i.e., CoM accelerations) as better estimates of bone load. Greater impulse during a leaping take-off in rhythmic gymnasts has been associated with higher BMD at the femur when compared to the contralateral side that imposed a lower force (Wu et al., 1998). Furthermore, the increment attained from impulse during a CMJ strongly correlated to an increase in hip and lumbar spine bone mass accretion (Vicente-Rodriguez et al., 2004), demonstrating that impulse has been associated to bone characteristics in research. To understand the effects of neuromuscular performance as an estimate of bone load, healthy athletes should be examined alongside an age-matched, less active population.

Contrastingly, GRF's have been shown to be misleading for monitoring load in relation to bone. Research has shown that GRF does not correlate with tibial load as it does not account for muscular contraction (Matijevich et al., 2019). GRF's are not representative of internal multi-axial stress and may have little influence on the mechanical behaviour of bone relative to load magnitude (Loundagin et al., 2018). Although muscular force applies the highest load on bone, GRF's account for ~30% of bone load (Matijevich et al., 2019), thus it is argued that it can be used as a guide for the relative intensity of internal bone load during hopping exercises (Allison et al., 2013). Overall, GRF is associated to changes in bone within exercise interventions, however, studies are often performed on nonathletic populations (i.e., post-menopausal women or adolescents), meaning there is limited data on active athletes. The impracticality of the method mean that it cannot be implemented in day-to-day training for most sports. Based on the current literature, those who have access to force plates should consider using them to monitor bone adaptation, as higher GRF's have been associated to greater bone accrual (Rantalainen et al., 2009).

5.4.4 IMU and bone

IMUs were the second most used method to estimate bone load (Table 5.2). IMUs are small, moveable devices that can be used in an applied setting, due to their small size and light weight, and provide site-specific measurements for segmental external load (Rojas-

Valverde et al., 2019). This allows segmental information, such as specific tibial sites, to be assessed, which is not the case with GPS or force plates. Segmental accelerations are shown to have a weak relationship with centre of mass accelerations (Nedergaard et al., 2017), which highlights the importance of knowing what is required of the measurement so the correct measurement tool can be applied. IMUs have been shown to reliably monitor impact load, step count, and step intensity during dynamic team sport tasks (Armitage et al., 2021), although no studies have investigated IMU metrics in relation to bone. Proxy bone-specific metrics, such as bone stimuli, have been developed for IMU devices to represent the cumulative nature of impacts and predict the mechanical stimuli responsible for bone remodelling (Armitage et al., 2021; Besier, 2019). However, there is no published evidence of the metric to suggest they are associated with changes in bone. Using IMUs on anatomical positions unspecific to the area of interest may lead to less accurate results for the movements being performed (Tan et al., 2019). Therefore, it is essential that the location of the IMU devices is site-specific when measuring load to understand the effects of sport-specific tasks on bone adaptation. Overall, a small amount (17%) of support staff use IMUs, however, the prevalence of those that use IMUs to estimate bone load is high (Table 5.2), highlighting the practicality of the method. As further research is undertaken, the relevance of IMU devices for monitoring external load in the field, and their association to bone characteristics, will become clearer.

5.4.5 Motion capture and bone

The application of motion capture, particularly 3D analysis techniques, can create predictive models of movement patterns that may reduce the likelihood of injury (Hewett et al., 2005). Motion capture is not widely employed to monitor external load by support staff, however when it is used, the prevalence of use in relation to bone is high (Table 5.2). In theory, this technique can offer the greatest insight into the load being applied to bone due to its ability to create internal models of the musculoskeletal system. The application, however, is limited in most sporting environments as it is time consuming and requires expertise.

Torque (n=2; 9.1%), moments (n=2; 9.1%) and stiffness (n=3; 13.6%) were used by support staff to monitor external load with motion capture. Higher knee joint stiffness create higher loading rates and have been strongly associated with the estimation of bone

load (Groothausen et al., 1997; Laughton et al., 2003; Milner et al., 2006), which may be why support staff use stiffness as an informative metric. BMD in elderly women has been correlated with hip power-time during walking and maximal hip power can predict 25.4% of femoral neck BMD (Choi et al., 2021). This finding was supported by a decrease in hip power correlating with a decrease in BMD in postmenopausal women (El Deeb & Khodair, 2014). These studies only assessed habitual walking, therefore it could be suggested that dynamic, high intensity movements may produce additional increases in BMD as running creates a greater mechanical load than walking (Meardon et al., 2021). Comparatively, these increases are shown to be 2-9% higher in bone compression and tension, and 10-26% higher in shear stress, when running compared to walking (Meardon et al., 2021). This increase can be used to harness a positive adaptation in bone and minimise injury risk by creating training programmes that expose athletes to gradual increases in load. Therefore, the findings of Choi et al. (2021) and El Deeb & Khodair. (2014) suggest metrics derived from motion capture may offer informative data on bone adaptations and the beneficial effect load can have on special populations. The use of motion capture by support staff to inform on bone related outcomes is low. This is likely due to the time-consuming nature and expertise required for both data collection and data analysis. Motion capture is, therefore, mainly used by researchers rather than support staff. However, the recent development of markerless systems may widen the opportunity to integrate motion capture systems into applied environments in the future.

5.4.6 Limitations

The responses collected offer a general consensus for support staff working alongside athletes in an applied setting. The support staff were responsible for a large number of athletes (1000+) across 5 continents with the majority (85%) competing at a national/international level, therefore the authors are confident the sample is representative of support staff working in an elite environment. Recall bias may have impacted the answers supplied by the support staff but as those who completed the survey were all currently active at their organisations the recall bias should be minimal, given they would have been referring to current practices. This study was exploratory in attempting to understand how external load is used to estimate bone load and as such the survey tool used was not validated, although this is offset by the novelty of the research approach.

5.4.7 Practical applications

Despite the commonality of BSI being acknowledged (71%) by support staff in the survey, few support staff use external load to estimate bone load. Excessive load is known to contribute to BSI, therefore monitoring and acting upon an athlete's external load as a proxy of bone load may help reduce the burden of BSI. This study shows a cohort of support staff monitoring external load, what equipment/metrics they use and if they use those metrics to estimate bone load. There are a variety of methods that are available to support staff to inform on bone characteristics in an applied environment, however, each of these technologies are acknowledged to have their limitations which should be considered by support staff before implementing their preferred choice.

5.4.8 Conclusion

The findings show external load monitoring is commonly used in sporting environments but is seldom used as a proxy of bone load. There is no consistent measurement tool or metric that is used to monitor bone load by support staff or research. GPS is the most commonly used method to estimate bone load by support staff but there is not much evidence associating GPS metrics with bone load. Accelerometry-derived data was shown to be the most prevalent method to assess bone load, but this method lacked generic popularity amongst support staff. Force plates were reported to be the second most popular method for monitoring external load (48%), but the least prevalent to estimate bone load. Measuring external load as a proxy of bone load within applied environments is challenging due to the cost, time and expertise of the methods. Research exploring methods for monitoring external load as a proxy of bone load in applied environments are recommended.

6 Associations between Football-Specific Training Characteristics, and Changes in Bone Characteristics in Male Academy Football Players

6.1 Introduction

Physical activities and exercises that subject bones to significant mechanical stress tend to enhance bone density and strength more effectively than those that apply lesser stress (Nilsson et al., 2013). Although an optimal exercise regimen for bone health has not been fully established, activities involving varied movements and high mechanical stress are associated with improved bone mass, stiffness, and geometry (Vlachopoulos et al., 2017; Maïmoun et al., 2013; Seabra et al., 2017). Team sports such as football consist of turns, jumps, and sprints, with accelerations and decelerations causing high rates of force application and large GRFs, reported to be osteogenic (Clemente et al., 2019). Studies have linked football participation with greater bone size, density, and cortical thickness compared to non-weight-bearing sports (Greene et al., 2012; Varley et al., 2022). Furthermore, increases in BMD, BMC as well as tibial bone mass and area are observed over a season in adult elite footballers (Varley et al., 2022), whilst training volume is also associated with increased bone density and cortical area (Varley et al., 2017). A recent meta-analysis reported football produced improvements in lower limb and whole-body BMD as well as positive anabolic changes in bone turnover markers PINP, osteocalcin and CTX (Milanović et al, 2022).

Despite research on athletic populations and bone adaptation, the specific training loads, such as accelerating and decelerating, necessary for optimal bone health remain unclear. Studies indicate that both moderate and vigorous intensity activities, as measured by accelerometer-based monitors, are associated with increased bone strength (Tobias et al., 2007; Gabel et al., 2017). Categorising external load metrics into predetermined thresholds and not accounting the type of exercise being performed hinder the specificity of load and therefore limit the insight into the effects of load on bone. When exercise is specified, peak speed was observed to positively relate to increases in trabecular density, distance covered was positively related to increases in cortical density and tibial strength increased following 12 weeks of training in academy footballers (Varley et al., 2023). Furthermore, Varley et al. (2023) have demonstrated GPS-derived variables, such as accelerations and decelerations, correlate with pQCT-derived bone metrics in 12-year-old academy footballers. This study, however, did not track changes in muscle strength characteristics which are also known to influence bone adaptation (Montgomery et al., 2019).

Higher external loads are not a direct measurement of the mechanical stress imposed upon bone as muscles dissipate the mechanical energy during exercise (Decker et al., 2003). A consistent association between muscular strength, BMD and BMC suggests a cause-effect relationship of muscle contractions acting as powerful osteogenic stimuli (Torres-Costoso et al., 2020). The dynamic interplay between muscle and bone, represents an important aspect of bone accrual that is not often considered. It is known that this relationship is essential for maintaining skeletal health and function (Nilsson et al., 2013) however, the contribution that resistance exercise and muscle forces have on bone accrual is not often taken into consideration in an applied environment (Almstedt et al., 2011; Candow et al., 2021). Therefore, understanding the exercise variables responsible for positive bone adaptations is crucial for the prevention and treatment of bone disorders. This knowledge is particularly relevant for athletes, given that stress fracture risk in footballers has been linked to high volume pre-season training (Ekstrand & Torstveit, 2012). Identifying the specific training characteristics in football that promote bone accrual is crucial for a deeper understanding of how exercise and strength training influences bone adaptation.

Therefore, this study examined the relationship between football-specific external training load and changes in bone characteristics and body composition in elite academy footballers across 14 weeks.

6.2 Methods

6.2.1 Participants

Fifteen first year full-time male academy footballers (aged 19.2 ± 1.4 years old, 75.1 ± 6.1 kg and 1.82 ± 0.06 m) were recruited from a professional football academy through previously established relationships. Participants were deemed eligible for the study if they were aged 18 years or above, injury free, not currently taking any medication that influenced bone metabolism and had not received a joint replacement or prostheses. After reading the participant information sheet and having the opportunity to ask questions, participants signed a statement of informed consent, completed a pre-scan screening form (ensuring they met the inclusion criteria) and completed a health screen questionnaire. Forms were scrutinised by the lead investigator before the study commenced to confirm that participants met the inclusion/exclusion criteria. The study conformed to Ionising

Radiation (Medical Exposure) Regulations and was approved by the National Health Service Research Ethics Committee (Ref 15/EM/0037).

6.2.2 Design

The study was a 14-week prospective longitudinal study. Participants underwent whole-body DXA scans (iDXA, GE Healthcare, UK) on two occasions across the study period. Scan 1 occurred at the start of the pre-season training period (n = 18) and scan 2 occurred after 14 ± 1 weeks. This allowed changes in DXA variables to be calculated during the early stage of the football season that is of particular importance to the applied practitioner in preparation for the competitive season. Players were monitored using GPS devices during outdoor football training sessions and matches, which allowed external load to be quantified. To assess external training load for strength and conditioning sessions, changes in strength measures were monitored across the 14 weeks. This allowed for relationships between external load indicators and changes in body composition/bone characteristics to be explored.

6.2.3 Procedures

Whole-body DXA scans assessed participant BMD, BMC, area, lean and fat mass. Participants were positioned supine on the DXA bed within the scanner range, with ankles and knees held in place by Velcro straps to minimise unintended movements. The participants laid with their arms by their sides and asked to remain motionless for the duration of the scan. Subsequent analysis for all scans was completed by the same trained operator. Coefficients of variation for the model of scanner used were 0.08–1.30 % (BMD) and 0.6 % (fat mass) (Norcross and Van Loan, 2004; Ward et al., 2007). If any movement artefacts (inaccuracies in the measurement caused by motion) were present following the scan, the image was classed as invalid, and a repeat measure was performed. To assess for artefacts, the image was visually inspected by the researcher performing the scan. If an artefact was thought to exist, the image was viewed by a second researcher that was also trained in the scanning procedure.

6.2.4 Training and Match load

Participants engaged in their normal training and competitions associated with being academy professional footballers. The physical demands for all outdoor matches and

training sessions were monitored using a 10 Hz GPS (Viper, STATSports, Ireland). This system has been validated for use by team sport players, demonstrating a bias of 1.80 ± 1.93 % in peak speed during a 20 m sprint, when assessed by GPS (26.3 ± 2.4 km·h^{-1}) and radar gun (26.1 ± 2.6 km·h^{-1}) (Beato et al., 2018). Each player wore a harness containing a GPS unit positioned between the shoulder blades. Post-session, each GPS unit was downloaded and analysed using commercially available software (Viper, STATSports, Ireland). The training variables assessed included: total distance covered (m), high speed (>5.5 m/s) distance covered (m), very high speed (>7.0 m/s) distance covered (m), number of accelerations above 0.5 m/s^2 for >0.5 s, and number of decelerations below −0.5 m/s^2 for >0.5 s. High Metabolic Load Distance (HMLD) was also measured as the total amount of high speed running coupled with the total distance of accelerations and decelerations (STATSports, 2023). Acceleration zones were preset as: Z1 = 0.5-1 m/s^2, Z2 = 1-2 m/s^2, Z3 = 2-3 m/s^2, Z4 = 3-4 m/s^2, Z5= 4-5 m/s^2 and Z6 = 5-10 m/s^2. All training was conducted and supervised by qualified coaches, as part of their habitual practice. Sessions consisted of gym sessions (*e.g.,* mobilisation, fixed cycle ergometer work, weight training), high-intensity running drills, small-sided games, technique-based drills, and matches. To assess physical demands in the 14-week period, players wore GPS units during outdoor training sessions.

6.2.5 Gym-based measurements

To assess external training load during gym-based sessions, changes in the following strength measures were monitored across the 14 weeks: Isometric mid-thigh pull (IMTP), Nordic hamstring, 1RM bench, and 1RM squat. All strength tests were administered by the same support staff to ensure consistent protocols as these tests were used as an indicator of gym-based load. IMTP was performed using ForceDecks (Vald Performance, Brisbane, Australia). An immovable bar was positioned at the mid-thigh position. The bar height was fixed at various heights to accommodate for different sized athletes as long as the bar was placed between the iliac crest and patella (Guppy et al., 2019) and the rack was anchored to the floor. Once a position was established, the athlete performed two warm-up pulls of 50% and 75%, respectively based on their perceived maximum effort. Maximum effort pulls were subject to a countdown of "3, 2, 1, Pull". Athletes performed 3 maximal IMTP's, holding each repetition for 5 seconds and given strong verbal encouragement throughout. Two minutes of rest were given between each effort (Thomas

et al., 2015) and maximal force was recorded. The Nordic hamstring exercise was performed using a NordBord (Vald Performance, Brisbane, Australia) assisted by a member of the clubs' support staff. Participants began in a kneeling position with the support staff ensuring the feet were in contact with the floor throughout the exercise by applying pressure to the lower legs. The player lowered their body to the ground, as slowly as possible to maximise eccentric loading. Upon completion the participant used their hands to break their fall and push them back up from the ground. Three submaximal efforts were performed as a warmup before conducting one maximal repetition. Peak force was recorded. Participants were also measured for their 1-repetition maximum (1RM) for back squat and bench press. Following Papla et al. (2020) the protocol involved a progressive increase in load and decrease in reps per set until the participant was unable to complete a full lift. The 1RM was recorded. Time spent in Strength & Conditioning was also recorded.

6.2.6 Statistical analysis

Analyses were conducted using IBM SPSS (v.29). Prior to the main analyses data were checked for parametric assumptions and screened for outliers. Inspection of histograms revealed that residuals were not normally distributed for several variables. Moreover, inspection of Z-scores revealed some outliers ($z \geq 3.29$) (Field, 2017). Thus, bootstrapping was applied to analyses, as this technique is robust to these violations (Field & Wilcox, 2017). Bootstrapping works by estimating the shape of the sampling distribution by sampling with replacement from the observed data (Field & Wilcox, 2017). In the present study, pre- and post- changes in bone and body composition characteristics and the training period were estimated with paired samples t-tests, using bias-corrected and accelerated bootstrapped confidence intervals with a 95% confidence level (BCa 95% CI) and 2000 resamples (Field & Wilcox, 2017). Bootstrapped p-values were used to estimate statistical significance at $P<0.05$. Cohen's d was used to estimate size of effects.

For any body composition characteristics that did demonstrate statistically significant change across the 14-week period, we subsequently assessed relationships between the delta change in these body composition characteristics and a range of training load indicators using bootstrapped bivariate Pearson's correlations BCa 95% CI and 2000 resamples. Correlations were interpreted with regards to r-values and whether the

bootstrapped BCa 95% confidence intervals crossed zero (Field & Wilcox, 2017). Mean and standard deviation were used to describe average and variability of data (Mean ± SD).

6.3 Results

Descriptives statistics of weekly training load indicators across 14 weeks of pre-season are displayed in Tables 6.1, as well as gym-based measurements in Table 6.2. Over the course of 14 weeks, the academy footballers averaged taking part in 7.1 ± 3.1 hours of football training and matches a week and 1.3 ± 0.7 hours of strength & conditioning sessions per week.

Table 6.1. GPS training load indicators across 14 weeks of training in academy footballers

	Weekly load (M ± SD)
Total Distance (km)	31.54 ± 8.31
High Speed Distance (km)	1.80 ± 0.51
Zone 6 Distance (km)	0.30 ± 0.11
Higher Metabolic Load Distance (km)	4.95 ± 2.31
Number of Accelerations in Zone 4-6	297 ± 145
Number of Accelerations in Zone 6	16 ± 9
Number of Decelerations in Zone 4-6	218 ± 120
Number of Decelerations in Zone 6	19 ± 13

Table 6.2. Changes in gym-based measurements across 14 weeks of training in academy footballers

	Baseline (M ± SD)	% Change
IMTP peak force (Nm/kg)	43.08 ± 5.08	-1.24 ± 10.76
Nordic peak force (Nm/kg)	5.63 ± 0.88	2.49 ± 10.36
1RM bench press (kg)	80.53 ± 8.41	1.21 ± 0.15
1RM back squat (kg)	115.75 ± 12.45	1.75 ± 0.17

Bootstrapped paired samples t-tests were conducted to examine statistically significant changes in body composition characteristics (Table 6.3). Analysis revealed significant increases in legs BMC (P<0.01, d=0.70), total BMC (P<0.05, d=0.66), and total lean mass (P<0.05, d=0.63). There was a significant decrease in body fat percentage (P<0.05, d=0.62). No other significant changes were detected.

Table 6.3. Changes in body composition characteristics in academy footballers following the first 14 weeks of training in a new football season.

					Pre	Post
	p	t	df	Cohen's d		
Legs BMD (g/cm^2)	0.094	-1.806	17	-0.43	1.56 ± 0.10	1.59 ± 0.12
Total BMD (g/cm^2)	0.113	-1.697	17	-0.40	1.36 ± 0.09	1.37 ± 0.10
Legs BMC (g)	0.008*	-2.958	17	-0.70	1459 ± 153	1475 ± 149
Total BMC (g)	0.022*	-2.802	17	-0.66	3582 ± 373	3606 ± 360
Legs Area (cm^2)	0.932	-0.091	17	-0.02	929 ± 55	930 ± 55
Total Area (cm^2)	0.959	-0.055	17	-0.01	2620 ± 157	2621 ± 133
Total Lean Mass (kg)	0.030*	-2.672	17	-0.63	62.13 ± 4.23	63.18 ± 5.41
Total %Fat	0.048*	2.642	17	0.62	14 ± 4	12 ± 3

*depicts a significant value (P<0.05), BMD = bone mineral density, BMC = bone mineral content.

Subsequently, we conducted bootstrapped bivariate Pearson's correlations to examine relationships between training load indicators and the delta change of those body composition characteristics that had significantly changed. There were significant positive correlations between the changes in total BMC and HMLD (r=0.36, 95%CI=0.01-0.66), distance decelerating in Zones 4-6 (r=0.39, 95%CI=0.04-0.67), distance accelerating in Zone 6 (r=0.42, 95%CI=0.03-0.74), and distance decelerating in Zone 6 (r=0.43, 95%CI=0.14-0.67). For lean body mass, there were significant negative correlations between the changes in total lean mass and high speed running distance (r=-0.49, 95%CI=-0.76-0.10), and zone 6 running distance (r=-0.45, 95%CI=-0.76-0.11). No other significant relationships were detected.

6.4 Discussion

This study is the first to assess the relationship between football-specific external training load and bone and body composition characteristics amongst elite academy footballers during a 14-week period of the season. Significant positive associations were observed between total HMLD, accelerations and decelerations in zone 6 and decelerations in zone 4-6 combined, with changes in total BMC. Additionally, high-speed running and high-speed running in zone 6 demonstrated a negative correlation with a change in lean mass. Changes in muscle strength performance measures were not related to any of the body composition or bone characteristics measured.

The associations between GPS-derived performance variables and changes in bone characteristics offer insights into specific load components of football training that trigger bone adaptation. The positive correlations between accelerations, decelerations, and changes in total BMC suggest that high impact mechanical loading, produced during football, contribute to bone accrual. This premise is supported by previous studies linking high impact movements, such as jumping, with increases in BMD and bone strength (Lambert et al, 2020). No associations, however, were observed between GPS-derived variables and leg BMC. This is surprising when the load experienced at distal limbs is greater than that experienced at the proximal sites (Milgrom et al., 2022). One explanation for this may be related to habitual loading experienced within the legs. The areas regularly experiencing less load (*e.g.,* upper body) can respond at an increased rate compared to accustomed weight-bearing sites, therefore trivial bone accrual adaptation may occur in comparison to the whole body (Hind & Burrows., 2007). Associating external load metrics with changes in bone are scarce in research, and often dismissed as the measures are not capable of representing bone load. However, if it is acknowledged that external load is not being used as a form of bone load but as a superficial measure to understand the effects of mechanical loading then it can be a useful tool alongside bone adaptation. Studies have shown positive associations between increased accelerations, GRF and bone accrual (Marin-Puyalto et al., 2019; Rogers & Hinton, 2010). This has also been observed in research where GPS-derived metrics such as accelerations, decelerations and total distance are positively associated with bone strength and BMC in footballers over a season (Varley et al., 2023). The positive correlations between high magnitude GPS-derived variables and bone characteristics underscores the importance of dynamic actions in fostering osteogenesis during football participation.

Despite changes in bone characteristics, these alterations were not uniform within all skeletal measures as no changes were shown in bone area or BMD. As football encompasses multidirectional movement patterns, bone is expected to be accrued in a site-specific manner. This is demonstrated by Zouch et al. (2015) where BMD and BMC increased at the femoral neck and lumbar spine in footballers, whereas a nonsignificant change was observed in the legs. This site-specific response is shown by Söderman et al. (2000) in adolescent footballers showing higher bone mass at the hip and lumbar spine compared to controls. Adaptations in bone reflect the direction and force transmission of mechanical loading. Therefore, within long bone especially a particular site-specific

location, such as the distal tibia, may strengthen from the loads experienced whilst the diaphysis shows no changes. As long bones are curved they bend when loaded, resulting in different exposures of internal strains distributed within the tissue meaning the regions experiencing heightened loads will experience greater adaptations (Warden, 2006). As a result, particular sites may have increased in BMD, while other sites may have decreased leaving the region to show no changes. Further investigations employing segmental analysis of bone structures are recommended to gain a comprehensive understanding of site-specific bone adaptations.

The present study showed HMLD, number of accelerations and number of decelerations were associated with BMC. Employing GPS as a method of estimating bone load has shown speed and distance metrics to be both positively and negatively associated with pQCT-derived bone metrics in 16 year old academy footballers (Varley et al., 2023). The lack of association between speed metrics and bone characteristics in the present study may be due to the footballers being ~3 years older than the academy footballers previously studied and therefore already accustomed to professional training. The mechanosensitivity of bone reduces when it becomes accustomed to monotonous, habitual load (Robling et al., 2001). Therefore, although the movements produced during football are shown to be osteogenic stimuli the additional ~3 years of participation from the current study's population may have caused a suppressed adaptation in bone characteristics. Accelerations and decelerations were not captured in the previous research by Varley et al. (2023), therefore the similarity of findings in external load cannot be assessed. Accelerations and decelerations have, however, been captured by Varley et al. (2022) within elite adult footballers and are shown to positively correlate BMC and tibial strength (accelerations only). These findings taken together with the findings in the present study, show that accelerations and decelerations can be important for bone accrual in both adult and academy footballers.

The present study showed a negative correlation between lean mass and high-speed running. The present findings showed that distinct training variables (high speed running) are correlated with a decrease in lean mass. This may relate to the GPS-derived speed zones used by the football club not being individualised which is common due to it being a laborious process (Akenhead & Nassis, 2016). Greater insights into the external load of individuals would be more comparable if metrics were created relative to the athlete. For

example, a comparison between two professional footballers performing the same role within the same matches showed trivial differences (~5%), however, when metrics were individualised there was a 41% difference shown in high intensity running between the players (Lovell & Abt, 2013). Therefore, applying individualised measures to the player are advantageous to allow for specific relative loads to be compared between individuals. Although between-unit variation has previously been reported to be consistent, metrics calculated at higher speeds are likely to have more errors due to rapid changes in the velocity profiles (Jennings et al., 2010). To minimise this variability, as was done in the present study, the same unit was used by the athletes during all session. It is acknowledged, however, that the variability of GPS-derived metrics between athletes may have contributed to some associations not being shown (i.e., leg BMC).

6.4.1 Limitations

The monitoring of external load in the present study is an extension of previous work looking into the association between changes in bone and external load (Varley et al., 2023; Varley et al., 2022), however, it is still acknowledged GPS are a proxy measure of bone load. External load estimated from GPS serves as a superficial measure of bone load and may not accurately reflect the GRF and internal loading on bones. Similarly, changes in muscle strength were used as a proxy for the muscular load exerted on bone, therefore, the specific forces that muscle elicited on bone were not measured. The loading experienced by participants prior to the study may have influenced their bone characteristics and subsequent adaptations. However, given that all participants were professional footballers with over a decade of training experience, it is expected that the pre-study habitual loading was similar in type and volume among all participants.

6.4.2 Conclusion

In conclusion, high magnitude football-specific actions, such as accelerations and decelerations, exhibited positive associations with changes in whole body BMC among elite academy footballers, highlighting the pivotal role of these actions in driving osteogenesis during football training and match-play. These findings underscore the importance of considering such dynamic actions when prescribing exercise to promote bone health in athletes.

7 The association between external load and bone structural characteristics within asymmetrical athletes.

7.1 Introduction

Sports, such as cricket, produce different magnitudes of load between limbs. For example, during cricket bowling, the front foot of a medium to fast bowler can experience a peak vertical GRF between 2 and 5 body weights (BW) higher than the back foot (Hurrion et al., 2000). These 'asymmetrical' sports people are an interesting population to study in the context of bone adaptation, as the imbalanced movement patterns they habitually perform produce loads that can create a difference in bone response. The asymmetrical nature and force of the cricket bowling technique are shown to result in greater torsional and shear forces within the contralateral side of the body (Always et al., 2019). This increase has been observed to create greater BMD and BMC in the non-dominant vertebra (Alway et al., 2019). The load experienced at the lumbar spine, however, may not represent the load experienced at distal parts of the skeleton, such as the distal tibia. There is only one study known to the present authors that has investigated lower extremity bone characteristics in relation to the asymmetrical application of load in cricket bowlers. Despite differences in vertical GRF and loading rate between legs, there were no bilateral differences in femur bone characteristics (Lees et al., 2018), which is surprising as load magnitude is influential on the osteogenic response (Guadalupe-Grau et al., 2009).

Asymmetrical sports have examined the effects of divergent load on skeletal characteristics (Krahl et al., 1994; Warden et al., 2009; Bogenschutz et al., 2011). Limb-specific loading of the forearm in professional tennis players has been shown to lengthen the ulna and second metacarpal in the racket arm (Krahl et al., 1994). Baseball and softball pitchers also display greater bone mass, cortical area, cortical thickness and BMC in the humerus of the throwing arm compared to the non-throwing arm (Warden et al., 2009; Bogenschutz et al., 2011; Warden et al., 2019). Upper extremity models suggest the asymmetrical differences are likely caused by the habitual mechanical load experienced in the active arm, as no external forces are exerted as in the lower extremity. External forces (*e.g.,* GRF) are prominent in the lower extremities during daily activity, therefore understanding the influence of mechanical load is more difficult due to the external interferences. The aforementioned studies have offered insight into how bone can react to its mechanical environment and how exercise can influence bone adaptation within an isolated bone-load interaction.

Fast bowlers can perform a bowling action up to 324 times per week (Orchard et al., 2009), meaning they habitually experience an imbalanced distribution of load within their lower extremities. This could have an impact on the bone characteristics of the lower legs, specifically the tibia, as forces are heightened at the distal most part of the body (Milgrom et al., 2022). An insight into how exercise variables relate to bone adaptation is important for elite athletes, as the risk of stress fracture injuries in cricketers has been associated with excessive load (Alway et al., 2019). Knowledge of loading metrics that may contribute to bone adaptation is important for practitioners wanting to prevent or treat bone conditions, since they may use this information to prescribe exercise thresholds.

The novelty of studying bilateral differences in the tibiae of cricket bowlers offers insight into the most distal long bone that is likely experiencing the greatest magnitude of load during bowling. How this translates to tibial bone characteristics is unknown and could offer insight into tibial adaptation from external load during habitual high-load exercise. IMUs can be used as a practical, non-invasive measurement and they can provide site-specific information on the acceleration and direction of movement to assess external load during unrestricted exercise (Ueberschär et al., 2019). Research has suggested measures such as tibial acceleration can act as surrogate measures of impact force experienced on bone (Sheerin et al., 2019) and moderate correlations have been shown between accelerations, GRF metrics (*e.g.,* impact peak) and tibial acceleration during running (Greenhalgh et al., 2012).

Assessing sporting activity can provide valuable insights that may not be possible to obtain in a laboratory setting, where exercise is typically controlled and restricted. By studying athletes in applied environments, researchers and practitioners can gain a better understanding of how different types of external load and training regimes affect bone health. The aim of this study was to assess the association between external load and bone characteristics in cricket fast bowlers. It is hypothesised that cricket fast bowlers will produce greater external load and bone characteristics in their dominant leg compared to the non-dominant leg. It is also hypothesised that higher external load metrics will be associated with greater BMD and bone strength characteristics.

7.2 Methods

7.2.1 Participants and study design

11 male elite right-armed cricket fast bowlers (aged 24 ± 5 years old, height 1.91 ± 0.08 m, body mass 91.1 ± 12.1 kg, participation 12.9 ± 3.8 years) and 14 right-footed male elite footballers (aged 19 ± 1 years old, height 1.81 ± 0.04 m, body mass 77.0 ± 6.3 kg, overall participation 10.6 ± 2.9 years) were recruited from professional clubs via pre-existing professional networks. Each athlete group was used as an independent sample to investigate the differences between legs. Fast bowlers typically bowled 35 overs, or 210 balls, per week whilst footballers typically trained for ~12 hours per week and played competitive matches for 1 - 2 hours per week. In fast bowlers, the front planting leg during a bowl is referred to as the dominant leg and the back or trailing leg is referred to as non-dominant. In footballers, the preferred kicking leg is referred to as dominant and the standing or supporting leg is referred to as non-dominant. The inclusion criteria for the study required participants to be aged between 18 and 40 years old, competing at an elite level (elite being defined as a professional athlete contracted to and competing in their chosen sport), a fast bowler (cricket only) or an outfield player (football only), injury free, not currently taking any medication that influenced bone metabolism and had not received a joint replacement or prostheses. Before taking part in the study, participants completed informed consent, a health screen questionnaire, a pre-scan screening and an athletic and injury status questionnaire. Height (Stadiometer, Seca, Hamburg, Germany) and body mass (Seca, Birmingham, UK) were recorded wearing minimal clothing. The study was approved by the ethics committee (Ref 604) and the National Health Service Research Ethics Committee (Ref 260817).

7.2.2 Protocol

The external load was assessed in the fast bowlers whilst they performed six overs (36 balls) at a wicket and batsperson during a pre-season training session at the intensity they would during a competitive match. The external load was assessed in footballers whilst they performed a habitual warm-up that replicated the movements performed during match-play (*e.g.,* acceleration/deceleration, change of direction, hopping and jumping). Athletes attended a lab for DXA and pQCT scans within 2 weeks of their loading assessment during training.

7.2.3 External load monitoring

Prior to the activity, IMUs (dimensions 42 x 27 x 11 mm, mass 9.5 grams, operating range 200g; Blue Trident, Vicon Motion Systems Ltd, Oxford, UK), recording at 1600 Hz were secured with a self-adhesive overwrap (Lightpast Pro, Vivomed) to each leg at the 14% site of the tibial length measured from the distal end to match the 14% site of the pQCT scan. IMUs have been shown to reliably monitor impact load, bone stimulus and step count during running-based team sport tasks (Armitage et al., 2021), peak acceleration and loading rate during fast bowling (Senington et al., 2020). They may also be used as a better estimation of bowling load than bowling frequency alone as not all deliveries exert the same level of load (McGrath et al., 2021). Tibial length was measured with a ruler between the medial aspect of the tibial plateau and the medial malleolus. Raw acceleration data were exported into Python (version 3.10) to calculate resultant peak acceleration, resultant peak positive acceleration (PPA), cumulative load and relative load. Resultant peak acceleration was calculated using the three-dimensional Pythagoras' Theorem formula, meaning the maximum acceleration in each axis (vertical, anteroposterior, mediolateral) were used. Resultant peak positive acceleration was calculated as the average of the 10 highest resultant peaks identified. Cumulative load was calculated as the sum of the number of foot plants multiplied by the sum of peak accelerations adapted from Besier (2019). A step was identified when the acceleration trace surpassed 5 gravitational units (Schmidt et al., 2016) with one gravitational unit (g) being equal to 9.81 m/s. The maximum acceleration observed within 90 ms of the 5 g threshold was taken as the resultant peak acceleration during each step. Relative load was calculated by dividing the cumulative load by their body mass.

7.2.4 Bone characteristics

DXA scans (iDXA, GE Healthcare, UK) were used to assess whole body BMD (g/cm^2), BMC (g) and bone area (cm^2). DXA is the gold standard technique to measure bone parameters and body composition due to its precision and reproducibility and has wide clinical use (Lodder et al., 2004) with a coefficient of variation of <3% (El Maghraoui & Roux., 2008). Participants were positioned supine on the scanner bed with their ankles and knees strapped to restrict involuntary movement. The participants lay motionless for the duration of the scan with their arms by their sides. All scans and analyses were performed by the same manufacturer-trained operator to keep the scans consistent. If any

movement artifacts were present, the image was classified as invalid and repeated. No participants were removed from the analysis due to artifacts.

pQCT scans (XCT2000L, Stratec Medizintechnik) were used to assess Trabecular density (g/cm^3) at the 4% site. Stress strain index (SSI) X (axial anteroposterior bone strength), Y (axial mediolateral bone strength) and polar (torsional bone strength), cortical thickness (mm) and periosteal circumference (mm) at 14% and 38% site and cortical density (g/cm^3) at 14%, 38% and 66% site of the right and left tibia were measured. PQCT allows high quality, accurate imaging and morphological assessment of bone (Stagi et al., 2016) with a coefficient of variation of <2.19% (Rinaldi et al., 2011). The participant's tibial length was measured to the nearest mm, determined as the medial aspect of the tibial plateau to the medial malleolus. The participant's leg was placed in the scanner with their foot secured in a purpose-built attachment. The leg was aligned with an integral laser and clamped at the knee to restrict movement whilst the participant was directed to remain as still as possible during the scan. A reference point locating the scan was performed to confirm the location of the distal end plate, which acts as a positioning line. Sectional images were obtained at the distal sites (4%, 14%) and the diaphysis of the tibia (38%, 66%) from the positioning line. A voxel size of 0.5 mm and slice thickness of 2.5 mm was used for all measurements. A contour mode, with a threshold of 180 mg·cm3, was used to separate soft tissue and bone. If any movement artifacts (inaccuracies in the measurement caused by motion) were present following the scan, the image was classed as invalid, and a repeat measure was performed. No participants were removed from the analysis due to artifacts.

7.2.5 Statistical analyses

Data were checked for normality of distribution with Shapiro-Wilks tests (IBM, SPSS Statistics, v.29). To determine differences in external load and bone characteristics between legs, paired samples t-tests were performed. To test the differences of change between legs across athlete groups independent samples t-tests were performed. Statistical significance was accepted at the 95% confidence level (P<0.05). If data were nonparametric then a Wilcoxon signed Rank test was performed. Pearson correlations were performed to assess correlations between bone characteristics and external load metrics for each leg. Correlations were interpreted as negligible (.00 to .30), low (.30 to

.50), moderate (.50 to .70), high (.70 to .90) and very high (.90 to 1.00) following the guidelines of Hinkle et al. (2003). Coefficients of variation (CV%) were reported to display IMU-derived within-person variance.

7.3 Results

7.3.1 Physical characteristics

There was no difference in leg fat mass within fast bowlers (*P*=.76) or footballers (*P*=.60). Leg lean mass was higher in the dominant leg for footballers (*P*=.03) but no difference was shown in lean mass between legs in fast bowlers.

7.3.2 Cricket fast bowlers

Fast bowlers displayed significantly higher resultant peak accelerations and resultant PPA in the dominant leg compared to the non-dominant leg (Table 7.1; *P*<.01). No differences were shown in cumulative or relative load. Fast bowlers had greater BMC (*P*=.02) and tibial strength (X and Polar) (Figure 7.1, Table 7.2; *P*<.04) in the dominant leg compared to the non-dominant leg. No differences were shown in BMD, bone area, trabecular density, cortical thickness, periosteal circumference or tibial strength (Y). Within-person dominant leg variability of peak resultant acceleration was 30%, PPA was 29%, cumulative load was 42% and relative load was 45%. The non-dominant leg variability peak resultant acceleration was 35%, PPA was 32%, cumulative load was 52% and relative load was 56%.

Table 7.1. Fast bowler's external load metrics

Variable	Fast bowlers			
	Dominant leg	Non-dominant leg	**% Difference**	***P***
Resultant peak acceleration (g)	26.9* ± 7.9	17.6 ± 6.1	53%†	.00
Resultant PPA (g)	21.1* ± 6.3	15.5 ± 4.9	38%	.00
Cumulative load	94756 ± 40019	90609 ± 47036	5%	.58
Relative load	1038 ± 469	997 ± 556	4%	.62

Values are represented as mean (±1SD). *depicts a significant difference between the dominant leg and the non-dominant leg (P>0.05). †depicts a significant difference in change between legs across athlete group.

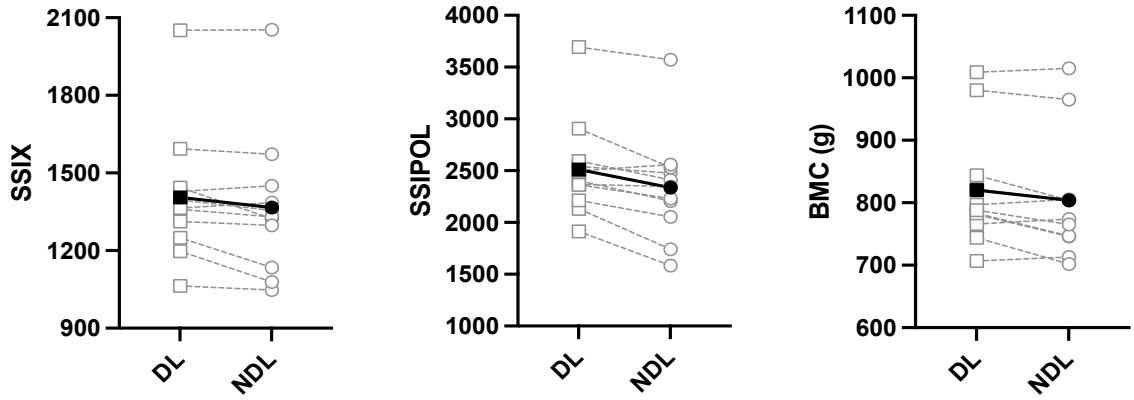


Figure 7.1. Individual fast bowler bone characteristics between dominant leg (DL - □) and non-dominant leg (NDL - ○). Black depicts group mean. SSIX and SSIPOL were assessed from pQCT. BMC assessed from DXA.

7.3.3 Footballers

No differences in any external load metrics were shown between legs (Appendix XI). Footballers showed no differences in bone characteristics between legs (Table 7.2). Within-person variance was higher in footballers compared to fast bowlers. The dominant leg variability of peak resultant acceleration was 50%, PPA was 39%, cumulative load was 78% and relative load was 77%. The non-dominant leg variability peak resultant acceleration was 43%, PPA was 38%, cumulative load was 75% and relative load was 73%.

Table 7.2. DXA and pQCT-derived bone measurements from fast bowlers and footballers. pQCT measurements taken at the 4%, 14%, 38% and 66% sites of the tibia.

Variables	Fast bowlers				Footballers			
	Dominant leg	Non-dominant leg	**% Difference**	***P***	Dominant leg	Non- dominant leg	**% Difference**	***P***
DXA								
BMD (g/cm^2)	1.62 ± 0.12	1.59 ± 0.42	1.9%†	.13	1.59 ± 0.16	1.57 ± 0.17	1.3%	.19
BMC (g)	820* ± 94	804± 220	2.0%	.02	738 ± 81	736 ± 81	0.3%	.47
Total bone area (cm^2)	507 ± 43	504 ± 40	0.6%	.20	466 ± 24	469 ± 24	0.6%	.27
pQCT								
4%								
Trabecular density (g/cm^3)	276 ± 22	269 ± 32	2.5%	.13	304 ± 45	298 ± 42	2.0%	.24
14%								
Cortical density (g/cm^3)	1106 ± 14	1100 ± 19	0.5%	.07	1091 ± 18	1094 ± 17	0.3%	.33
Cortical thickness (mm)	3.11 ± 0.46	3.07 ± 0.38	1.3%	.73	3.11 ± 0.39	3.16 ± 0.46	1.6%	.35
Periosteal circumference (mm)	90 ± 7.9	90 ± 6.9	0.0%	.92	88.3 ± 6.1	88.3 ± 5.3	0.0%	.94
SSIX	1405 ± 255	1366 ± 277	2.8%†	.06	1339 ± 199	1315 ± 159	1.8%	.67
SSIY	1441 ± 259	1424 ± 245	1.2%	.65	1404 ± 206	1439 ± 181	2.4%	.35

SSIPOL	2511* ± 470	2337 ± 516	7.0%	.00	2386 ± 352	2408 ± 284	0.9%	.83
38%								
Cortical density (g/cm^3)	1139 ± 17	1136 ± 16	0.3%	.52	1138 ± 20	1142 ± 18	0.4%	.22
Cortical thickness (mm)	6.35 ± 0.79	6.34 ± 0.66	0.2%	.98	6.64 ± 0.35	6.54 ± 0.51	1.5%	.41
Periosteal circumference (mm)	86.9 ± 4.6	84.9 ± 5.7	2.4%	.11	81.7 ± 3.3	81.1 ± 3.0	0.7%	.52
SSIX	1768 ± 295	1657 ± 382	6.3%	.06	1513 ± 171	1473 ± 147	2.7%	.36
SSIY	1500 ± 223	1482 ± 290	1.2%	.53	1346 ± 164	1303 ± 157	3.3%	.35
SSIPOL	2746 ± 326	2619 ± 506	4.6%	.13	2448 ± 305	2424 ± 284	1.0%	.73
66%								
Cortical density (g/cm^3)	1108 ± 20	1104 ± 16	0.4%	.61	1086 ± 18	1098 ± 18	1.1%	.09

Values are represented as mean (±1SD). *depicts a significant difference between the dominant leg and the non-dominant leg (P>0.05). †depicts a significant difference in change between legs across athlete groups (P>0.05).

7.3.4 External load and bone characteristic correlations

In fast bowlers, cumulative load showed moderate positive correlations with SSIX (r=.638) and SSIPOL (r=.638) at the 14% site of the tibia in the dominant leg (*P*=0.035) (Figure 7.2). No correlations were shown between any other external load metrics and bone characteristics of the fast bowlers and footballers.

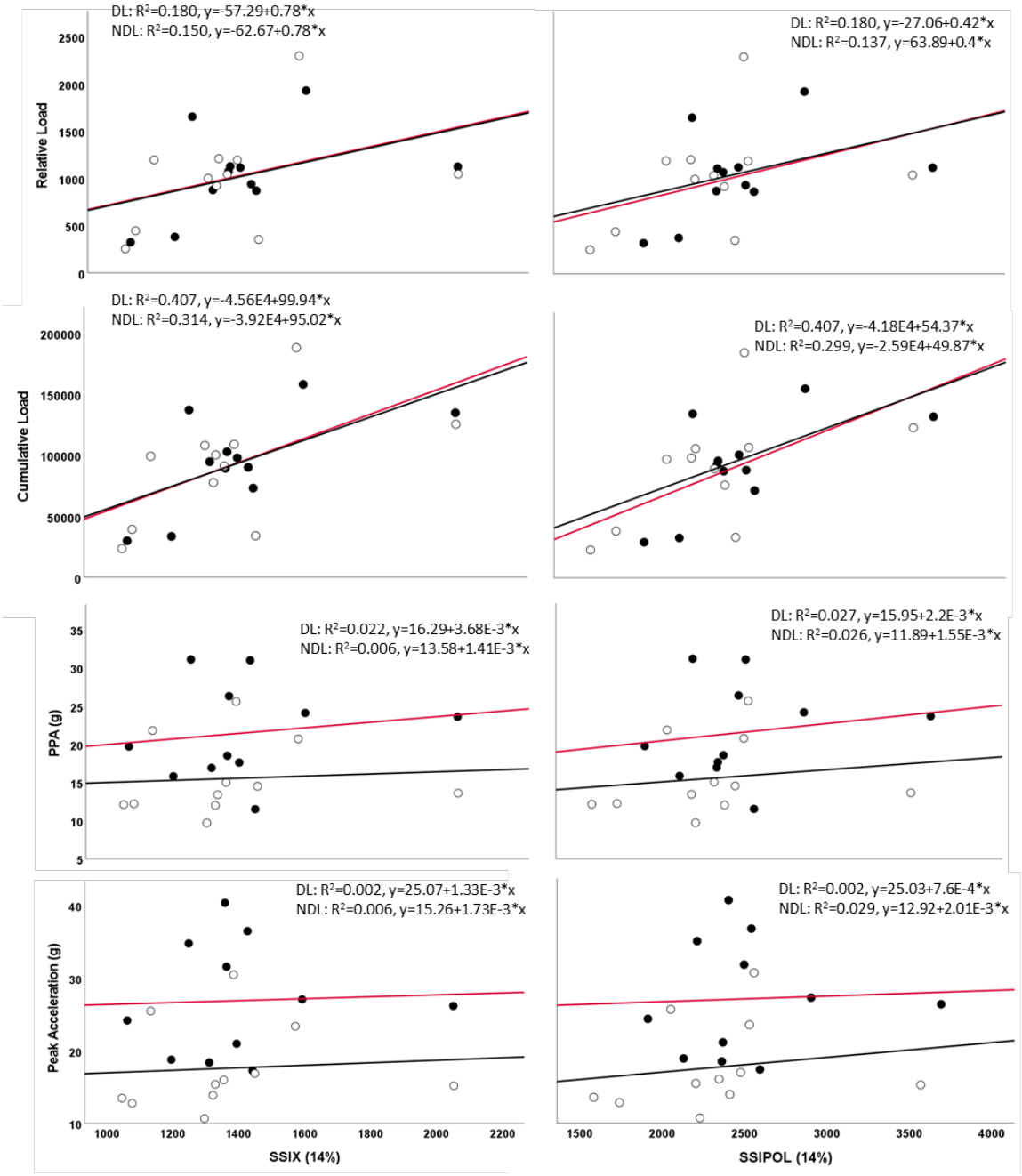


Figure 7.2. Pearson correlations between external load metrics and bone characteristics within legs of fast bowlers. Black dots and red line depict dominant leg. White dots and black line depict non-dominant leg.

7.4 Discussion

The present study is the first study to show an association between tibial strength and cumulative load in fast bowlers**.** The association with cumulative load, but not peak load is surprising based on research demonstrating that high magnitude loading is important for bone accrual (Guadalupe-Grau et al., 2009) and the current study showing a 53% difference in peak acceleration in the fast bowlers' dominant leg compared to the non-dominant leg. The mechanisms that drive bone response have shown that high load creates large rates of deformation in the bone matrix, which promotes osteogenesis (Turner & Robling., 2005). Most of the research exploring mechanical load and bone accrual, however, has used rhythmic loading (replication of running, jumping; Weatherholt & Warden 2016; Weeks et al., 2008; Varley et al., 2017), whereas fast bowling has unique loading cycles (run-up, pre-delivery stride, delivery stride, follow through; Bartlett et al., 1996). The intermittent nature of the foot planting cycles may help promote bone adaptation as the use of rest periods has been shown to regain bone mechanosensitivity (Burr et al., 2002). Fifty loading cycles in a single bout promote osteogenesis, with bone becoming refractory to loading cycles beyond this (Burr et al., 2002). It could be speculated the intermittent nature of cricket bowling may be used by support staff in cricket to help with bone adaptation. Using the guidelines of Burr et al. (2002) for bone adaptation to load, it may be possible to assess the load magnitude and recovery times during cricket training as a method of understanding bone accrual in an applied environment.

Fast bowlers experienced more peak acceleration and PPA in the dominant leg compared to the non-dominant leg, whereas no differences in external load were shown in footballers (Table 7.1). This is comparable to other studies that have shown high tibial accelerations during fast bowling (Epifano et al., 2022) and the action creates a heightened impact force of 5 - 9 times the athlete's body weight when using force plates (Johnstone et al., 2014; Hurrion et al., 2000). In comparison, footballers only generate ~2.5 times their body weight during sprinting (Meijer et al., 2006). Despite the dominant leg of fast bowlers having greater tibial strength (SSIPOL, BMC) and greater external load (peak acceleration and PPA) than the non-dominant leg, no correlations were shown between bone characteristics and peak external

load parameters (Figure 7.2). Furthermore, although this was not a comparative study between groups, we analysed if there was a difference between the dominant and non-dominant legs between the athlete groups. There was a significant difference between dominant leg and non-dominant leg resultant PPA, peak acceleration, BMC and SSIPOL (14%) in fast bowlers, but no differences in external load or bone characteristics in the dominant and non-dominant leg of footballers. The technique of cricket bowling creates a large shift in the linear velocity of the centre of mass as the dominant leg produces a braking force when planted (Worthington et al., 2013). This heightened load may contribute to the stronger bone characteristics shown within the tibia of the dominant leg as a higher magnitude of load is known to initiate a greater osteogenic response (Guadalupe-Grau et al., 2009).

An advantage of the present study is the site-specific measurement of external load and the environment in which it took place. Ideally, a direct measurement of bone strain (*e.g.,* tibial mounted strain gage) during activity would be applied, but this is invasive and impractical, particularly in an elite athlete population (Burr et al., 1996). Therefore, practical measurements are necessary to capture natural movements with GPS being commonly used by practitioners to estimate bone load (Study 2). Correlations have been shown between total distance, accelerations and decelerations derived from GPS and bone strength characteristics in football players across a season (Varley et al., 2022), suggesting quantifying load from wearable technology can assist in monitoring external load alongside bone during exercise. The current study, however, assessed bone characteristics at the tibia, whereas Varley et al. (2022) assessed external load at the upper back therefore it is not site-specific. The athletes in the present study were monitored using IMUs during habitual training rather than in a controlled laboratory setting. IMUs are capable of providing information specific to the area (Armitage et al., 2021), in this case the tibia. Changes in bone mass and geometry are sensitive to change at different sites (anterior, posterior, medial or lateral) of the same bone (Zernicke et al., 2006). The current study reported load experienced at the anteromedial distal site of the tibia where loads are observed to be highest (Milgrom et al., 2022). This is an improvement on GPS placement as it is not an approximate load transferred through the body (Varley et al., 2022).

Previous studies have shown that accumulated impact-based loads incite positive adaptations in bone strength and can act as predictors for bone characteristics at the tibia (Ducher et al., 2011; Nikander et al., 2006). No correlation, however, was shown between peak acceleration and tibial strength in the present study. The reasons for this could be (1) only using IMU-derived metrics, whereas other external load measures and metrics are available and (2) no segmental analysis was performed using the pQCT scans. If additional time and IMUs were available then segmental analysis would have been performed which may have produced correlations at specific areas of the tibia, however this was not possible for the current body of work. The lack of correlation in the present study may be due to the placement of the IMUs. The present study placed the IMUs at the 14% site of the distal tibia, therefore, the accelerations and inferred load were only measured at the anteromedial site of the tibia. It may be hypothesised that the loading experienced at other tibial sites (*e.g.,* posterolateral) differ from the site measured. Tibial accelerations can fluctuate across different locations of the tibia and loading applied across bone does not act uniformly (Lucas-Cuevas et al., 2017). The action of cricket bowling necessitates linear movement patterns so that bone accrual may occur at a specific location (*e.g.,* anteriorly) in response to the load experienced. Therefore, movement can cause an excessive load on bone in one direction whilst simultaneously unloading the other (Weatherholt & Warden, 2016).

Although BMC and torsional tibial strength (polar) were different, there were no differences in BMD, bone area, trabecular density, cortical density, cortical thickness, periosteal circumference, and tibial strength (X and Y) between the dominant and non-dominant legs of fast bowlers. This may be explained by each leg being habitually exposed to mechanical loading on a daily basis. Unlike previous studies (Krahl et al., 1994; Warden et al., 2009; Bogenschutz et al., 2011), the observations in the present study were made in the lower limbs, which means habitual load occurred outside of the bowling sport-specific actions. This habitual load may create a higher baseline load threshold for adaptation, whereas the upper limbs do not have any regular exposure to GRF, which has been associated with bone accrual (Hind & Burrows., 2007).

External load was monitored during a single training session due to the time constraints of the participants, therefore the one-off measurement may not represent the athletes overall load exposure. It does, however, offer an insight into the exercise intensity experienced at the distal limbs of matchlike fast bowling. It is recognised using a warm-up as a representation of load during football does not replicate all of the movements and intensity of match play, however, it does offer a snapshot of the physical match demands of football. No differences in bone characteristics were shown between legs within footballers, although there was a difference in leg lean mass, which may be ascribed to being the kicking foot. Injury history was recorded by the participants during this study, however, no dietary tracking was performed even though it is well-established that diet can influence bone health. As the study aimed to make inter-limb comparisons in the same individuals, diet is unlikely to have significantly influenced the findings. It should, however, be noted that dietary differences between the fast bowlers and footballers could influence inter-group comparisons. The present study was cross-sectional in nature, therefore it cannot distinguish whether the sport alone or maturation influenced the bone characteristics measured. Therefore, longitudinal study designs monitoring external load would offer insight into the relationship between high-impact exercise and bone characteristics.

7.4.1 Conclusion

High cumulative load may be associated with an increase in anteroposterior and torsional tibial bone strength. This offers applied practitioners' insight into how bone accrues to habitual high-load activity. Using IMUs as a method to estimate bone load and subsequent bone adaptation may offer an alternative solution to using GPS for measuring external load for applied practitioners. It would be insightful to observe the relationship between external load and bone during interventions where load magnitude differs between groups. This would help to observe the effects of quantified repetitive loading on bone characteristics in human exercise protocols, where groups are habitually exposed to different magnitudes during the same type of activity.

Acknowledgement: Thank you to Thomas Parsons for developing the IMU data processing within this study.

8 The effect of 16 weeks impact exercise on tibial adaptation in young adults

8.1 Introduction

Dynamic loading is important for bone adaptation with evidence suggesting high-impact exercise can minimise bone loss and promote bone accrual (Dasarathy & Labrador, 2018; Barry & Kohrt, 2008). Jumping interventions are recommended as an accessible, non-pharmacological alternative that may assist in improving bone health (Florence et al., 2024). This high-impact activity has consistently been shown to result in increases in BMD (Simoes et al., 2021; Yu et al., 2019; Vainionpää et al., 2005) and bone geometry (Allison et al., 2013; Ferry et al., 2013; Ducher et al., 2011). This is because jumping is thought to apply a sufficient magnitude of strain to support bone maintenance and stimulate accretion (Al Nazer et al., 2012). There is limited knowledge, however, as to whether there is an optimal load magnitude to increase BMD and whether any specific loading thresholds to promote bone adaptation can be identified. Studies in pre-pubescent youth cohorts have shown impact exercise produces gains in BMD of 3.5 – 8% (Gunter et al., 2008; Fuchs et al., 2001) which is considerably higher than gains reported in studies accessing bone accrual in response to interventions in later life (Hind & Burrows., 2007). This premise is also supported whereby a 9-month jumping intervention in adolescent male swimmers and cyclists reported ~5% gains in leg BMC compared to non-jumping controls (Vlachopoulos et al., 2018). The heightened response from habitual exercise during youth is thought to reduce the risk of fragility fractures by 50% compared to age-matched non-athletic participants (Tveit et al., 2013) exemplifying the importance of accruing bone mass in the early years for later life. Therefore, it may be better to use exercise as a preventative intervention for increasing bone strength when young rather than as a treatment for weaker bones in old age.

Progressively increasing load magnitude during jumping using weighted vests has been shown to cause positive changes in leg BMC in adolescent athletes compared to sport-matched non-jumping controls (Vlachopoulos et al., 2018). Allison et al. (2013) showed unilateral jumping increases femoral neck BMD and BMC after 12 months in the jumping leg. In addition, both Lambert et al. (2020) and Weeks et al. (2008) found jumping interventions enhance lower extremity adaptation over ten and eight months in young adults and adolescents. Diagonal drop jumps are thought to be beneficial to bone due to their multi-

directional component which is suggested to be more osteogenic than linear movements, such as countermovement jumps (Nikander et al., 2010). Recently, diagonal drop jumps have been shown to stimulate the development of bone (Prawiradilaga et al., 2020) with the multidirectional landing mechanics of diagonal drop jumps distributing strains more widely across the tissue (Yan et al., 2022). Although these studies provide useful insights into the osteogenic effects of jumping, they do not attempt to monitor load magnitude. As a result, there is an inability to prescribe jumping as an osteogenic activity as loading parameters are scarce.

It is understood that habitual, high-impact exercise can result in bone accrual, although research lacks accurate load data during exercise protocols. The relationship between external load and bone adaptation is not well understood (Study 2), mainly due to external load being a proxy measure of bone load and not accounting for muscular activity that also imposes load onto bone. Although the load required to stimulate bone adaptation is unclear, it is thought that higher load magnitudes produce greater bone strains resulting in positive changes (Warden et al., 2004). External load is commonly measured in sports and clinical settings and, therefore, has the potential to be used to understand bone adaptation. When load is monitored the loading metrics used are often nonspecific, such as using exercise time (Weeks et al., 2008; Vainionpää et al., 2005), exercise quantity (Daly et al., 2021; Bailey & Brooke-Wavell., 2010) or non-site-specific measures *e.g.,* GPS (Varley et al., 2023) and hip-mounted accelerometers (Vainionpää et al., 2006). More appropriate methods of monitoring external load during osteogenic interventions are IMU, force plates or motion capture that have all been used to quantify load (Choi et al., 2021; Willy, 2018; Rogers & Hinton., 2010). For example, increases in GRF are shown to associate with increases in bone strength (Yuan et al., 2016), whilst kinetic data, such as joint moments, are predictors of BMD and BMC (Moisio et al., 2004). These measures, however, are subjected to criticism as they do not account for muscular force (Matijevich et al., 2019). It is not clear how external load can be monitored as a superficial measure of bone load, therefore it is difficult to prescribe exercise thresholds for bone accrual. To advance our knowledge of bone health mechanical loading should be monitored to develop more definitive exercise guidelines.

Therefore, the primary aim of this study is to assess if a dose-response relationship exists between load magnitude and bone adaptation. The primary outcome measures of the present study are BMD and pQCT-derived bone strength measurements. The secondary outcome measures are load-derived (force plates, IMU, motion capture) variables produced during the jumping intervention. It is hypothesised that external load measurements will be higher from greater diagonal drop jump heights, and this will result in greater bone adaptation. The null hypothesis states there will be no changes in external load or bone characteristics between jumping conditions.

8.2 Methods

8.2.1 Participants and study design

Forty-eight low activity young adults (aged 22 ± 2 years old, height 1.73 ± 0.17 m, body mass 71.0 ± 17.4 kg) were recruited to take part in the present study at Nottingham Trent University (Figure 8.1). Low activity being defined as someone who partakes in physical activity ≤2 times per week (Evans et al., 2012), including no more than once per week in exercise reported to be osteogenic (*e.g.,* weight-bearing, multidirectional exercise). The study a required a sample size of n=48, calculated using G*Power (Faul et al., 2009) with an alpha value of 0.05, estimated power of 0.85 and effect size of 0.655 based off previous a previous RCT applying high-impact interventions (Bailey & Brooke-Wavell., 2010). The inclusion criteria for the study required participants to be between 18 and 25 years old and not regularly participating (more than once a week) in dynamic weight-bearing exercise that may influence bone adaptation. Exclusion criteria included, any current unresolved cardiovascular complaints, taking any medication that is known to influence bone metabolism and have had a recent or current injury, joint replacement, or prostheses. Before taking part in the study, participants completed informed consent, a health screen questionnaire, and a pre-scan screening. Height (Stadiometer, Seca, Hamburg, Germany) and body mass (Seca, Birmingham, UK) were recorded wearing minimal clothing. The study was a sixteen-week diagonal drop jump intervention comprised of three drop height groups: 0cm, 40cm, 60cm and a control. These drop heights were decided based upon previous research displaying differences in external load magnitude between the heights (Makaruk & Sacewicz, 2011).

Participants were initially randomised to an exercise group, however as recruitment advanced participants were matched within groups depending on sex, height, and weight. The study was approved by the ethics committee (Ref 737) and the National Health Service Research Ethics Committee (Ref 306347).

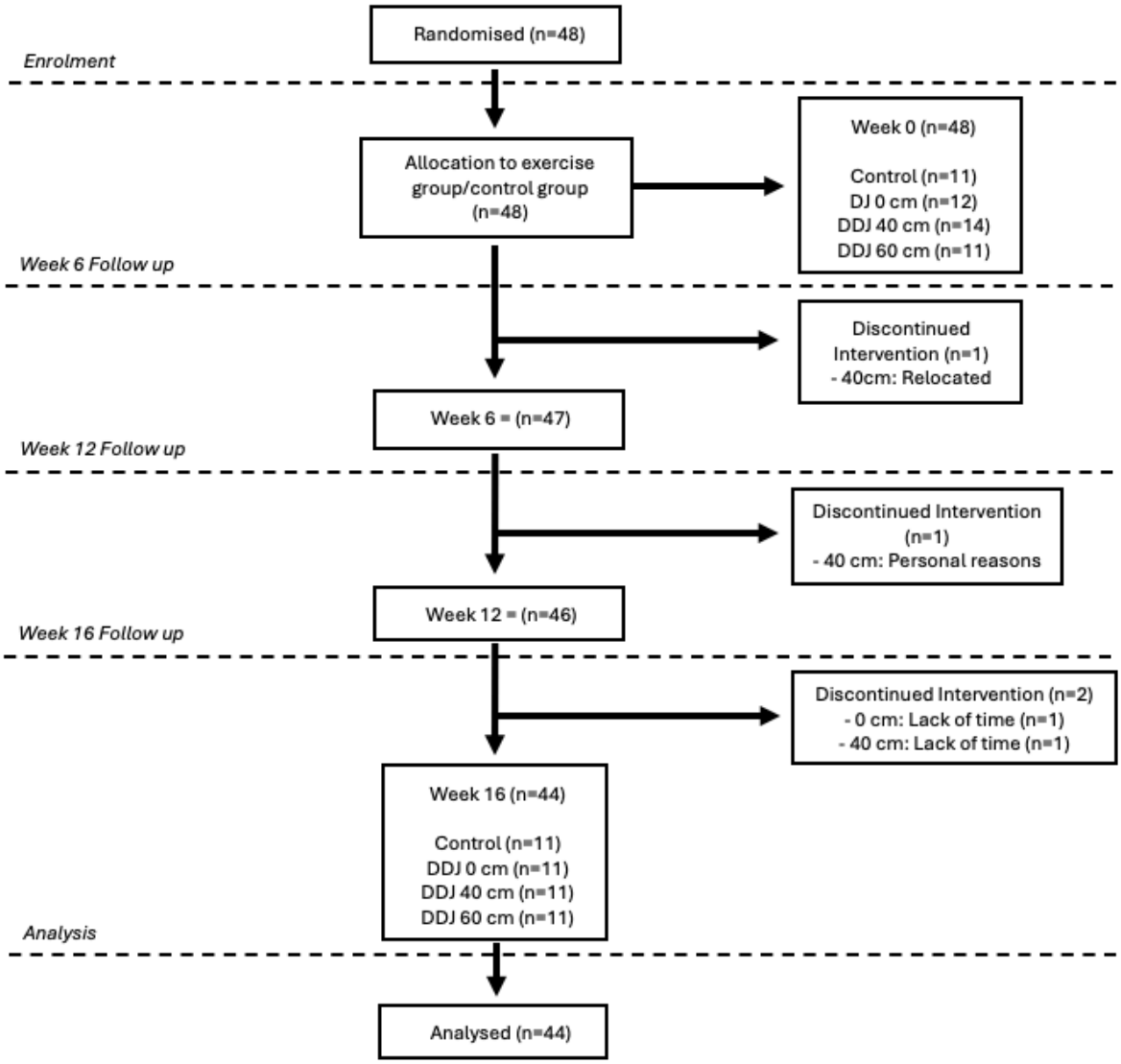


Figure 8.1. Consort diagram of study sample

8.2.2 Protocol

A sixteen-week intervention study was performed. Upon acceptance to take part, participants were invited to a laboratory-based pre-study screening on the initial visit. This involved participants recording their current diet (food frequency questionnaire), sleep quality (Pittsburgh sleep quality index: Buysse et al., 1989) and current physical activity status

(Bone-specific physical activity questionnaire: Weeks & Beck, 2008) as a way of monitoring individuals did not make any changes to their lifestyle throughout the intervention. Historical physical activity was also recorded using the BPAQ to understand the historical activity status of participants (www.fthdysign.com/BPAQ/). During the first visit, the protocol (Figure 8.2) was explained to the participants to ensure they understood the requirements. Participants were put into a diagonal drop jump group (0cm, 40cm, 60cm) or the control group on their first visit using a covariate adaptive randomisation method. Covariate adaptive randomization is where a new participant is sequentially assigned to a group by taking into account the specific covariates and previous assignments of participants. In this study, initial recruitment in wave one randomised participants to a group and wave two participants were assigned based on anthropometrics (sex, height, weight) to minimise an imbalance amongst groups. Following the initial screening all drop jumps were performed at home. During the intervention, four visits were required to the laboratory at pre-intervention (week 0), week 6, week 12 and post-intervention (week 16).

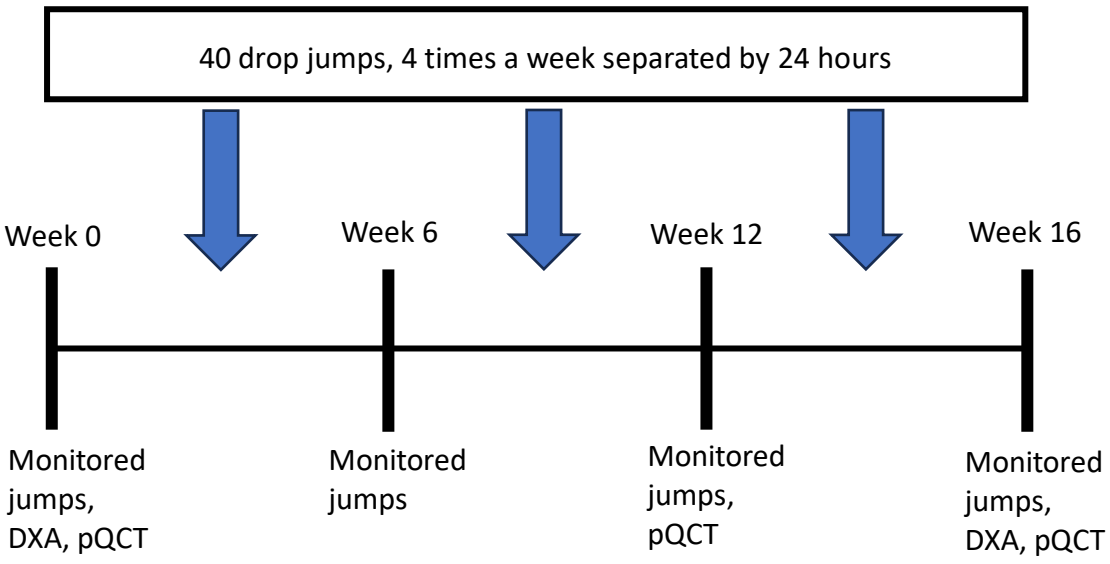


Figure 8.2. Intervention protocol design

During the intervention, four participants withdrew, leaving forty-four participants to complete the study. Those assigned to an intervention group were required to perform forty diagonal drop jumps from a predetermined height four days a week, with each session

separated by 24 hours. This rest period is recommended to restore mechanosensation, allowing subsequent sessions to provide osteogenic loading benefits (Turner & Robling, 2003). Participants performed four sets of ten alternating diagonal drop jumps (10 to the left and 10 to the right) per session, similar to the frequency used by Bailey & Brooke-Wavell (2010). Participants jumping from 40cm or 60cm were provided with a plyometric box (Decathlon, 40x50x60cm, France). All jumps, both in the laboratory and at home, were performed barefoot on non-carpeted flooring to eliminate any cushioning effects (Malisoux et al., 2017). Control group participants did not perform any jumps but underwent DXA and pQCT scans. Upon completing the study, participants filled out an exit survey on intervention compliance and applicability. The survey inquired about the difficulty of the intervention, the number of jump sessions missed at home, and whether the intervention was realistic for low-active individuals (Appendix XXV).

8.2.3 Load measurements

During the laboratory-based monitoring of diagonal drop jumps, participants were assessed via motion capture (Nexus, v2.14, Vicon Motion Systems Ltd, Oxford, UK), force plates (AMTI, Massachusetts, USA) and IMUs (dimensions 42 x 27 x 11 mm, mass 9.5 grams, operating range 200g; Blue Trident, Vicon Motion Systems Ltd, Oxford, UK). The measures were all calibrated through the Vicon motion capture software to ensure they were synchronised during data collection. Upon entering the laboratory, a lower extremity marker system was attached to the participant (Figure 8.3) to create a skeletal model developed from a combination of lower extremity marker systems by List et al. (2013) and Leardini et al. (2007). Reflective markers were placed at the anterior superior iliac spine, posterior superior iliac spine, 50% of the lateral thigh, 20% of the lateral thigh, medial and lateral epicondyle of the knee, head of the fibula, tibial tuberosity, 50% of the tibia, 30% of the lateral fibula, medial and lateral malleolus, calcaneus, medial apex of the sustentaculum tali, lateral apex of the peroneal tubercle, base of the first, second and fifth metatarsal, head of the first, second and fifth metatarsal and the most distal point of the head of the proximal phalanx of the hallux.

Figure 8.3. Lower extremity marker system.

IMUs recording at 1600 Hz were secured with a self-adhesive overwrap (Lightpast Pro, Vivomed) to each leg at the 14% site of the tibial length measured from the distal end to match the 14% site of the pQCT scan. Participants were instructed to walk onto the force plates (1000 Hz) and make a static T-pose to ensure the markers were visible within the Vicon motion capture (200 Hz). The participants were instructed and shown a demonstration of the diagonal drop jump technique by the same investigator on each visit. They were instructed to perform diagonal drop jumps from their pre-selected height with hands placed on their hips and aim to land with one foot on each force plate. After each jump, they were required to wait for instruction before leaving the force plates and were asked to repeat the process until completion. After each jump there was a fourteen second rest and a one-minute rest between each set of ten jumps to limit mechanosensory habituation (Robling et al., 2001).

Data were synchronised within Vicon during each jump (Figure 8.4) and transferred to Visual3D for processing (v10.1, C-motion, Maryland, USA). Force data was filtered using a 25 Hz lowpass Butterworth filter (Robertson & Dowling, 2003) and motion capture filtered using a 10 Hz lowpass Butterworth filter. Kinetic and kinematic data from the skeletal model was used to calculate knee and ankle stiffness and moments. Once uploaded force plate and IMU data were processed by identifying the peak value on the relevant force/acceleration

trace, for force data this was the vertical force whereas IMU data this was identified on the x, y and z traces. IMU data was processed as a resultant value to reduce the variability that can occur when using single axis due to device orientation (Sheerin et al., 2016). Vertical impact load, vertical impulse, vertical load rate, CoM velocity and jump height were calculated from force plates, and resultant peak acceleration was calculated from the IMUs.

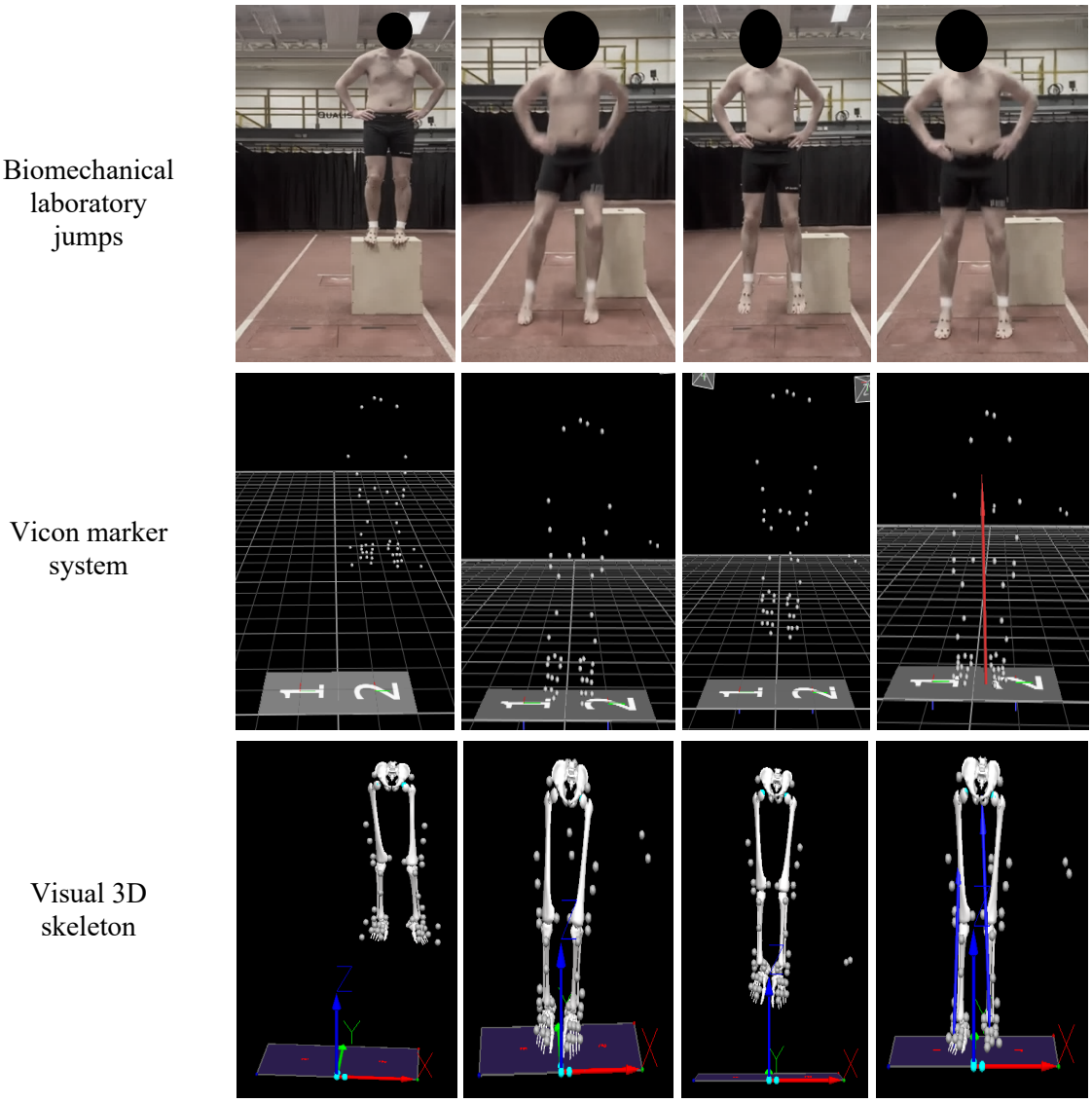


Figure 8.4. Visualisation of diagonal drop jump protocol (60cm jump to the right) and processing from testing to marker system and skeleton creation.

Resultant peak acceleration was calculated from the IMUs using the three-dimensional Pythagoras' Theorem formula. This was calculated as the sum of the maximum accelerations

in the three axis; vertical, anteroposterior, mediolateral. Ankle moments were processed using inverse dynamics with GRF and anthropometric data (Dempster, 1955). All kinetic and kinematic data were normalised to body weight (BW). All metrics were calculated within each of the jumping intervention groups at weeks 0, 6, 12 and 16. Vertical impact loads were taken as the peak GRF from the force data. Impact load 1 was determined as the peak GRF during the initial landing. Impact load 2 was determined as the peak GRF during the second landing (Figure 8.5). The average impact load was taken as an average between the legs. Vertical impulse 1 was calculated as the area under the curve between the initial landing and initial take-off from the drop jump (Figure 8.5). Impulse 2 was calculated by removing the vertical impulse exerted through acceleration due to gravity (Kirby et al., 2011). The vertical load rate was calculated as the peak GRF divided by the time to peak GRF. These were calculated separately for the left leg and right leg within each group. CoM velocity at take-off was determined from the vertical force data between the initial force plate landing and take-off. Jump height was calculated using CoM velocity at take-off as:

$$Jump\ Height = TOV^2/2g$$

where TOV = take-off velocity, g = 9.81 (Moir. 2008).

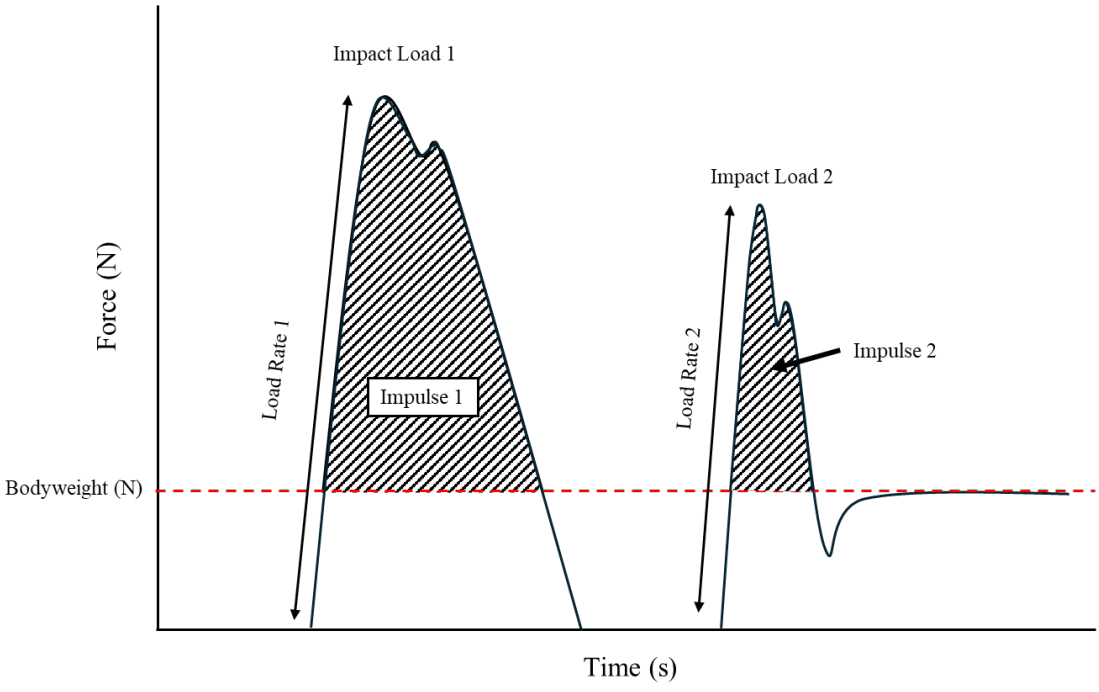


Figure 8.5. Kinetic data derived from Visual 3D.

8.2.4 *Bone measurements*

For a more detailed explanation of the bone measurements please see chapter 3 (Section 3.1.1, 3.1.2). DXA scans were performed pre-intervention and post-intervention to assess whole body BMD (g/cm^2), BMC (g) and bone area (cm^2). Participants were positioned supine on the scanner bed with their ankles and knees strapped to restrict involuntary movement. The participants lay motionless for the duration of the scan with their arms by their sides. All scans and analyses were performed by the same manufacturer-trained operator to keep the scans consistent.

Tibial pQCT scans were performed at pre-intervention, week 12 and post-intervention to assess trabecular density (g/cm^3) at the 4% site. Stress strain index (SSI) X (axial anteroposterior bone strength), Y (axial mediolateral bone strength) and polar (torsional bone strength), cortical thickness (mm) and periosteal circumference (mm) at 14% and 38% site and cortical density (g/cm^3) at 14%, 38% and 66% site of the right and left tibia. Sectional images were obtained at the distal sites (4%, 14%) and the diaphysis of the tibia (38%, 66%) from the positioning line. The participant's tibial length was measured to the nearest mm, determined as the medial aspect of the tibial plateau to the medial malleolus. The participant's leg was placed in the scanner with their foot secured in a purpose-built attachment. The leg was aligned with an integral laser and clamped at the knee to restrict movement whilst the participant was directed to remain as still as possible during the scan. A reference point locating the scan was performed to confirm the location of the distal end plate, which acts as a positioning line.

8.2.5 Statistical analyses

All statistical analyses were conducted using SPSS software (IBM, SPSS Statistics, v.28) with the significance level set at $P<0.05$ and presented as Mean ± 1 SD. A one-way Analysis of Covariance (ANCOVA) with Bonferroni post hoc analysis controlling for baseline bone status to detect differences in week 12 (pQCT) and post intervention (DXA and pQCT) adjusted gains between the intervention and control groups were performed. Bonferroni post hoc analysis were used with ANCOVA as it reduces the likelihood of type 1 error (false positive) during multiple comparisons that were tested. Two Way Mixed Repeated Measures

Analysis of Variance (RM-ANOVA) with Tukey post hoc analysis were used to assess the difference in load measurements (time x intervention). Tukey analyses were applied post ANOVA as it is a more robust test when testing a large amount of means. Paired samples T-tests were performed on significant interactions to observe where the differences occurred. Any significant changes in bone characteristics were followed up with Pearson correlations. That is, Pearson correlations were conducted to examine relationships between external load variables and DXA or pQCT variables. Coefficients of variation (CV%) were reported for external load variables within-person variance for each intervention group (Appendix XXIV).

8.3 Results

No significant differences were shown in age, body mass, height or past BPAQ scores between groups at pre-intervention. There were no differences in lean body mass and body fat % at pre- to post-intervention. There were also no significant changes in lean body mass or body fat % from pre- to post-intervention (Table 8.1). Participant compliance was ~90% (average 58 out of 64 days of jumps) across all intervention groups.

Table 8.1. Anthropometric data for control and diagonal drop jump groups.

	Control (n=11)		0cm (n=11)		40cm (n=11)		60cm (n=11)	
Age (years)	22 ± 2		21 ± 2		22 ± 2		22 ± 2	
Body mass (kg)	66.9 ± 13.6		72.3 ± 10.8		69.8 ± 11.6		68.1 ± 14.6	
Height (m)	1.72 ± 0.09		1.72 ± 0.06		1.74 ± 0.11		1.74 ± 0.12	
Past BPAQ	36		24		42		48	
Sex (M/F)	6/5		7/4		7/4		8/3	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Lean body mass (kg)	46.7 ± 10.2	45.1 ± 10.0	51.2 ± 11.6	51.9 ± 12.7	48.3 ± 10.6	48.7 ± 10.2	50.0 ± 12.0	49.8 ± 12.0
Body fat (%)	26 ± 7	26 ± 7	30 ± 10	31 ± 8	27 ± 8	26 ± 7	23 ± 7	23 ± 7

M/F – Male/Female. BPAQ – Bone-specific physical activity questionnaire. Values are represented Mean (±1SD).

8.3.1 Bone response to diagonal drop jump height

There was a significant difference in change in cortical density at the 14% site of the right distal tibia at week 12. The 40cm and control group increased by 0.5% and 0.4%, respectively, whereas the 60cm group decreased by 1.5% (P=0.006; P=0.027) (Table 8.3; Table 8.5-8.6). Furthermore, there was also a significant difference in change in cortical density at the 38% site of the right tibial diaphysis at week 12. The 40cm group increased by 0.9% whereas the control group decreased by 0.7 (P=0.033) (Table 8.3; Table 8.5). There was a significant difference in change in axial strength at the 14% site of the left distal tibia post-intervention. The 60cm group increased axial strength by 3% whilst the 0cm group decreased by 1% (P=0.024) (Table 8.4; Table 8.6). All other bone characteristics showed no significant differences (Table 8.2-8.6).

Table 8.2. DXA measurements taken pre-intervention and post-intervention.

		Control		0cm		40cm		60cm	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
WB BMD (g/cm^2)		1.18 ± 0.11	1.14 ± 0.11	1.26 ± 0.19	1.23 ± 0.21	1.18 ± 0.13	1.19 ± 0.13	1.20 ± 0.12	1.22 ± 0.12
WB BMC (g)		2798 ± 378	2557 ± 396	3052 ± 788	2941 ± 758	2850 ± 648	2863 ± 652	2849 ± 643	2839 ± 632
WB Bone Area (cm^2)		2368 ± 231	2242 ± 195	2399 ± 240	2382 ± 202	2381 ± 303	2386 ± 307	2340 ± 339	2315 ± 309
Leg BMD (g/cm^2)	Right	1.25 ± 0.09	1.21 ± 0.14	1.35 ± 0.21	1.31 ± 0.22	1.29 ± 0.19	1.28 ± 0.19	1.32 ± 0.14	1.33 ± 0.17
	Left	1.27 ± 0.11	1.23 ± 0.17	1.36 ± 0.21	1.31 ± 0.22	1.28 ± 0.20	1.27 ± 0.19	1.31 ± 0.17	1.33 ± 0.20
Leg BMC (g)	Right	532 ± 78	481 ± 93	567 ± 168	548 ± 161	534 ± 150	534 ± 152	538 ± 139	540 ± 139
	Left	532 ± 87	481 ± 93	576 ± 176	558 ± 169	538 ± 155	536 ± 153	538 ± 145	535 ± 142
Leg Bone Area (cm^2)	Right	427 ± 45	395 ± 40	414 ± 56	416 ± 56	405 ± 60	408 ± 60	404 ± 72	399 ± 63
	Left	419 ± 45	388 ± 34	417 ± 59	420 ± 56	411 ± 62	414 ± 64	406 ± 68	396 ± 59

WB – Whole body. Values are represented as Mean (±1SD).

Table 8.3. Control group pQCT measurements at pre-intervention, week 12 and post-intervention.

	Pre		Week 12		Post	
	Left leg	Right leg	Left leg	Right leg	Left leg	Right leg
4%						
Trabecular density (g/cm^3)	228 ± 42	237 ± 42	224 ± 41	237 ± 42	223 ± 42	229 ± 39
14%						
Cortical density (g/cm^3)	1124 ± 18	1116 ± 22	1132 ± 14	**1121 ± 16****	1130 ± 15	1128 ± 16
Cortical thickness (mm)	2.58 ± 0.35	2.45 ± 0.46	2.58 ± 0.32	2.49 ± 0.39	2.61 ± 0.39	2.49 ± 0.34
Periosteal circumference (mm)	78 ± 8	79 ± 8	77 ± 9	79 ± 9	77 ± 9	78 ± 8
SSIX	901 ± 235	901 ± 233	902 ± 237	902 ± 228	899 ± 233	909 ± 226
SSIY	930 ± 249	958 ± 232	942 ± 260	946 ± 223	938 ± 244	947 ± 232
SSIPOL	1640 ± 440	1609 ± 382	1619 ± 455	1649 ± 366	1624 ± 435	1634 ± 377
38%						
Cortical density (g/cm^3)	1167 ± 24	1170 ± 27	1166 ± 24	**1162 ± 26***	1166 ± 30	1155 ± 23
Cortical thickness (mm)	5.31 ± 0.54	5.15 ± 0.65	5.34 ± 0.51	5.31 ± 0.51	5.32 ± 0.53	5.29 ± 0.62
Periosteal circumference (mm)	74 ± 6	74 ± 6	74 ± 6	74 ± 6	74 ± 6	75 ± 5
SSIX	1153 ± 274	1148 ± 270	1156 ± 268	1156 ± 233	1143 ± 279	1155 ± 288
SSIY	977 ± 239	993 ± 280	992 ± 219	1001 ± 216	980 ± 217	1007 ± 259
SSIPOL	1832 ± 417	1806 ± 400	1841 ± 416	1792 ± 271	1799 ± 350	1789 ± 309
66%						
Cortical density (g/cm^3)	1106 ± 22	1096 ± 45	1102 ± 24	1109 ± 29	1099 ± 32	1088 ± 75

*depicts a significant difference in change from pre-intervention to 40cm group (P<0.05).**depicts a significant difference in change from pre-intervention to 60cm group (P<0.05).

Table 8.4 0cm diagonal drop jump group pQCT measurements at pre-intervention, week 12 and post-intervention.

	Pre		Week 12		Post	
	Left leg	Right leg	Left leg	Right leg	Left leg	Right leg
4%						
Trabecular density (g/cm^3)	264 ± 31	267 ± 31	264 ± 33	264 ± 31	262 ± 34	265 ± 27
14%						
Cortical density (g/cm^3)	1117 ± 20	1117 ± 24	1120 ± 24	1116 ± 20	1123 ± 18	1120 ± 23
Cortical thickness (mm)	2.79 ± 0.55	2.82 ± 0.49	2.85 ± 0.58	2.80 ± 0.52	2.92 ± 0.61	2.87 ± 0.26
Periosteal circumference (mm)	79 ± 7	77 ± 7	79 ± 7	78 ± 7	79 ± 7	77 ± 7
SSIX	870 ± 154	920 ± 255	860 ± 135	935 ± 279	**860 ± 137***	906 ± 220
SSIY	1010 ± 285	970 ± 303	1014 ± 288	975 ± 297	1016 ± 302	994 ± 311
SSIPOL	1595 ± 264	1569 ± 223	1573 ± 246	1563 ± 218	1602 ± 258	1575 ± 234
38%						
Cortical density (g/cm^3)	1151 ± 18	1154 ± 24	1157 ± 18	1155 ± 22	1154 ± 19	1153 ± 22
Cortical thickness (mm)	5.62 ± 0.72	5.71 ± 0.80	5.57 ± 0.65	5.74 ± 0.74	5.54 ± 0.65	5.71 ± 0.46
Periosteal circumference (mm)	76 ± 8	75 ± 7	76 ± 8	74 ± 7	77 ± 8	74 ± 6
SSIX	1217 ± 358	1278 ± 507	1274 ± 497	1177 ± 388	1232 ± 408	1293 ± 516
SSIY	1010 ± 369	955 ± 295	1002 ± 295	982 ± 291	1003 ± 283	986 ± 293
SSIPOL	1880 ± 671	1723 ± 608	1926 ± 678	1756 ± 519	1864 ± 529	1798 ± 720
66%						
Cortical density (g/cm^3)	1098 ± 24	1096 ± 29	1110 ± 19	1106 ± 32	1104 ± 24	1096 ± 24

*depicts a significant difference in change to 60cm group (P<0.05).

Table 8.5. 40cm diagonal drop jump group pQCT measurements at pre-intervention, week 12 and post-intervention.

	Pre		Week 12		Post	
	Left leg	Right leg	Left leg	Right leg	Left leg	Right leg
4%						
Trabecular density (g/cm^3)	255 ± 36	256 ± 36	250 ± 44	259 ± 41	251 ± 40	253 ± 39
14%						
Cortical density (g/cm^3)	1123 ± 29	1122 ± 20	1132 ± 18	**1128 ± 13****	1133 ± 14	1127 ± 22
Cortical thickness (mm)	2.82 ± 0.35	2.62 ± 0.45	2.82 ± 0.35	2.62 ± 0.43	2.80 ± 0.34	2.60 ± 0.44
Periosteal circumference (mm)	78 ± 7	78 ± 6	78 ± 7	78 ± 6	79 ± 6	78 ± 7
SSIX	904 ± 258	901 ± 249	923 ± 255	887 ± 232	920 ± 243	888 ± 236
SSIY	977 ± 292	930 ± 236	985 ± 278	927 ± 244	1009 ± 303	940 ± 249
SSIPOL	1673 ± 485	1600 ± 469	1686 ± 464	1598 ± 479	1679 ± 471	1603 ± 453
38%						
Cortical density (g/cm^3)	1149 ± 28	1148 ± 18	1153 ± 22	**1158 ± 24***	1155 ± 23	1153 ± 24
Cortical thickness (mm)	5.45 ± 0.64	5.21 ± 0.98	5.50 ± 0.67	5.26 ± 0.89	5.47 ± 0.70	5.30 ± 0.92
Periosteal circumference (mm)	76 ± 6	75 ± 7	76 ± 6	74 ± 8	77 ± 6	74 ± 8
SSIX	1161 ± 359	1169 ± 345	1200 ± 343	1183 ± 336	1182 ± 346	1177 ± 329
SSIY	989 ± 319	978 ± 307	1006 ± 294	1005 ± 301	984 ± 307	988 ± 284
SSIPOL	1804 ± 585	1777 ± 533	1825 ± 570	1831 ± 521	1802 ± 534	1841 ± 514
66%						
Cortical density (g/cm^3)	1104 ± 19	1099 ± 36	1109 ± 20	1112 ± 24	1107 ± 15	1114 ± 24

*depicts a significant difference in change from pre-intervention to control group (P<0.05).**depicts a significant difference in change from pre-intervention to 60cm group (P<0.05).

Table 8.6. 60cm diagonal drop jump group pQCT measurements at pre-intervention, week 12 and post-intervention.

	Pre		Week 12		Post	
	Left leg	Right leg	Left leg	Right leg	Left leg	Right leg
4%						
Trabecular density (g/cm^3)	272 ± 63	283 ± 32	274 ± 30	280 ± 28	269 ± 34	278 ± 26
14%						
Cortical density (g/cm^3)	1124 ± 19	1125 ± 23	1128 ± 19	**1109 ± 26*†**	1122 ± 24	1111 ± 25
Cortical thickness (mm)	2.77 ± 0.39	2.75 ± 0.32	2.78 ± 0.39	2.68 ± 0.29	2.77 ± 0.34	2.66 ± 0.29
Periosteal circumference (mm)	77 ± 10	78 ± 10	76 ± 10	79 ± 11	77 ± 11	79 ± 10
SSIX	907 ± 327	954 ± 303	919 ± 307	933 ± 307	**933 ± 317****	943 ± 308
SSIY	975 ± 325	1011 ± 319	1000 ± 322	1024 ± 319	997 ± 319	1007 ± 310
SSIPOL	1680 ± 593	1700 ± 522	1707 ± 596	1719 ± 552	1776 ± 607	1695 ± 535
38%						
Cortical density (g/cm^3)	1163 ± 25	1163 ± 24	1165 ± 35	1164 ± 25	1169 ± 42	1159 ± 25
Cortical thickness (mm)	5.46 ± 0.73	5.51 ± 0.70	5.46 ± 0.73	5.47 ± 0.74	5.51 ± 0.74	5.44 ± 0.64
Periosteal circumference (mm)	74 ± 9	74 ± 9	74 ± 10	73 ± 8	73 ± 9	74 ± 9
SSIX	1193 ± 414	1208 ± 390	1185 ± 429	1202 ± 390	1197 ± 422	1189 ± 385
SSIY	1014 ± 355	1008 ± 333	1015 ± 365	1015 ± 349	1020 ± 353	986 ± 336
SSIPOL	1851 ± 618	1820 ± 568	1864 ± 645	1815 ± 570	1859 ± 612	1799 ± 578
66%						
Cortical density (g/cm^3)	1106 ± 20	1106 ± 24	1108 ± 27	1109 ± 24	1104 ± 23	1110 ± 23

*depicts a significant difference in change from pre-intervention to control group (P<0.05).**depicts a significant difference in change from pre-intervention to 0cm group (P<0.05). †depicts a significant difference in change from pre-intervention to 40cm group (P<0.05).

8.3.2 External load differences between drop jump height

Load variables recorded at pre-intervention, week 6, week 12 and post-intervention are displayed in Table 8.7. In summary, the 60cm diagonal drop jump group displayed greater impact peak 1 at pre-intervention and week 12, greater impulse 1 at pre-intervention, week 12 and post-intervention, and greater load rate 1 at pre-intervention and week 12 in both legs compared to the 0cm group (P<0.05). The 60cm group also showed greater impact peak 1 at week 12, greater peak acceleration 1 at pre-intervention and greater impulse 1 at week 6 than the 40cm group in both legs (P<0.05). The right leg of the 60cm group created higher ankle moment 1 at week 12 and post-intervention, higher load rate 1 at week 6 and post-intervention, and higher peak acceleration 1 at week 12 compared to the 0cm group (P<0.05), and higher ankle moment 1 at pre-intervention, week 6 and post-intervention, and peak acceleration 1 at pre-intervention and week 12 compared to the 40cm group. Left leg peak acceleration 1 was also greater in the 60cm group at week 6 compared to the 0cm group (P<0.05; Table 8.7).

The 40cm diagonal drop jump group displayed greater impact peak 1 at week 6 and post-intervention, and greater load rate 1 at pre-intervention and week 12 compared to the 0cm group in both legs (P<0.05). The left leg of the 40cm group created higher peak acceleration 1 at week 12 and load rate at post-intervention compared to the 0cm group. Right ankle moment 1 at week 12 and load rate 1 at week 6 and post-intervention were greater in the 40cm group than the 0cm group (P<0.05; Table 8.7).

8.3.3 External load differences over time

There were significant differences in peak acceleration within groups over time. The 60cm group created higher peak acceleration 1 at pre-intervention compared to week 12 and post-intervention in the right leg, and at week 6 compared to week 12 in the left leg (P<0.05). Peak acceleration 2 was also higher at pre-intervention compared to week 12 in the left leg and post-intervention in both legs in the 60cm group (P<0.05). The 40cm group created higher peak acceleration 1 at pre-intervention and week 6 compared to week 12 in the right leg (P<0.05). The 0cm group displayed higher peak acceleration 1 at week 6 compared to

post-intervention in the right leg and peak acceleration 2 at pre-intervention compared to post-intervention in the left leg (P<0.05).

Table 8.7. Load variables recorded at pre-intervention, week 6, week 12 and post-intervention.

	Pre			Week 6			Week 12			Post		
	0cm	40cm	60cm	0cm	40cm	60cm	0cm	40cm	60cm	0cm	40cm	60cm
Left IP1 (BW)	1.64±0.34*,**	2.37±0.35	2.22±0.22	1.84±0.46**	2.43±0.39	2.49±0.56	1.48±0.57*,**	2.56±0.49	2.55±0.68	1.79±0.47**	2.49±0.40	2.63±0.77
Left IP2 (BW)	1.95±0.39	2.38±0.80	2.06±0.34	2.20±0.88	2.04±0.42	2.05±0.34	1.93±0.50	2.17±0.72	1.99±0.36	1.98±0.66	2.26±0.76	2.05±0.34
Right IP1 (BW)	1.56±0.22*,**	2.34±0.46	2.37±0.39	1.86±0.58**	2.41±0.24	2.45±0.42	1.65±0.30*,**	2.41±0.42	2.57±0.58	1.79±0.40**	2.44±0.76	2.49±0.59
Right IP2 (BW)	2.02±0.46	2.30±0.75	2.07±0.45	2.12±0.84	2.01±0.44	1.94±0.33	1.77±0.48	2.18±0.90	2.02±0.29	1.96±0.68	2.24±0.83	2.04±0.39
Left I1 (BW)	3.43±0.46*	3.85±1.14	4.15±0.60	3.32±0.80	3.43±0.37*	4.26±0.62	3.10±0.37*	3.72±0.79	4.42±0.83	3.16±0.43*	3.69±0.69	4.32±0.79
Left I2 (BW)	2.34±0.39	2.15±0.53	2.34±0.59	2.37±0.53	2.05±0.40	2.54±0.71	2.60±0.57	2.18±0.56	2.67±0.70	2.49±0.35	2.18±0.60	2.58±0.77
Right I1 (BW)	3.32±0.80*	3.76±1.04	4.49±0.75	3.41±0.76	3.45±0.29*	4.23±0.85	3.22±0.38*	3.62±0.85	4.17±0.76	3.23±0.36*	3.60±0.91	4.32±0.79
Right I2 (BW)	2.52±0.55	2.07±0.53	2.45±0.46	2.37±0.43	2.05±0.41	2.59±0.72	2.37±0.40	2.28±0.63	2.56±0.83	2.52±0.56	2.31±0.65	2.50±0.73
Left LR1 (BW/s)	25±28*,**	45±14	52±11	25±28*,**	45±14	52±11	18±13*,**	45±14	53±15	23±28**	43±13	53±17
Left LR2 (BW/s)	89±37	95±18	83±35	91±37	97±26	78±38	78±26	89±21	69±17	73±26	90±23	77±33
Right LR1 (BW/s)	19±11*,**	41±10	47±10	20±15*,**	43±10	49±16	19±15*,**	38±10*	48±5	21±16*,**	47±20	56±12
Right LR2 (BW/s)	32±11	33±13	3±20	25±11	27±8	28±7	23±14	29±16	26±4	31±29	32±17	29±6
Left PA1 (g)	3.09±1.09	6.47±3.46*	13.86±5.21	2.92±1.57*	6.37±4.17	16.80±8.94$^{\#}$	2.12±1.05**	4.44±2.83	7.96±4.04	1.83±0.55	6.34±3.42	9.48±3.81
Left PA2 (g)	7.86±3.21$^{\#\#}$	7.75±4.55	10.89±3.14$^{\#,\#\#}$	6.63±2.79	7.81±4.25	9.92±5.69	5.57±2.44	4.02±0.61	4.50±1.58	3.83±1.72	5.38±2.66	6.67±2.89

Right PA1 (g)	4.88±6.22	8.32±2.95$^{\#,*}$	15.63±5.40$^{\#}$	4.42±2.56$^{\#\#}$	7.25±3.77$^{\#}$	12.00±9.82	3.24±2.06*	4.21±1.93*	8.20±2.11	2.03±1.38	7.08±3.24	8.51±5.96
Right PA2 (g)	8.42±6.15	7.73±4.18	10.92±2.95$^{\#\#}$	7.05±4.20	6.40±4.18	8.87±5.54	5.46±3.37	3.72±0.94	6.01±2.88	4.09±2.54	5.68±3.27	6.21±3.71
Left Ankle Moment 1 (Nm.kg)	2.32±0.70	2.88±0.60	2.29±0.92	2.32±0.74	2.96±1.07	3.24±1.35	2.26±0.47	2.95±0.71	2.90±0.94	2.66±1.18	2.52±1.23	2.76±0.97
Left Ankle Moment 2 (Nm.kg)	2.11±0.74	2.48±0.83	1.74±0.39	2.10±0.81	2.41±1.22	2.23±0.77	1.94±0.32	2.40±0.66	2.13±0.47	2.35±0.99	2.72±1.13	2.07±0.45
Right Ankle Moment 1 (Nm.kg)	1.77±0.47	1.80±0.61*	2.73±0.64	2.02±0.73	1.85±0.62*	2.84±0.51	1.92±0.59*	1.98±0.65	3.00±0.81	2.02±0.91*	1.95±0.52*	3.35±0.47
Right Ankle Moment 2 (Nm.kg)	2.07±0.68	2.12±0.72	2.62±0.44	1.83±0.75	1.83±0.52	2.60±0.63	1.80±0.82	2.08±0.66	3.06±0.60	2.07±0.91	2.13±0.59	3.25±0.45

BW – Bodyweight. G – gravitational units (1g = 9.81 m/s). IP – Impact Peak. I – Impulse. LR – Load Rate. PA – Peak acceleration. *depicts a significant difference to 60cm (P<0.05). **depicts a significant difference to 40cm (P<0.05). $^{\#}$depicts a significant difference to week 12 (P<0.05). $^{\#\#}$depicts a significant difference to post-intervention (P<0.05).

8.3.4 External load variability

There was a large individual variability within all groups; impact peak and impulse had the lowest variability being 10-40% in the 0cm group, 8-34% in the 40cm group and 10-29% in the 60cm group. Load rate and ankle moments were between 31-107% at all time points in the 0cm group, 19-53% in the 40cm group and 14-56% in the 60cm group. The variability of IMU-derived peak acceleration was 42-140% in the 0cm group, 35-67% in the 40cm group and 25-82% in the 60cm group (Appendix XXIV).

8.3.5 External load and bone characteristic correlations

The 60cm group displayed positive correlations between right leg cortical density (38%) and impact peak 1 (r=.435, P=.035). Impact peak 1 (r=-.457, P=.021) and peak acceleration 1 (r=-.553, .006) negatively correlated with axial strength of the left leg in the 60cm group (Figure 8.6).

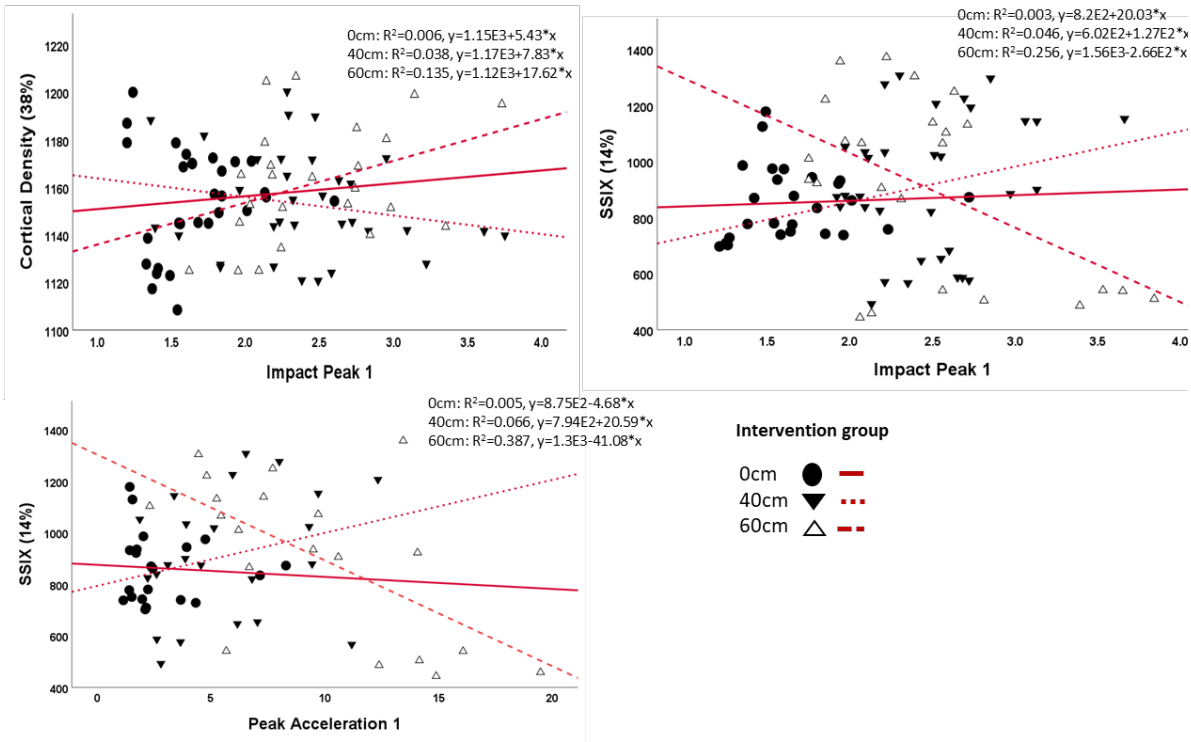


Figure 8.6. Pearson correlations between external load metrics and bone characteristics subcategorised into drop jump heights.

Due to the variability of individual data within groups the correlations were also performed on the data without sub categorising into drop height groups. No further correlations were found between bone characteristics and load metrics when accounting for this.

8.4 Discussion

This is the first study to employ a dose-response methodology to quantify load magnitude during an intervention designed to assess bone adaptation in healthy young adults. Diagonal drop jumping from 60cm resulted in a detrimental effect of cortical density in the 14% distal tibia site compared to those jumping from 40cm and the control group. Furthermore, cortical density in the tibial diaphysis (38% site) was improved in the 40cm group compared to the control group after 12 weeks. Drop jumping from 60cm, however, showed a greater improvement in axial bone strength compared to the 0cm group across 16 weeks.

The skeletal response to load is suggested to be threshold-driven (Christen et al., 2014) and therefore, the 60cm drop height could be above the threshold for bone adaptation in the present study. This is emphasised by the 40cm group, and even control participants, showing a greater improvement in cortical density than the 60cm group over 12 weeks. The observation could be explained by the greater load experienced in the 60cm group stimulating a more profound bone remodelling response. As microdamage acts as a stimulant for bone remodelling, the higher magnitude of load within the 60cm group could reflect higher rates of remodelling, leading to incomplete mineralisation and increased intracortical porosity (Rantalainen et al., 2011; Wilks et al., 2009). These proposed effects have been shown to occur in as little as 8 weeks in army recruits where distal tibia cortical density decreased by 0.34% and the diaphyseal site decreased 0.71% in cortical density (Hughes et al., 2018). Microdamage is the microstructural consequence of fatigue in bone and although it is essential for the maintenance of its structural quality, excessive microdamage consequently generates material damage (Herman et al., 2010). Where typical peak mechanical strains exceed the microdamage threshold, microdamage can escape usual repair and accumulation, increasing fragility and the likelihood of sustaining stress fractures (Alway et al., 2019). Therefore, the current study's data suggest the effects of 12 weeks of 60cm drop jumps causes

a detrimental effect in cortical density, especially highlighted by the significant decrease compared to the control group who did not perform jumps. In this study 40cm drop jumps may be the optimal loading threshold for an osteogenic response before excessive microdamage occurs. It could be speculated, however, that if the study was performed for a further 3-6 months then the microdamage would have been repaired and the 60cm group would have seen the greatest increase.

Despite right leg cortical density being less in the 60cm group than the 40cm and control groups in response to the intervention, left leg axial bone strength increased highlighting the complex nature of bone remodelling. The reason for this may be due to landing technique. Similarly, Hardcastle et al. (2014) observed increased peak force and power resulted in a positive adaptation in tibial strength despite being inversely associated with endocortical expansion. As increased mechanical loading is associated with bone strength (Allison et al., 2013; Jämsä et al., 2006; Vainionpää et al., 2005), it is a surprise that there was no significant difference in bone strength between the 40cm and control group. Greater load magnitude increases the strain on bone cells, accounting for an increase in the bone remodelling response and subsequentially bone strength (Ireland et al., 2011), indicating the beneficial effects of high magnitude jumping (60cm) compared to low magnitude jumping (0cm) and control group on bone strength at 16 weeks. As the 40cm drop height displayed similar load values to the 60cm group it is not clear why the bone strength of the 40cm group is not higher than the low magnitude jumpers (0cm) and control group. Based on the notion that higher load magnitude results in greater bone adaptation (Guadalupe-Grau et al., 2009) it would be anticipated that those producing higher loads would improve bone strength. Similarly, no differences were found between the right leg and left leg external load metrics suggesting adaptations between legs which may be due to metrics not measured during the landing mechanics of the dominant and non-dominant leg. Although the same researcher demonstrated and instructed the diagonal drop jump technique to participants at each laboratory visit, individuals are likely to have different landing mechanics, therefore, affecting the load imposed on bone. This is observed by Lim et al. (2020) whereby drop jumps from 31cm, 46cm and 61cm elicited higher lower extremity kinetics and kinematics as drop height increased. Differences in jump height mechanics correspond to fatigue with

higher drop jumps inducing a faster rate of fatigue than lower heights thereby causing a more abrupt absorption of force during landing and imposing a quicker increase of energy in fatigued muscle (Chappell et al., 2005). Joint power absorption is also shown to be greater in jumps over 40cm and maximised at 60cm which puts a higher mechanical strain on muscles and tendons during landing (Peng et al., 2011). Although higher external loads do not necessitate the mechanical stress imposed upon bone as muscle dissipates the mechanical energy during impacts (Decker et al., 2003), the differences in landing mechanics are shown to influence muscle activity (Howe et al., 2020). Therefore, it is possible there was a difference in neuromuscular activity when landing on either leg. Neuromuscular control is suggested to differ between the dominant and non-dominant limb during landing tasks, indicating internal loads generated by muscle are likely to differ between limbs (Bates et al., 2013). Such differences in muscle-derived load could explain the distinct adaptations within each leg in the 60cm group.

Most bone characteristics measured were not observed to change. Although studies have shown positive adaptation following impact exercise there are also examples of interventions producing no improvements in bone. An 8-month intervention performing 28cm single leg drop jumps did not elicit any osteogenic adaptations in prepubertal girls (Greene et al., 2009). This was also shown by Wiebe et al. (2008) in the same population, concluding that 28cm drop jumps do not provide an effective stimulus for bone adaptation. Significant increases in tibial cortical density are often not shown over time from impact exercise in young adults (Evans et al, 2012; Liu et al., 2003), with the exception of an increase in cortical density shown from short-term arduous training (Izard et al., 2016). As the current study did not observe changes in bone characteristics over time, this contradicts the mechanistic understanding that greater load intensity enhances bone adaptation (Rubin & Lanyon, 1985). Although BMD and geometry were mainly unaffected by the interventions over time, there may have been changes within other factors of bone quality, such as changes in type I collagen in the bone matrix (Sundh et al., 2018). For example, the bone matrix consists mainly of type I collagen which gives the tissue its ductility and strength. It may be there was an effect from the current interventions on collagen fibrils which contribute to the structure

of bone as observed in mice (McNerny et al., 2015). Therefore, any subresolution structural changes not possible to measure with the DXA or pQCT technique may have occurred.

The high variability in individual load shown in the present study strengthens the case that mechanical loading requires quantification when assessing impact activity and bone adaptation for exercise prescription. A large intra-group variance in load measurements (Figure 8.6, Appendix XXIV) meant some participants in the 0cm group experienced greater load than those in the 60cm group, exemplifying that loading cannot be assumed from activity alone and emphasises the need to quantify loading when assessing activities designed to stimulate bone load. For example, the intra-variability of drop jump outcomes, such as the reactive strength index and contact time, have been shown to produce unacceptable reliability (CV>10%) during a study on female volleyball players (González-García et al., 2024). This indicates the drop jump technique is a difficult reproducible jumping task even when performed from the same height. Previous studies have lacked specific measurements of load and opted to assume the load produced from impact exercise is uniform within participants. For example, Allison et al. (2015) did not quantify load during a hopping intervention and inferred the participants were subjected to 2.7-3.0 times their bodyweight based on a previous study they conducted performing the same impact exercise in different individuals (Allison et al., 2013). This was also the method adopted by Kim et al. (2021) using a similar approach to estimating load during jumping activity based on the data of Zhao et al. (2014). These studies assume that each individual is performing identical techniques during the movement and imposing the same magnitude of load, which we have shown is not the case. It is suggested that impact loads of >2 times bodyweight are enough to create a positive osteogenic response (Florence et al., 2024; Kistler-Fischbacher et al., 2021). This implies that the 40cm and 60cm groups imposed the required impact load (2.3 BW) to promote bone accrual. As there were no changes in bone over time in either of the groups, this supports the notion that relying on previous studies exercise recommendations is not suitable. Therefore, to observe the relationship between load magnitude and bone adaptation it is suggested load should be monitored and specific to the intervention performed. This will acquire a better understanding of bone adaptation and mechanical load during intended osteogenic protocols and help inform on the applied measures suitable for use within clinical settings.

It is argued that bone formation induced by high-impact mechanical loading might not be completed at 4 months (Hughes et al., 2020). Therefore, prolonged interventions, 6 months or longer, may be necessary to detect significant adaptations in mineralised bone (Kast et al., 2022). However, the bone remodelling cycle can produce changes in BMD at 4 months (Kenkre & Bassett, 2018) and short term changes in bone have been observed in as little as 8 weeks (Hughes et al., 2018). Therefore, the authors are satisfied the current study's timeframe (16 weeks) can elicit bone adaptation. Those with lower bone mass are more likely to experience notable changes in response to mechanical loading compared to those with average bone mass (Winters-stone & Snow, 2003). As the participants in the current study were young low-active individuals it was hypothesised that the population would be responsive to impact interventions based on the understanding of age-dependant bone adaptation and mechanical loading (Javaheri & Pitsillides, 2019). There may have been a lack of bone response due to the historical exercise status of participants in the present study. Physical activity during childhood and adolescence is associated with higher BMD in later life (Robling et al., 2019; Tervo et al., 2008) meaning past exercise may have influenced the findings as past activity participation was not controlled for. The Bone-specific Physical Activity Questionnaire (BPAQ) was used to gain insight into participants past activity (Weeks & Beck., 2008). There were no significant differences in past BPAQ scores in the current study, although the 60cm height displayed double the score of the 0cm group, suggesting changes in bone may have been less sensitive within the 60cm population due to past activity participation. However, the z-scores within all groups ranged between 0.37 and 0.69, meaning the overall bone health of each group was above average (>0.0) compared to their population. Therefore, habitual exercise promoting bone accrual may have been performed before the current study (Kim et al., 2018).

8.4.1 Limitations

The intervention groups were accountable for completing an unsupervised jumping intervention following the guidelines they received during laboratory visits. Compliance was monitored with regular check-ups, as well as in person check-ups at each laboratory visit (every 6 weeks). Post-intervention an exit survey was completed that provided the opportunity for participants to recall jumping compliance throughout the 16-week

intervention. Based upon the exit there was a ~90% (average 58 out of 64 days of jumps) compliance across all intervention groups. As the drop jumps were unsupervised, however, the researchers cannot be certain if the participants performed the jumps required and whether they replicated the technique performed during laboratory visits. Furthermore, the variability of the study load data makes it difficult to interpret the effect of quantified load on bone adaptation. The high variability amongst individuals during drop jumps, therefore, suggests further studies should examine external load monitoring during high-impact exercise. Performing the intervention beyond 16 weeks, which was not possible due to time restrictions in the current body of work, may have also produced more significant changes in bone characteristics than already provided.

8.4.2 Conclusion

In conclusion, this study is the first to utilize a dose-response methodology to quantify the relationship between load magnitude and bone adaptation in healthy young adults. The findings reveal that jumping from 60cm results in a detrimental effect on cortical density compared to the 40cm and control groups. The 60cm drop height, however, improved axial bone strength relative to the 0cm group. Despite previous evidence (section 2.7.1) stating greater load magnitude induces greater bone adaptation, the current study, showed no differences over time in any diagonal drop jump group. The variability between individual load responses, possibly as a result of landing techniques, underscores the necessity of quantifying mechanical loading rather than relying on assumed uniformity from previous impact exercises. Future studies should focus on precise load monitoring to better understand bone adaptation mechanisms and optimise exercise protocols for clinical applications.

9 General Discussion

This thesis aimed to investigate the relationship between quantified mechanical loading and bone adaptation. The research was conducted through the following approaches: (1) evaluating the osteoblast response to varying loading cycles, (2) establish the extent to which bone loading is monitored/estimated in the applied environment, (3) analysing the changes in bone characteristics in relation to GPS-derived performance metrics and strength measures, (4) examining the relationship between external load and bone characteristics in asymmetrical sport, and (5) exploring the dose-response relationship between jump height and bone accrual. This work contributes to the field by providing insights into how bone responds to specific loading patterns and advances methodologies for quantifying bone load (see Table 9.1 for key findings). It has been reported that certain exercise interventions have an osteogenic effect on bone (see section 2.6). The specific characteristics of these exercises, however, are rarely quantified accurately. Previous studies have attempted to associate external load to bone load using force plates (Rogers & Hinton, 2010; Turner & Robling, 2003; Wu et al., 1998; Kohrt et al., 1997), accelerometry (Kelley et al., 2014; Ahola et al., 2009; Vainionpää et al 2009), motion capture (Choi et al., 2021; Wang & Dueball, 2018; El Deeb & Khodair, 2014) and GPS (Varley et al., 2023; Varley et al., 2022), however, the mechanical load necessary for optimal bone health remains unclear. Furthermore, studies that have assessed the relationship between bone characteristics and factors such as exercise magnitude (Rowland et al., 2020; Vlachopoulos et al., 2018; Allison et al., 2013), frequency (Daly et al., 2021; Kemmler & Von Stengel., 2014; Bailey & Brooke-Wavell., 2010), duration (Marin-Puyalto et al., 2019; Varley et al., 2017) and type (Du et al., 2021; Min et al., 2019; Robling et al., 2019) offer some insight, however, they do not accurately capture the bone load and fail to account for individual variability in how participants perform the same exercise. Additionally, previous research has shown that incorporating rest periods between exercise bouts may enhance bone accrual by restoring mechanosensitivity (section 2.6.3). These studies often fail to control for load magnitude across conditions, leading to ambiguity about whether the positive effects are due to the rest periods or differences in load. Without precise quantification of bone load, it is challenging to determine the optimal exercise regime needed to maximise bone accrual. This lack of accurate measurement complicates exercise prescription for the prevention and treatment of bone conditions and

poses a significant obstacle in the fight against bone diseases, such as osteoporosis and osteopenia.

9.1.1 Key findings

Key findings from chapters 4 through 8 are summarised in Table 9.1. The findings summarised in Table 9.1 have been interpreted and discussed in detail within each corresponding chapter.

Table 9.1. A summary of the key findings of study chapters of this thesis.

Chapter 4 (Study 1): *In vitro* study investigating the effects of intermittent load (rest periods) on bone formation when load magnitude is matched. Cell line MC3T3 osteoblasts used.	• ALP activity was higher in response to intermittent load compared to continuous load (↓30-59%, $P<0.035$) and control conditions (↓70-90%, $P<0.001$) following 1-, 3- and 12 days. • PINP was higher at day 3 when continuously loaded compared to intermittent load (↓112%, $P=0.007$). • ARS did not change over time in the loaded conditions but displayed higher levels at day 12 in the control condition compared to day 1 ($P<0.001$) and day 3 ($P=0.007$).
Chapter 5 (Study 2): A hybrid study consisting of a survey and narrative review to compare perspectives of applied practitioners and academic research on bone load monitoring. Data from 71 currently active sport support staff.	• 92% of support staff stated they monitor external load, but, in those that did, only 28% claimed to monitor external load to estimate bone load. • 40% of support staff stated the main barrier to relating external load with bone is a lack of knowledge. • GPS was the most common method to monitor external load (n=55, 85%) and bone load (n=11, 50%) amongst support staff, however, the most prevalent methods to monitor bone load were IMUs and motion capture. • External load may be used as a proxy measure of monitoring bone load, however, there are no recommendations regarding the best methods associated to bone in the applied environment.
Chapter 6 (Study 3): A prospective longitudinal study assessing the relationship between football-specific training load and bone characteristics over 14 weeks. Data from 15 full time academy footballers, aged 19 ± 1.	• Leg BMC (↑1.9%, $P=0.008$), total body BMC (↑0.7%, $P=0.022$) and lean mass (↑1.7%, $P=0.030$) increased over 14 weeks of football-specific training. • Positive correlations were shown between the change in BMC and HMLD (r=.36), accelerations (r=.42) and decelerations (r=.39). • Using GPS, during football training, to measure external load showed high magnitude movements, such as accelerations and decelerations were positively associated to changes in total body BMC.

Chapter 7 (Study 4): A cross-sectional study associating external load with bone characteristics in habitual, high impact athletes (fast bowlers). Data from 11 male elite fast bowlers, aged 24 ± 5.	• The dominant leg of fast bowlers produced higher peak acceleration (↑53%, P<0.01) and PPA (↑38%, P<0.01) than the non-dominant leg. • The dominant leg had greater BMC (↑2%, P=0.02) and tibial strength (↑7%, P<0.04) than the non-dominant leg. • Cumulative load was positively associated to axial bone strength (r=.633, P=0.035) and polar bone strength (r=.638, P=0.035). • Site-specific IMUs, attached to the tibia, showed the leg producing higher load metrics, such as peak acceleration and PPA, had greater bone characteristics.
Chapter 8 (Study 5): Randomised control trial investigating the dose-response relationship of drop jump height and bone adaptation across 16-weeks. Data from 44 young adults, aged 22 ± 2 (60cm, n=11; 40cm, n=11; 0cm, n=11; control, n=11).	• The 40cm (↑0.5%, P=0.006) and control group (↑0.4%, P=0.027) displayed greater improvements in cortical density in the 14% site of the right distal tibia than the 60cm group (↓1.5%) over 12 weeks, whilst the 40cm group (↑0.9%) also had greater improvements in cortical density at the 38% site of the right tibia compared to the control group (↓0.7%, P=0.033). • The 60cm group (↑3%) showed a greater improvement in axial strength at the 14% site of the left leg distal tibia compared to the 0cm group (↓1%, P=0.024) post-intervention. • A positive correlation was observed between right leg cortical density at the 38% site and impact peak (r=.435) in the 60cm group. However, impact peak (r=-.457) and peak acceleration (r=-.553) negatively correlated with left leg axial strength at the 14% site in the 60cm group. • There was no dose-response effect on bone adaptation observed when increasing drop jump height, which may be due to the large variability (8-140%) in external load data between individuals. However, high magnitude load (40 and 60cm groups) were shown to induce positive changes in tibial characteristics compared to low magnitude and no load.

ALP, Alkaline Phosphatase; ARS, Alizarin Red Staining; BMC, Bone Mineral Content; GPS, Global Positioning System; HMLD, High Metabolic Load Distance; IMU, Inertial Measurement Unit; PINP, Procollagen I N-Terminal Propeptide; PPA, Peak Positive Acceleration.

This thesis has offered novel insights that will facilitate the work of future researchers and practitioners in seeking to optimise bone loading protocol. It was shown that following 12 days of matched load *in vitro*, the short-term effects of intermittent load showed increases in bone formation marker ALP compared to continuous load (Chapter 4). This supports the current literature suggesting rest periods can heighten osteogenic activity by restoring bone mechanosensitivity (Burr et al., 2002). Applying this concept to human studies is difficult

due to a lack of consensus on the optimal method to quantify bone load. Currently, the most prevalent method used by applied practitioners to measure bone load is GPS, however, the relationship between GPS loading metrics and bone adaptation isn't well established. There is a clear gap between research evidence and applied practice as highlighted in chapter 5, where multiple methods of measuring external load (GPS, accelerometry, force plates) were used by support staff. Support staff believe they lack the knowledge required for correctly assessing external load to monitor bone load, therefore research into applied measures would provide knowledge on the association between external load and bone accrual. Despite the association between GPS-derived metrics and bone accrual not being well established GPS-derived metrics, such as acceleration, deceleration and high metabolic load distance (HMLD) were associated with increases in BMC over 14 weeks (Chapter 6). The present study builds on previous literature as strength-derived measures were performed which is something that previous studies have not assessed, with no associations being observed between bone characteristics and change in strength. Although these findings are of interest, bone is known to adapt in a site-specific manner and as the GPS devices were placed on the upper back, it is unclear how accurately they represent the loads experienced in the lower limbs (Winters-Stone & Snow., 2006). IMUs were, therefore, utilised in chapter 7 and 8 to assess load as IMUs offer a site-specific method of capturing external load. This study showed that IMU-derived cumulative load captured from the 14% tibial site was associated to tibial strength (14% tibial site) in professional fast bowlers (Chapter 7). These findings were built upon in chapter 8, where an intervention study was conducted. In this study impact peak was positively associated to cortical density when performing 60cm drop jumps, although there was a large variability between individual load data (10 - 82%). Significant changes in bone characteristics were found in the groups with higher loading magnitudes (40cm and 60cm drop heights). The 40cm diagonal drop jump group displayed a positive response in cortical density of the distal tibia (14%) and tibial diaphysis (38%) across 12 weeks, whereas the 60cm group showed a positive adaptation in axial strength (14%) across 16 weeks.

9.1.2 Quantification of external load to monitor bone adaptation

In the present thesis, external load measurements were sought to monitor and assess the association with bone adaptation. This thesis produced the first study to examine if and how

external load measures are used in an applied environment to monitor bone load (Chapter 5). These findings highlight the disconnect between applied work and lab-based research when using external load as a measure of bone load, as support staff believe monitoring bone load is confusing or not possible. For example, one support staff stated monitoring bone load is 'too dependent on the individual rather than generalisable load characteristics' and they are 'unsure of the assumptions and validity' of external load measures. This is not surprising as research provides opposing views on using external load as a measurement of bone load (Besier., 2019; Matijevich et al., 2019). However, it is generically accepted that external load cannot infer internal bone load, yet that does not mean it cannot not be used as a proxy measure to monitor bone adaptation. Therefore, it is clear applied external load measures require investigating to understand if they can be associated to bone adaptation. The findings in relation to GPS-derived load and bone adaptation (Chapter 6) are complemented by previous research using GPS in football (Varley et al., 2023; Varley et al., 2022). Although the study by Varley et al. (2023) used a younger population of footballers (mean age ~16) compared to the present population (mean age ~19) meaning the training programmes may differ, the positive association between accelerations and changes in BMC suggest the potential of associating GPS-derived load (accelerations and declarations) to bone adaptation. However, as bone responds in a site-specific manner (Winters-Stone & Snow., 2006; Adami et al., 1999), site-specific measures of external load are needed to understand bone adaptation. The practicality of using GPS in applied environments is acknowledged and associations between GPS-derived metrics (accelerations and decelerations) and bone adaptation are observed. However, as GPS are not capable of assessing distal site-specific load (*e.g.,* tibia), IMUs may act as practical alternative.

IMUs can be attached to distal limbs and used to measure site-specific load (Armitage et al., 2021), hence tibial cumulative load was measured and associated to axial and polar tibial bone strength in elite fast bowlers (Chapter 7). No other studies have investigated the relationship between IMU-derived data and bone adaptation, meaning there is no comparative data, but as the load metrics in the dominant leg were greater than the non-dominant leg in chapter 7, it is unsurprising the bone characteristics were also higher. Therefore, IMUs may be useful for measuring site-specific load at distal sites to understand

the effect of distal mechanical load on bone adaptation. However, the IMU-derived data (peak acceleration) in chapter 8 was associated to a negative correlation in tibial axial strength during drop jumps. This lack of commonality between studies may be caused by the high variability (CV: 25-140%) in load metrics across multiple visits. Previous research has assumed exercise can repeatably produce similar loads (Kim et al., 2021; Allison et al., 2015; McKay et al., 2005), however, it is common for impact exercise to have divergent effects on external load during the same task (Unilateral hops - 2 BW, Hartley et al., 2020; 3 BW, Allison et al 2013). This is likely a result of various landing mechanics which produce differences in muscle-derived load that contribute largely to the load imposed on bone (Torres-Costoso et al., 2020; Montgomery et al., 2019). As was demonstrated with repeat measures in chapter 8, there can be a high load variability in external load measures (IMU, force plate, motion capture), research in an applied environment should be encouraged to perform longitudinal measures of external load and bone adaptation.

9.1.3 Mechanical load and bone adaptation

High-impact load was associated to increases in leg BMC (Chapter 6 and 7), total body BMC (Chapter 6), tibial strength at the 14% site of the tibia (Chapter 7), and cortical density and axial strength at the 14% site of the tibia (Chapter 8). This is due to bone optimising its structure to suit the functional needs of habitual load to prevent microdamage and adapt to the mechanical environment (Frost, 1987). These findings are supported by previous studies investigating impact exercise and bone accrual (Allison et al., 2013; Jämsä et al., 2006; Vainionpää et al., 2005). Incorporating rest periods intermittently between loading bouts is known to enhance the osteogenic stimulus in bone by restoring mechanosensitivity (Klein-Nuland et al., 2015; Srinivasan et al., 2007; Robling et al., 2002). Studies examining the topic, however, do not match total accumulated load, meaning it is not possible to conclude rest periods are the only reason for increases in bone activity. Study 1 (reported in chapter 4) was the first study to assess the effects of matching the total load magnitude *in vitro* when comparing intermittent and continuous load. Since this study determined that rest periods between loading bouts encouraged osteoblast activity when load magnitude was matched, it can be suggested intermittent load can facilitate an osteogenic stimulus. This was similar in chapter 7 and 8 when investigating high-impact load in fast bowlers and young adults.

Applying high-impact load in an intermittent nature showed higher leg BMC and tibial strength (fast bowling) and cortical density and axial strength (drop jumps).

High-impact activity was associated to positive bone adaptation compared to low-impact participants (Chapter 8). Previously, it has been reported bone adaptation is threshold-driven and a minimal loading threshold (>2BW) is required to initiate bone accrual (Hartley et al., 2020; Vlachopoulos et al., 2018; Allison et al., 2015; Bolam et al., 2015), however, an upper loading threshold that may be detrimental to bone is not known. Study 5 (reported in chapter 8) was the first to perform a dose-response impact intervention composed of multiple drop jump heights in young adults, whereas previous research often use single impact magnitude groups vs controls (Lambert et., 2020; Anliker et al., 2012). However, there were no findings to suggest a load magnitude dose-response exists during drop jumps. Since the 40cm and 60cm groups produced loads above the minimal loading threshold and showed increases in cortical density and axial strength, respectively, compared to low magnitude and control groups, it can be suggested high-impact load is greater for bone accrual. Furthermore, as the 60cm group displayed a decrease in cortical density whilst the 40cm increased, it can be speculated that an upper loading threshold limit may exist before load magnitude becomes overbearing and causes detrimental effects to bone geometry.

Habitual high-impact load was observed to have positive effects on bone accrual in fast bowlers (Chapter 7). This thesis is the first to investigate contralateral bone characteristics in the lower extremity of elite, asymmetrical athletes. Previously, studies have shown the dominant limb in upper extremity models has greater bone mass, cortical area, cortical thickness and BMC compared to the non-dominant limb (Warden et al., 2019; Bogenschutz et al., 2011; Warden et al., 2009). Although study 4 (Chapter 7) did not observe as many differences between legs as reported in the upper extremity models (only cortical density and axial strength), the effect mechanical load can have on bilateral bone adaptation is evident. The limited bilateral differences in chapter 7, compared to previous studies, are likely due to the lower extremity experiencing external forces during daily activity, whereas external forces are not exerted on the upper body. The high-impact, cumulative mechanical load on the dominant leg can heighten the osteogenic response, therefore causing greater bone accrual

compared to the contralateral limb. The current thesis suggests habitual, high-impact, intermittent load can act as a platform to improve bone accrual, although large cohorts of less active young people are required to further investigate the effects of load magnitude on bone adaptation.

9.1.4 Limitations

Specific limitations of each study are discussed in the relevant experimental chapters of the thesis. Overall, the samples of chapter 4 (*in vitro*) and 8 (dose-response) likely hindered the opportunity to observe further significant changes to bone. Lab restrictions and limited spacing during chapter 4 meant I was only able to conduct the experiments over a single summer during COVID. Unsurprisingly, not all experiments were successful as highlighted in chapter 3 (general methods), therefore, to account for unsuccessful attempts of cell culturing it meant it was only possible to produce three independent experiments per condition (unloaded, continuous and intermittent). Prior to chapter 8, a power sample was performed pre-recruitment indicating 49 subjects were required. This does not differ from other studies that are shown to recruit similar sample sizes during RCT's assessing impact exercise (Bolam et al., 2015; Bailey & Brooke-Wavell., 2010; Sugiyama et al., 2002). However, as indicated by Kistler-Fischbacher et al. (2021a), it is possible that intervention studies examining bone adaptation require a larger sample size (33+ subjects per group) to observe significant results, as changes in bone are often small. I believe everything possible was done to recruit the targeted population without offering incentives, which was not possible due to ethical restrictions.

9.1.5 Practical implications

The overarching practical implications of these findings are:

- Rest periods may be useful for bone accrual.
- There is a relationship between IMU and GPS-derived load and bone accrual.
- There is complexity in the dose-response relationship of load magnitude and bone adaptation.
- Variability in external load exists with people performing the same tasks.

As the findings related to BMC and bone strength demonstrate in chapter 6 and 7, GPS and IMUs can be used as a practical tool to monitor external load alongside bone adaptation. Currently, bone load is not commonly monitored in applied environments, however, this thesis has shown IMU and GPS-derived metrics are capable of measuring load associated with bone accrual in an applied environment. Applied practitioners may use either of these methods, however, to assess site-specific load, which is relevant to site-specific bone adaptation, IMUs should be favoured. Due to the variability in load metrics, it cannot be assumed that the external load experienced within individuals is the same when performing the same task. Therefore, it is recommended that load should be monitored during exercise in order to understand bone response to mechanical load. Short bouts of high-impact exercise, implemented with bouts of rest, is an effective method to optimise positive bone adaptation. This emphasises the current implications of the thesis, whereby high-impact mechanical load can be used as a means of improving bone characteristics, but to understand the effects of exercise the quantification of load is required.

9.1.6 Conclusion

Positive bone adaptation is commonly associated with high-magnitude impact exercise. It has been shown in this thesis that high-impact exercise can encourage bone adaptation in young, low active adults and professional athletes. In addition, external load can be quantified to inform on bone adaptation, however, a variability in load exists in individuals performing the same type of exercise. To my knowledge, this is the first time that external load has been prospectively studied and associated to bone adaptation. It is understood that applied measures of external load may be used as proxy measures to inform on bone adaptation. Furthermore, individualised load is necessary to understand the mechanisms of mechanical load and is of significant value to future research to develop optimal exercise programmes for bone accrual.

9.1.7 Future investigations

This thesis expands the knowledge base on quantifying mechanical load and understanding its association with bone adaptation during impact activity. The ability to monitor external load and associate this with skeletal changes can be of applied relevance, although the extent

to which external load can represent a proxy measure of bone load requires development. Future investigations should include but not be limited to:

- Developing applied measures and/or testing current external load measures to monitor bone, not just GRF-derived measures. This would expand the understanding into the associations between external load monitoring and bone adaptation.

- Longitudinal, site-specific load monitoring (IMUs) alongside bone measurements (imaging techniques) during high-impact movements should be explored to try and understand the association between habitual load and site-specific adaptation.

- A continuation of the dose-response study (Chapter 8) with a larger cohort to understand bone adaptation in response to load magnitude using multiple loading groups (not just exercise vs. control).

- Repeat measures testing of external load measurements to investigate the variability of load metrics during multidirectional, high-impact activity

10 References

Adami, S., Gatti, D., Braga, V., Bianchini, D. and Rossini, M., 1999. Site-specific effects of strength training on bone structure and geometry of ultradistal radius in postmenopausal women. *Journal of Bone and Mineral Research*, *14*(1), pp.120-124.

Adams, J.E., 2009. Quantitative computed tomography. *European journal of radiology*, *71*(3), pp.415-424.

Addison, W.N., Nelea, V., Chicatun, F., Chien, Y.C., Tran-Khanh, N., Buschmann, M.D., Nazhat, S.N., Kaartinen, M.T., Vali, H., Tecklenburg, M.M. and Franceschi, R.T., 2015. Extracellular matrix mineralization in murine MC3T3-E1 osteoblast cultures: an ultrastructural, compositional and comparative analysis with mouse bone. *Bone*, *71*, pp.244-256.

Ahmad, N., Ghazilla, R.A.R., Khairi, N.M. and Kasi, V., 2013. Reviews on various inertial measurement unit (IMU) sensor applications. *International Journal of Signal Processing Systems*, *1*(2), pp.256-262.

Ahola, R., Korpelainen, R., Vainionpää, A. and Jämsä, T., 2010. Daily impact score in long-term acceleration measurements of exercise. *Journal of biomechanics*, *43*(10), pp.1960-1964.

Ahola, R., Korpelainen, R., Vainionpää, A., Leppäluoto, J. and Jämsä, T., 2009. Time-course of exercise and its association with 12-month bone changes. *BMC musculoskeletal disorders*, *10*, pp.1-8.

Akenhead, R. and Nassis, G.P., 2016. Training load and player monitoring in high-level football: current practice and perceptions. *International journal of sports physiology and performance*, *11*(5), pp.587-593.

Almstedt, H.C., Canepa, J.A., Ramirez, D.A. and Shoepe, T.C., 2011. Changes in bone mineral density in response to 24 weeks of resistance training in college-age men and women. *The Journal of Strength & Conditioning Research*, *25*(4), pp.1098-1103.

Al Nazer, R., Lanovaz, J., Kawalilak, C., Johnston, J.D. and Kontulainen, S., 2012. Direct in vivo strain measurements in human bone—a systematic literature review. *Journal of biomechanics*, *45*(1), pp.27-40.

Allen, M.R. and Burr, D.B., 2014. Bone modeling and remodeling. In *Basic and applied bone biology,* pp.75-90. Academic Press.

Allison, S.J., Folland, J.P., Rennie, W.J., Summers, G.D. and Brooke-Wavell, K., 2013. High impact exercise increased femoral neck bone mineral density in older men: a randomised unilateral intervention. *Bone*, *53*(2), pp.321-328.

Allison, S.J., Poole, K.E., Treece, G.M., Gee, A.H., Tonkin, C., Rennie, W.J., Folland, J.P., Summers, G.D. and Brooke-Wavell, K., 2015. The influence of high-impact exercise on cortical and trabecular bone mineral content and 3D distribution across the proximal femur in older men: a randomized controlled unilateral intervention. *Journal of Bone and Mineral Research*, *30*(9), pp.1709-1716.

Althubaiti, A., 2016. Information bias in health research: definition, pitfalls, and adjustment methods. *Journal of multidisciplinary healthcare*, pp.211-217.

Alway, P., Peirce, N., King, M., Jardine, R. and Brooke-Wavell, K., 2019. Lumbar bone mineral asymmetry in elite cricket fast bowlers. *Bone*, *127*, pp.537-543.

Anliker, E., Dick, C., Rawer, R. and Toigo, M., 2012. Effects of jumping exercise on maximum ground reaction force and bone in 8-to 12-year-old boys and girls: a 9-month randomized controlled trial. *J Musculoskelet Neuronal Interact*, *12*(2), pp.56-67.

Armitage, M., Beato, M., and McErlain-Naylor, SA., 2021. Inter-unit reliability of IMU Step metrics using IMeasureU Blue Trident inertial measurement units for running-based team sport tasks. *J Sports Sciences*, 39(13):1512-18.

Ashe MC, Liu-Ambrose TYL, Cooper DML, Khan KM, McKay HA., 2008. Muscle power is related to tibial bone strength in older women. *Osteo Int*, 19(12):1725-32.

Augat, P. and Schorlemmer, S., 2006. The role of cortical bone and its microstructure in bone strength. *Age and ageing*, *35*(2), pp.27-31.

Bacabac, R.G., Smit, T.H., Mullender, M.G., Dijcks, S.J., Van Loon, J.J. and Klein-Nulend, J., 2004. Nitric oxide production by bone cells is fluid shear stress rate dependent. *Biochemical and biophysical research communications*, *315*(4), pp.823-829.

Bacabac, R.G., Smit, T.H., Mullender, M.G., Van Loon, J.J. and Klein-Nulend, J., 2005. Initial stress-kick is required for fluid shear stress-induced rate dependent activation of bone cells. *Annals of Biomedical Engineering*, *33*, pp.104-110.

Bacon, CS., and Mauger, AR., 2017. Prediction of overuse injuries in professional u18-u21 footballers using metrics of training distance and intensity. *J Strength & Con Research*, 31(11):3067-76.

Baecker, N., Tomic, A., Mika, C., Gotzmann, A., Platen, P., Gerzer, R. and Heer, M., 2003. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. *Journal of applied physiology*, *95*(3), pp.977-982.

Bailey, C.A. and Brooke-Wavell, K., 2010. Optimum frequency of exercise for bone health: randomised controlled trial of a high-impact unilateral intervention. *Bone*, *46*(4), pp.1043-1049.

Barrett S, Midgley A, Lovell R., 2014. PlayerLoad™: reliability, convergent validity, and influence of unit position during treadmill running. *Int J of Sports Phys and Perform*, 9(6):945-52.

Barry, D.W. and Kohrt, W.M., 2008. Exercise and the preservation of bone health. *Journal of cardiopulmonary Rehabilitation and Prevention*, *28*(3), pp.153-162.

Bartlett, R.M., Stockill, N.P., Elliott, B.C. and Burnett, A.F., 1996. The biomechanics of fast bowling in men's cricket: A review. *Journal of sports sciences*, *14*(5), pp.403-424.

Bass, S., Eser, P. and Daly, R., 2005. The effect of exercise and nutrition on the mechanostat.

Bassey, E.J., Littlewood, J.J. and Taylor, S.J.G., 1997. Relations between compressive axial forces in an instrumented massive femoral implant, ground reaction forces, and integrated electromyographs from vastus lateralis during various 'osteogenic' exercises. *Journal of biomechanics*, *30*(3), pp.213-223.

Bassey, E.J. and Ramsdale, S.J., 1994. Increase in femoral bone density in young women following high-impact exercise. *Osteoporosis international*, *4*, pp.72-75.

Bates, N.A., Ford, K.R., Myer, G.D. and Hewett, T.E., 2013. Timing differences in the generation of ground reaction forces between the initial and secondary landing phases of the drop vertical jump. *Clinical biomechanics*, *28*(7), pp.796-799.

Batra, N.N., Li, Y.J., Yellowley, C.E., You, L., Malone, A.M., Kim, C.H. and Jacobs, C.R., 2005. Effects of short-term recovery periods on fluid-induced signaling in osteoblastic cells. *Journal of biomechanics*, *38*(9), pp.1909-1917.

Baudequin, T., Legallais, C. and Bedoui, F., 2019. In vitro bone cell response to tensile mechanical solicitations: Is there an optimal protocol?. *Biotechnology Journal*, *14*(1), p.1800358.

Bazzocchi, A., Ponti, F., Albisinni, U., Battista, G. and Guglielmi, G., 2016. DXA: Technical aspects and application. *European journal of radiology*, *85*(8), pp.1481-1492.

Beaupré, G.S., Orr, T.E. and Carter, D.R., 1990. An approach for time-dependent bone modeling and remodeling—application: A preliminary remodeling simulation. *Journal of orthopaedic research*, *8*(5), pp.662-670.

Beck, B., Daly, R., Singh, M. and Taaffe, R. 2017. Exercise and Sports Science Australia (ESSA) position statement on exercise prescription for the prevention and management of osteoporosis. *Journal of science and medicine in sport*, *20*(5), pp.438-445.

Besier T., 2019. Bone stimulus. Available at: https://imeasureu.com/wp-content/uploads/2019/10/IMU_Bone_Stimulus.pdf.

Bhattoa, H., Cavalier, E., Eastell, R., Heijboer, A., Jørgensen, N., Makris, K., Ulmer, C., Kanis, J., Cooper, C., Silverman, S. and Vasikaran, S., 2021. Analytical considerations and plans to standardize or harmonize assays for the reference bone turnover markers PINP and β-CTX in blood. *Clinica Chimica Acta*, *515*, pp.16-20.

Blake, G. and Fogelman, I., 1997. Technical principles of dual energy x-ray absorptiometry. *Seminars in nuclear medicine*, *27*(3), pp.210-228.

Blake, G.M. and Fogelman, I., 2010. An update on dual-energy x-ray absorptiometry. *Seminars in nuclear medicine*, *40*(1), pp.62-73.

Bogenschutz, E.D., Smith, H.D. and Warden, S.J., 2011. Mid-humerus adaptation in fast pitch softballers and the impact of throwing mechanics. *Medicine and science in sports and exercise*, *43*(9), p.1698.

Bolam, K.A., Skinner, T.L., Jenkins, D.G., Galvão, D.A. and Taaffe, D.R., 2015. The osteogenic effect of impact-loading and resistance exercise on bone mineral density in middle-aged and older men: a pilot study. *Gerontology*, *62*(1), pp.22-32.

Bonewald, L.F., Harris, S.E., Rosser, J., Dallas, M.R., Dallas, S.L., Camacho, N.P., Boyan, B. and Boskey, A., 2003. von Kossa staining alone is not sufficient to confirm that mineralization in vitro represents bone formation. *Calcified tissue international*, *72*, pp.537-547.

Bonnet, N. and Ferrari, S.L., 2010. Exercise and the skeleton: how it works and what it really does. *IBMS BoneKEy*, *7*, p.235.

Borresen, J., and Lambert, MI., 2009. The quantification of training load, the training response and the effect on performance. *Sports Med*, 39(9):779-95.

Bourdon, P.C., Cardinale, M., Murray, A., Gastin, P., Kellmann, M., Varley, M.C., Gabbett, T.J., Coutts, A.J., Burgess, D.J., Gregson, W. and Cable, N.T., 2017. Monitoring athlete training loads: consensus statement. *International journal of sports physiology and performance*, *12*(s2), pp.S2-161.

Bowtell, J.L., Jackman, S.R., Scott, S., Connolly, L.J., Mohr, M., Ermidis, G., Julian, R., Yousefian, F., Helge, E.W., Jørgensen, N.R. and Fulford, J., 2016. Short duration small sided football and to a lesser extent whole body vibration exercise induce acute changes in markers of bone turnover. *BioMed Research International*.

Brady, R., Brown, W.J. and Mielke, G.I., 2023. Day-to-day variability in accelerometer-measured physical activity in mid-aged Australian adults. *BMC Public Health*, *23*(1), p.1880.

Bredt S, Chagas M, Peixoto G, Menzel H, de Andrade A., 2020. Understanding player load: Meanings and limitations. *J Human Kin*, 71(1):5-9.

Brooke-Wavell, K., Skelton, D.A., Barker, K.L., Clark, E.M., De Biase, S., Arnold, S., Paskins, Z., Robinson, K.R., Lewis, R.M., Tobias, J.H. and Ward, K.A., 2022. Strong, steady and straight: UK consensus statement on physical activity and exercise for osteoporosis. *British journal of sports medicine*, *56*(15), pp.837-846.

Burgess DJ., 2017. The research doesn't always apply: practical solutions to evidence-based training-load monitoring in elite team sports. *Int J Sports Phys and Perform*. 12(2):2-136.

Burr, D.B. and Akkus, O., 2014. Bone Morphology and Organization, Chapter 1. *Basic and Applied Bone Biology*, pp.3-25.

Burr, D.B., 1997. 7 Bone, Exercise, and Stress Fractures. *Exercise and sport sciences reviews*, *25*(1), pp.171-194.

Burr, D.B., Milgrom, C., Fyhrie, D., Forwood, M., Nyska, M., Finestone, A., Hoshaw, S., Saiag, E. and Simkin, A., 1996. In vivo measurement of human tibial strains during vigorous activity. *Bone*, *18*(5), pp.405-410.

Burr, D.B., Robling, A.G. and Turner, C.H., 2002. Effects of biomechanical stress on bones in animals. *Bone*, *30*(5), pp.781-786.

Burr, D.B., Schaffler, M.B., Yang, K.H., Wu, D.D., Lukoschek, M., Kandzari, D., Sivaneri, N., Blaha, J.D. and Radin, E.L., 1989. The effects of altered strain environments on bone tissue kinetics. *Bone*, *10*(3), pp.215-221.

Buysse, D.J., Reynolds III, C.F., Monk, T.H., Berman, S.R. and Kupfer, D.J., 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry research*, *28*(2), pp.193-213.

Caetano-Lopes, J., Canhao, H. and Fonseca, J.E., 2007. Osteoblasts and bone formation. *Acta reumatológica portuguesa*, *32*(2), pp.103-110.

Candow, D.G., Chilibeck, P.D., Gordon, J.J. and Kontulainen, S., 2021. Efficacy of creatine supplementation and resistance training on area and density of bone and muscle in older adults. *Medicine & Science in Sports & Exercise*, *53*(11), pp.2388-2395.

Carbuhn, A.F., Fernandez, T.E., Bragg, A.F., Green, J.S. and Crouse, S.F., 2010. Sport and training influence bone and body composition in women collegiate athletes. *The Journal of Strength & Conditioning Research*, *24*(7), pp.1710-1717.

Castillo D, Weston M, McLaren SJ, Cámara J, Yanci J., 2017. Relationships between internal and external match-load indicators in soccer match officials. *Int J sports Phys & Perform*, 12(7):922-27.

Cervinka, T., 2014. Bone structural analysis with pQCT: image preprocessing and cortical bone segmentation.

Cervinka, T., Hyttinen, J. and Sievanen, H., 2010. Enhanced bone structural analysis through pQCT image preprocessing. *Medical engineering & physics*, *32*(4), pp.398-406.

Chambon, N., Delattre, N., Guéguen, N., Berton, E. and Rao, G., 2014. Is midsole thickness a key parameter for the running pattern?. *Gait & posture*, *40*(1), pp.58-63.

Chappell, J.D., Herman, D.C., Knight, B.S., Kirkendall, D.T., Garrett, W.E. and Yu, B., 2005. Effect of fatigue on knee kinetics and kinematics in stop-jump tasks. *The American journal of sports medicine*, *33*(7), pp.1022-1029.

Chavassieux, P., Portero-Muzy, N., Roux, J.P., Garnero, P. and Chapurlat, R., 2015. Are biochemical markers of bone turnover representative of bone histomorphometry in 370 postmenopausal women?. *The Journal of Clinical Endocrinology & Metabolism*, *100*(12), pp.4662-4668.

Chen, K.Y. and Bassett, J.R., 2005. The technology of accelerometry-based activity monitors: current and future. *Medicine & Science in Sports & Exercise*, *37*(11), pp.S490-S500.

Chen, P., Satterwhite, J.H., Licata, A.A., Lewiecki, E.M., Sipos, A.A., Misurski, D.M. and Wagman, R.B., 2005. Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis. *Journal of bone and mineral research*, *20*(6), pp.962-970.

Chen, Z., Bemben, D. and Brooke-Wavell, K., 2023. Exercise and bone: loading characteristics, mechanisms and adaptation. *Frontiers in Physiology*, *14*, p.1335456.

Cheng, S., Sipilä, S., Taaffe, D.R., Puolakka, J. and Suominen, H., 2002. Change in bone mass distribution induced by hormone replacement therapy and high-impact physical exercise in post-menopausal women. *Bone*, *31*(1), pp.126-135.

Choi, W., Choi, JH., Chung, CY., Sung, KH., and Lee, KM., 2021. Can gait kinetic data predict femoral bone mineral density in elderly men and women aged 50 years and older?. *J Biomech*, 123:110520.

Christen, P., Ito, K., Ellouz, R., Boutroy, S., Sornay-Rendu, E., Chapurlat, R.D. and Van Rietbergen, B., 2014. Bone remodelling in humans is load-driven but not lazy. *Nature communications*, *5*(1), p.4855.

Civil, R., Dolan, E., Swinton, P.A., Santos, L., Varley, I., Atherton, P.J., Elliott-Sale, K.J. and Sale, C., 2023. P1NP and β-CTX-1 responses to a prolonged, continuous running bout in young healthy adult males: a systematic review with individual participant data meta-analysis. *Sports medicine-open*, *9*(1), p.85.

Civitelli, R., Armamento-Villareal, R. and Napoli, N., 2009. Bone turnover markers: understanding their value in clinical trials and clinical practice. *Osteoporosis international*, *20*, pp.843-851.

Clemente, F.M., Rabbani, A., Conte, D., Castillo, D., Afonso, J., Truman Clark, C.C., Nikolaidis, P.T., Rosemann, T. and Knechtle, B., 2019. Training/match external load ratios in professional soccer players: A full-season study. *International journal of environmental research and public health*, *16*(17), p.3057.

Clevers, H. and Nusse, R., 2012. Wnt/β-catenin signaling and disease. *Cell*, *149*(6), pp.1192-1205.

Clynes, M.A., Harvey, N.C., Curtis, E.M., Fuggle, N.R., Dennison, E.M. and Cooper, C., 2020. The epidemiology of osteoporosis. *British medical bulletin*, *133*(1), pp.105-117.

Cohen, H., Kugel, C., May, H., Medlej, B., Stein, D., Slon, V., Brosh, T. and Hershkovitz, I., 2017. The influence of impact direction and axial loading on the bone fracture pattern. *Forensic science international*, *277*, pp.197-206.

Cointry, G.R., Ferretti, J.L., Reina, P.S., Nocciolino, L.M., Rittweger, J. and Capozza, R.F., 2014. The pQCT "Bone Strength Indices" (BSIs, SSI). Relative mechanical impact and diagnostic value of the indicators of bone tissue and design quality employed in their calculation in healthy men and pre-and post-menopausal women.

Courteix, D., Lespessailles, E., Peres, S.L., Obert, P., Germain, P. and Benhamou, C.L., 1998. Effect of physical training on bone mineral density in prepubertal girls: a comparative study between impact-loading and non-impact-loading sports. *Osteoporosis international*, *8*, pp.152-158.

Coutts, AJ., and Duffield, R., 2010. Validity and reliability of GPS devices for measuring movement demands of team sports. *J Sci & Med in Sport*. 13(1):133-35.

Currey, J.D., 2012. The structure and mechanics of bone. *Journal of Materials Science*, *47*, pp.41-54.

Cussler, E.C., Going, S.B., Houtkooper, L.B., Stanford, V.A., Blew, R.M., Flint-Wagner, H.G., Metcalfe, L.L., Choi, J.E. and Lohman, T.G., 2005. Exercise frequency and calcium intake predict 4-year bone changes in postmenopausal women. *Osteoporosis International*, *16*, pp.2129-2141.

Daly, R.M., Dalla Via, J., Duckham, R.L., Fraser, S.F. and Helge, E.W., 2019. Exercise for the prevention of osteoporosis in postmenopausal women: an evidence-based guide to the optimal prescription. *Brazilian journal of physical therapy*, *23*(2), pp.170-180.

Daly, R.M., Dalla Via, J., Fyfe, J.J., Nikander, R. and Kukuljan, S., 2021. Effects of exercise frequency and training volume on bone changes following a multi-component exercise intervention in middle aged and older men: secondary analysis of an 18-month randomized controlled trial. *Bone*, *148*, p.115944.

Dasarathy, J. and Labrador, H., 2018. Bone health in women. *Primary Care: Clinics in Office Practice*, *45*(4), pp.643-657.

Deere, K., Sayers, A., Rittweger, J. and Tobias, J.H., 2012. Habitual levels of high, but not moderate or low, impact activity are positively related to hip BMD and geometry: results from a population-based study of adolescents. *Journal of bone and mineral research*, *27*(9), pp.1887-1895.

De Margerie, E., Sanchez, S., Cubo, J. and Castanet, J., 2005. Torsional resistance as a principal component of the structural design of long bones: comparative multivariate evidence in birds. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists*, *282*(1), pp.49-66.

Dempster, W.T., 1955. *Space requirements of the seated operator: geometrical, kinematic, and mechanical aspects of the body with special reference to the limbs* (Vol. 159). Wright-Patterson Air Force Base, Ohio: Wright Air Development Center.

De Souza, R.L., Matsuura, M., Eckstein, F., Rawlinson, S.C., Lanyon, L.E. and Pitsillides, A.A., 2005. Non-invasive axial loading of mouse tibiae increases cortical bone formation and modifies trabecular organization: a new model to study cortical and cancellous compartments in a single loaded element. *Bone*, *37*(6), pp.810-818.

Decker, M.J., Torry, M.R., Wyland, D.J., Sterett, W.I. and Steadman, J.R., 2003. Gender differences in lower extremity kinematics, kinetics and energy absorption during landing. *Clinical biomechanics*, *18*(7), pp.662-669.

Detter, F., Rosengren, B.E., Dencker, M., Lorentzon, M., Nilsson, J.Å. and Karlsson, M.K., 2014. A 6-year exercise program improves skeletal traits without affecting fracture risk: a prospective controlled study in 2621 children. *Journal of bone and mineral research*, *29*(6), pp.1325-1336.

Di Medio, L. and Brandi, M.L., 2021. Advances in bone turnover markers. In *Advances in Clinical Chemistry* (Vol. 105, pp. 101-140). Elsevier.

Dolan, E., Dumas, A., Keane, K.M., Bestetti, G., Freitas, L.H.M., Gualano, B., Kohrt, W.M., Kelley, G.A., Pereira, R.M.R., Sale, C. and Swinton, P.A., 2022. The bone biomarker response to an acute bout of exercise: a systematic review with meta-analysis. *Sports Medicine*, *52*(12), pp.2889-2908.

Donahue, S.W., Donahue, H.J. and Jacobs, C.R., 2003. Osteoblastic cells have refractory periods for fluid-flow-induced intracellular calcium oscillations for short bouts of flow and display multiple low-magnitude oscillations during long-term flow. *Journal of biomechanics*, *36*(1), pp.35-43.

Doyle, E.W., Doyle, T.L., Bonacci, J. and Fuller, J.T., 2024. Sensor location influences the associations between IMU and motion capture measurements of impact landing in healthy male and female runners at multiple running speeds. *Sports Biomechanics*, pp.1-15.

Dror, N., Carbone, J., Haddad, F., Falk, B., Klentrou, P. and Radom-Aizik, S., 2022. Sclerostin and bone turnover markers response to cycling and running at the same moderate-to-vigorous exercise intensity in healthy men. *Journal of Endocrinological Investigation*, *45*(2), pp.391-397.

Du, J., Hartley, C., Brooke-Wavell, K., Paggiosi, M.A., Walsh, J.S., Li, S. and Silberschmidt, V.V., 2021. High-impact exercise stimulated localised adaptation of microarchitecture across distal tibia in postmenopausal women. *Osteoporosis International*, *32*, pp.907-919.

Ducher, G., Bass, S.L., Saxon, L. and Daly, R.M., 2011. Effects of repetitive loading on the growth-induced changes in bone mass and cortical bone geometry: A 12-month study in pre/peri-and postmenarcheal tennis players. *Journal of bone and mineral research*, *26*(6), pp.1321-1329.

Duckham, R.L., Baxter-Jones, A.D., Johnston, J.D., Vatanparast, H., Cooper, D. and Kontulainen, S., 2014. Does physical activity in adolescence have site-specific and sex-specific benefits on young adult bone size, content, and estimated strength?. *Journal of bone and mineral research*, *29*(2), pp.479-486.

Ekstrand, J. and Torstveit, M.K., 2012. Stress fractures in elite male football players. *Scandinavian journal of medicine & science in sports*, *22*(3), pp.341-346.

El Deeb, AM., and Khodair, AS., 2014. Three-dimensional analysis of gait in postmenopausal women with low bone mineral density. *J Neuroengineering & Rehab*, 11(1):1-7.

El Maghraoui, A. and Roux, C., 2008. DXA scanning in clinical practice. *QJM: An International Journal of Medicine*, *101*(8), pp.605-617.

Elvin, N.G., Elvin, A.A. and Arnoczky, S.P., 2007. Correlation between ground reaction force and tibial acceleration in vertical jumping. *Journal of applied biomechanics*, *23*(3), pp.180-189.

Epifano, D.J., Ryan, S., Clarke, A.C., Huynh, M., Anderson, H. and Middleton, K.J., 2022. Comparing tibial accelerations between delivery and follow-through foot strikes in cricket pace bowling. *Sports biomechanics*, pp.1-14.

Eriksen, E.F., Charles, P., Melsen, F., Mosekilde, L., Risteli, L. and Risteli, J., 1993. Serum markers of type I collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. *Journal of Bone and Mineral Research*, *8*(2), pp.127-132.

Evans, R.K., Negus, C.H., Centi, A.J., Spiering, B.A., Kraemer, W.J. and Nindl, B.C., 2012. Peripheral QCT sector analysis reveals early exercise-induced increases in tibial bone mineral density. *J Musculoskelet Neuronal Interact*, *12*(3), pp.155-64.

Evans, W., Nevill, A., McLaren, S.J. and Ditroilo, M., 2020. The effect of intermittent running on biomarkers of bone turnover. *European Journal of Sport Science*. *20*(4), pp.505-515.

Farr, J.N., Blew, R.M., Lee, V.R., Lohman, T.G. and Going, S.B., 2011. Associations of physical activity duration, frequency, and load with volumetric BMD, geometry, and bone strength in young girls. *Osteoporosis international*, *22*, pp.1419-1430.

Fatihhi, S.J., Rabiatul, A.A.R., Harun, M.N., Kadir, M.R.A., Kamarul, T. and Syahrom, A., 2016. Effect of torsional loading on compressive fatigue behaviour of trabecular bone. *Journal of the mechanical behavior of biomedical materials*, *54*, pp.21-32.

Faul, F., Erdfelder, E., Buchner, A. and Lang, A.G., 2009. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. *Behavior research methods*, *41*(4), pp.1149-1160.

Fedde, K.N., Blair, L., Silverstein, J., Coburn, S.P., Ryan, L.M., Weinstein, R.S., Waymire, K., Narisawa, S., Millán, J.L., Macgregor, G.R. and Whyte, M.P., 1999. Alkaline phosphatase knock-out mice recapitulate the metabolic and skeletal defects of infantile hypophosphatasia. *Journal of bone and mineral research*, *14*(12), pp.2015-2026.

Ferry, B., Lespessailles, E., Rochcongar, P., Duclos, M. and Courteix, D., 2013. Bone health during late adolescence: effects of an 8-month training program on bone geometry in female athletes. *Joint Bone Spine*, *80*(1), pp.57-63.

Florence, G.E., Oosthuyse, T. and Bosch, A.N., 2024. Skeletal site-specific effects of jump training on bone mineral density in adults: a systematic review and meta-analysis. *Journal of Sports Sciences*, pp.1-14.

Fredericson, M., Jennings, F., Beaulieu, C., and Matheson, GO., 2006. Stress fractures in athletes. *Topics in Magnetic Resonance Imaging*. (2006) 17(5):309-25.

Frost, H.M., 1987. Bone "mass" and the "mechanostat": a proposal. *The anatomical record*, *219*(1), pp.1-9.

Fuchs, R.K., Bauer, J.J. and Snow, C.M., 2001. Jumping improves hip and lumbar spine bone mass in prepubescent children: a randomized controlled trial. *Journal of bone and mineral research*, *16*(1), pp.148-156.

Gabbett TJ., 2016. The training—injury prevention paradox: should athletes be training smarter and harder?. *Br J Sports Med*, 50(5):273-80.

Gabbett, TJ., and Ullah, S., 2012. Relationship between running loads and soft-tissue injury in elite team sport athletes. *J Strength & Con Research*. 26(4):953-60.

Gabel, L., Macdonald, H.M., Nettlefold, L. and McKay, H.A., 2017. Physical activity, sedentary time, and bone strength from childhood to early adulthood: a mixed longitudinal HR-pQCT study. *Journal of bone and mineral research*, *32*(7), pp.1525-1536.

García-Pérez, J.A., Pérez-Soriano, P., Llana Belloch, S., Lucas-Cuevas, Á.G. and Sánchez-Zuriaga, D., 2014. Effects of treadmill running and fatigue on impact acceleration in distance running. *Sports Biomechanics*, *13*(3), pp.259-266.

Gardner, M.J., van der Meulen, M.C., Demetrakopoulos, D., Wright, T.M., Myers, E.R. and Bostrom, M.P., 2006. In vivo cyclic axial compression affects bone healing in the mouse tibia. *Journal of orthopaedic research*, *24*(8), pp.1679-1686.
Gasser, J.A., 1995. Assessing bone quantity by pQCT. *Bone*, *17*(4), pp.S145-S154.

George, D., Pallu, S., Bourzac, C., Wazzani, R., Allena, R., Rémond, Y. and Portier, H., 2022. Prediction of cortical bone thickness variations in the tibial diaphysis of running rats. *Life*, *12*(2), p.233.

George, W.T. and Vashishth, D., 2005. Damage mechanisms and failure modes of cortical bone under components of physiological loading. *Journal of orthopaedic research*, *23*(5), pp.1047-1053.

George, W.T. and Vashishth, D., 2006. Susceptibility of aging human bone to mixed-mode fracture increases bone fragility. *Bone*, *38*(1), pp.105-111.

Godin, L.M., Suzuki, S., Jacobs, C.R., Donahue, H.J. and Donahue, S.W., 2007. Mechanically induced intracellular calcium waves in osteoblasts demonstrate calcium fingerprints in bone cell mechanotransduction. *Biomechanics and modeling in mechanobiology*, *6*, pp.391-398.

Gokhale, J.A., Boskey, A.L. and Robey, P.G., 2001. The biochemistry of bone. In *Osteoporosis* (pp. 107-188). Academic Press.

Gómez-Bruton, A., González-Agüero, A., Gómez-Cabello, A., Matute-Llorente, A., Casajús, J.A. and Vicente-Rodríguez, G., 2015. The effects of swimming training on bone tissue in adolescence. *Scandinavian journal of medicine & science in sports*, *25*(6), pp.e589-e602.

González-García, J., Conejero, M. and Gutiérrez-Hellín, J., 2024. Assessing Jump Performance: Intra-and Interday Reliability and Minimum Difference of Countermovement Jump and Drop Jump Outcomes, Kinetics, Kinematics, and Jump Strategy. *Applied Sciences*, *14*(6), p.2662.

Gracia-Marco, L., Moreno, L.A., Ortega, F.B., León, F., Sioen, I., Kafatos, A., Martinez-Gomez, D., Widhalm, K., Castillo, M.J., Vicente-Rodríguez, G. and HELENA Study Group, 2011. Levels of physical activity that predict optimal bone mass in adolescents: the HELENA study. *American journal of preventive medicine*, *40*(6), pp.599-607.

Greene, D.A., Naughton, G.A., Bradshaw, E., Moresi, M. and Ducher, G., 2012. Mechanical loading with or without weight-bearing activity: influence on bone strength index in elite female adolescent athletes engaged in water polo, gymnastics, and track-and-field. *Journal of Bone and Mineral Metabolism*, *30*, pp.580-587.

Greene, D.A., Wiebe, P.N. and Naughton, G.A., 2009. Influence of drop-landing exercises on bone geometry and biomechanical properties in prepubertal girls: a randomized controlled study. *Calcified Tissue International*, *85*, pp.94-103.

Greenhalgh, A., Sinclair, J., Protheroe, L. and Chockalingam, N., 2012. Predicting impact shock magnitude: which ground reaction force variable should we use. *International Journal of Sports Science and Engineering*, *6*(4), pp.225-231.

Greenway, K.G., Walkley, J.W. and Rich, P.A., 2015. Impact exercise and bone density in premenopausal women with below average bone density for age. *European journal of applied physiology*, *115*, pp.2457-2469.

Grenander, U., 1959. The Nyquist frequency is that frequency whose period is two sampling intervals. *Probability and Statistics: The Harald Cramér Volume*, *434*.

Grissom, L.E. and Harcke, H.T., 2003. Radiographic features of bisphosphonate therapy in pediatric patients. *Pediatric radiology*, *33*, pp.226-229.

Groothausen J, Siemer H, Kemper HC, Twisk J, Welten DC., 1997. Influence of peak strain on lumbar bone mineral density: an analysis of 15-year physical activity in young males and females. *Pediatric Ex Sci*, 9(2):159-73.

Guadalupe-Grau, A., Fuentes, T., Guerra, B. and Calbet, J.A., 2009. Exercise and bone mass in adults. *Sports medicine*, *39*, pp.439-468.

Gunter, K., Baxter-Jones, A.D., Mirwald, R.L., Almstedt, H., Fuchs, R.K., Durski, S. and Snow, C., 2008. Impact exercise increases BMC during growth: an 8-year longitudinal study. *Journal of Bone and Mineral Research*, *23*(7), pp.986-993.

Gunter, K., Baxter-Jones, AD., Mirwald, RL., Almstedt, H., Fuller, A., Durski, S. and Snow, C., 2008. Jump starting skeletal health: a 4-year longitudinal study assessing the effects of jumping on skeletal development in pre and circum pubertal children. *Bone*, *42*, pp.710–718.

Guppy, S.N., Brady, C.J., Kotani, Y., Stone, M.H., Medic, N. and Haff, G.G., 2019. Effect of altering body posture and barbell position on the within-session reliability and magnitude of force-time curve characteristics in the isometric midthigh pull. *The Journal of Strength & Conditioning Research*, *33*(12), pp.3252-3262.

Haapala, E.A., Rantalainen, T., Hesketh, K.D., Rodda, C.P. and Duckham, R.L., 2022. Accelerometer-based osteogenic indices, moderate-to-vigorous and vigorous physical activity, and bone traits in adolescents. *Journal of Musculoskeletal & Neuronal Interactions*, *22*(4), p.514.

Hagman, M., Helge, E.W., Hornstrup, T., Fristrup, B., Nielsen, J.J., Jørgensen, N.R., Andersen, J.L., Helge, J.W. and Krustrup, P., 2018. Bone mineral density in lifelong trained male football players compared with young and elderly untrained men. *Journal of sport and health science*, *7*(2), pp.159-168.

Hale, L.V., Galvin, R.S., Risteli, J., Ma, Y.L., Harvey, A.K., Yang, X., Cain, R.L., Zeng, Q., Frolik, C.A., Sato, M. and Schmidt, A.L., 2007. PINP: a serum biomarker of bone formation in the rat. *Bone*, *40*(4), pp.1103-1109.

Halson, SL., 2014. Monitoring training load to understand fatigue in athletes. *Sports Med*, 44(2): 139-47.

Han, Y., Cowin, S.C., Schaffler, M.B. and Weinbaum, S., 2004. Mechanotransduction and strain amplification in osteocyte cell processes. *Proceedings of the national academy of sciences*, *101*(47), pp.16689-16694.

Hansen, K.T., Cronin, J.B. and Newton, M.J., 2011. The reliability of linear position transducer and force plate measurement of explosive force–time variables during a loaded jump squat in elite athletes. *The Journal of Strength & Conditioning Research*, *25*(5), pp.1447-1456.

Hardcastle, S.A., Gregson, C.L., Rittweger, J., Crabtree, N., Ward, K. and Tobias, J.H., 2014. Jump power and force have distinct associations with cortical bone parameters: findings from a population enriched by individuals with high bone mass. *The Journal of Clinical Endocrinology & Metabolism*, *99*(1), pp.266-275.

Hart, N.H., Nimphius, S., Rantalainen, T., Ireland, A., Siafarikas, A. and Newton, R.U., 2017. Mechanical basis of bone strength: influence of bone material, bone structure and muscle action. *Journal of musculoskeletal & neuronal interactions*, *17*(3), p.114.

Hartley, C., Folland, J.P., Kerslake, R. and Brooke-Wavell, K., 2020. High-Impact Exercise Increased Femoral Neck Bone Density With No Adverse Effects on Imaging Markers of Knee Osteoarthritis in Postmenopausal Women. *Journal of Bone and Mineral Research*, *35*(1), pp.53-63.

Havaldar, R., Pilli, S.C. and Putti, B.B., 2014. Insights into the effects of tensile and compressive loadings on human femur bone. *Advanced biomedical research*, *3*.

Heikkinen, R., Vihriälä, E., Vainionpää, A., Korpelainen, R. and Jämsä, T., 2007. Acceleration slope of exercise-induced impacts is a determinant of changes in bone density. *Journal of Biomechanics*, *40*(13), pp.2967-2974.

Heinemann, D.F., 2000. Osteoporosis. An overview of the National Osteoporosis Foundation clinical practice guide. *Geriatrics*, *55*(5), pp.31-6.

Heiniö, L., Nikander, R. and Sievänen, H., 2015. Association between long-term exercise loading and lumbar spine trabecular bone score (TBS) in different exercise loading groups. *Journal of musculoskeletal & neuronal interactions*, *15*(3), p.279.

Heinonen, A., Kannus, P., Sievänen, H., Oja, P., Pasanen, M., Rinne, M., Uusi-Rasi, K. and Vuori, I., 1996. Randomised controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. *The Lancet*, *348*(9038), pp.1343-1347.

Heinonen, A., Sievänen, H., Kannus, P., Oja, P., Pasanen, M. and Vuori, I., 2000. High-impact exercise and bones of growing girls: a 9-month controlled trial. *Osteoporosis international*, *11*, pp.1010-1017.

Heishman, AD., Curtis, MA., Saliba, E., Hornett, RJ., Malin, SK., Weltman, AL., 2018. Noninvasive assessment of internal and external player load: implications for optimizing athletic performance. *J Strength & Con Research*, 32(5):1280-87.

Henry, Y.M., Fatayerji, D. and Eastell, R., 2004. Attainment of peak bone mass at the lumbar spine, femoral neck and radius in men and women: relative contributions of bone size and volumetric bone mineral density. *Osteoporosis international*, *15*, pp.263-273.

Hermens, H.J., Freriks, B., Disselhorst-Klug, C. and Rau, G., 2000. Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of electromyography and Kinesiology*, *10*(5), pp.361-374.

Hernandez, C.J., Beaupre, G.S. and Carter, D.R., 2003. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis international*, *14*, pp.843-847.

Hernlund, E., Svedbom, A., Ivergård, M., Compston, J., Cooper, C., Stenmark, J., McCloskey, E.V., Jönsson, B.K.J.A. and Kanis, J.A., 2013. Osteoporosis in the European Union: medical management, epidemiology and economic burden: a report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Archives of osteoporosis*, *8*, pp.1-115.

Herman, B.C., Cardoso, L., Majeska, R.J., Jepsen, K.J. and Schaffler, M.B., 2010. Activation of bone remodelling after fatigue: differential response to linear microcracks and diffuse damage. *Bone*, *47*(4), pp.766-772.

Herrmann, M. and Seibel, M., 2008. The amino-and carboxyterminal cross-linked telopeptides of collagen type I, NTX-I and CTX-I: a comparative review. *Clinica Chimica Acta*, *393*(2), pp.57-75.

Hewett, TE., Myer, GD., Ford, KR., Heidt, J., Colosimo, AJ., McLean, SG., Van den Bogert, AJ., Paterno, MV., Succop, P., 2005. Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study. *American J Sports Med*, 33(4):492-501.

Hilkens, L., Boerboom, M., van Schijndel, N., Bons, J., van Loon, L.J. and van Dijk, J.W., 2023. Bone turnover following high-impact exercise is not modulated by collagen supplementation in young men: A randomized cross-over trial. *Bone*, *170*, p.116705.

Hind, K. and Burrows, M., 2007. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. *Bone*, *40*(1), pp.14-27.

Hinkle, D.E., Wiersma, W. and Jurs, S.G., 2003. *Applied statistics for the behavioral sciences,* 5(663). pp.

Hinton, P.S., Nigh, P. and Thyfault, J., 2015. Effectiveness of resistance training or jumping-exercise to increase bone mineral density in men with low bone mass: A 12-month randomized, clinical trial. *Bone*, *79*, pp.203-212.

Holtermann, A., Roeleveld, K., Vereijken, B. and Ettema, G., 2007. The effect of rate of force development on maximal force production: acute and training-related aspects. *European journal of applied physiology*, *99*, pp.605-613.

Horton, J.A., Murray, G.M., Spadaro, J.A., Margulies, B.S., Allen, M.J. and Damron, T.A., 2003. Precision and accuracy of DXA and pQCT for densitometry of the rat femur. *Journal of Clinical Densitometry*, *6*(4), pp.381-390.

Howe, L., Bampouras, T.M., North, J.S. and Waldron, M., 2020. Reliability of two-dimensional measures associated with bilateral drop-landing performance. *Movement & Sport Sciences*, (2), pp.39-47.

Hughes, J.M., Castellani, C.M., Popp, K.L., Guerriere, K.I., Matheny Jr, R.W., Nindl, B.C. and Bouxsein, M.L., 2020. The central role of osteocytes in the four adaptive pathways of bone's mechanostat. *Exercise and sport sciences reviews*, *48*(3), pp.140-148.

Hughes, J.M., Gaffney-Stomberg, E., Guerriere, K.I., Taylor, K.M., Popp, K.L., Xu, C., Unnikrishnan, G., Staab, J.S., Matheny Jr, R.W., McClung, J.P. and Reifman, J., 2018. Changes in tibial bone microarchitecture in female recruits in response to 8 weeks of US Army Basic Combat Training. *Bone*, *113*, pp.9-16.

Hurrion, P.D., Dyson, R. and Hale, T., 2000. Simultaneous measurement of back and front foot ground reaction forces during the same delivery stride of the fast-medium bowler. *Journal of sports sciences*, *18*(12), pp.993-997.

Hutson, M.J. and Varley, I., 2024. An Opinion on the Interpretation of Bone Turnover Markers Following Acute Exercise or Nutrition Intervention and Considerations for Applied Research. *International Journal of Sport Nutrition and Exercise Metabolism*, *1*(aop), pp.1-7.

Impellizzeri, F.M., Marcora, S.M. and Coutts, A.J., 2019. Internal and external training load: 15 years on. *Int J Sports Physiol Perform*, *14*(2), pp.270-273.

Ireland, A., Korhonen, M., Heinonen, A., Suominen, H., Baur, C., Stevens, S., Degens, H. and Rittweger, J., 2011. Side-to-side differences in bone strength in master jumpers and sprinters. *Journal of Musculoskeletal and Neuronal Interactions*, *11*.

Izard, R.M., Fraser, W.D., Negus, C., Sale, C. and Greeves, J.P., 2016. Increased density and periosteal expansion of the tibia in young adult men following short-term arduous training. *Bone*, *88*, pp.13-19.

Jaasma, M.J. and O'Brien, F.J., 2008. Mechanical stimulation of osteoblasts using steady and dynamic fluid flow. *Tissue Engineering Part A*, *14*(7), pp.1213-1223.

Jaasma, M.J., Jackson, W.M., Tang, R.Y. and Keaveny, T.M., 2007. Adaptation of cellular mechanical behavior to mechanical loading for osteoblastic cells. *Journal of Biomechanics*, *40*(9), pp.1938-1945.

Jagodzinski, M., Drescher, M., Zeichen, J., Hankemeier, S., Krettek, C., Bosch, U. and Van Griensven, M., 2004. Effects of cyclic longitudinal mechanical strain and dexamethasone on osteogenic differentiation of human bone marrow stromal cells. *Eur Cell Mater*. *7*(35-41), p.41.

Jämsä, T., Vainionpää, A., Korpelainen, R., Vihriälä, E. and Leppäluoto, J., 2006. Effect of daily physical activity on proximal femur. *Clinical biomechanics*, *21*(1), pp.1-7.

Jansen, L.E., Birch, N.P., Schiffman, J.D., Crosby, A.J. and Peyton, S.R., 2015. Mechanics of intact bone marrow. *Journal of the mechanical behavior of biomedical materials*, *50*, pp.299-307.

Janz, K.F., Letuchy, E.M., Burns, T.L., Gilmore, J.M.E., Torner, J.C. and Levy, S.M., 2014. Objectively measured physical activity trajectories predict adolescent bone strength: Iowa Bone Development Study. *British journal of sports medicine*, *48*(13), pp.1032-1036.

Javaheri, B. and Pitsillides, A.A., 2019. Aging and mechanoadaptive responsiveness of bone. *Current osteoporosis reports*, *17*, pp.560-569.

Jennings, D., Cormack, S., Coutts, A.J., Boyd, L.J. and Aughey, R.J., 2010. Variability of GPS units for measuring distance in team sport movements. *International journal of sports physiology and performance*, *5*(4), pp.565-569.

Jensen, R.L. and Ebben, W.P., 2007. Quantifying plyometric intensity via rate of force development, knee joint, and ground reaction forces. *The Journal of Strength & Conditioning Research*, *21*(3), pp.763-767.

Johannsen, N., Binkley, T., Englert, V., Neiderauer, G. and Specker, B., 2003. Bone response to jumping is site-specific in children: a randomized trial. *Bone*, *33*(4), pp.533-539.

Johnstone, J.A., Mitchell, A.C., Hughes, G., Watson, T., Ford, P.A. and Garrett, A.T., 2014. The athletic profile of fast bowling in cricket: A review. *The Journal of Strength & Conditioning Research*, *28*(5), pp.1465-1473.

Kanis, J.A., Norton, N., Harvey, N.C., Jacobson, T., Johansson, H., Lorentzon, M., McCloskey, E.V., Willers, C. and Borgström, F., 2021. SCOPE 2021: a new scorecard for osteoporosis in Europe. *Archives of osteoporosis*, *16*(1), p.82.

Kanterewicz, E., Peris, P., Puigoriol, E., Yáñez, A., Rosique, P., Del Rio, L. and FRODOS Research Group, 2013. Distribution of serum β CTX in a population-based study of postmenopausal women taking into account different anti-osteoporotic therapies (the FRODOS Cohort). *Journal of bone and mineral metabolism*, *31*, pp.231-239.

Karsenty, G., Kronenberg, H.M. and Settembre, C., 2009. Genetic control of bone formation. *Annual Review of Cell and Developmental*, *25*, pp.629-648.

Kast, S., Shojaa, M., Kohl, M., von Stengel, S., Gosch, M., Jakob, F., Kerschan-Schindl, K., Kladny, B., Klöckner, N., Lange, U. and Middeldorf, S., 2022. Effects of different exercise intensity on bone mineral density in adults: a comparative systematic review and meta-analysis. *Osteoporosis International*, *33*(8), pp.1643-1657.

Kato, Y., Boskey, A., Spevak, L., Dallas, M., Hori, M. and Bonewald, L.F., 2001. Establishment of an osteoid preosteocyte-like cell MLO-A5 that spontaneously mineralizes in culture. *Journal of Bone and Mineral Research*, *16*(9), pp.1622-1633.

Kato, T., Terashima, T., Yamashita, T., Hatanaka, Y., Honda, A. and Umemura, Y., 2006. Effect of low-repetition jump training on bone mineral density in young women. *Journal of applied physiology*, *100*(3), pp.839-843.

Kavanagh, J.J. and Menz, H.B., 2008. Accelerometry: a technique for quantifying movement patterns during walking. *Gait & posture*, *28*(1), pp.1-15.

Kelley, S., Hopkinson, G., Strike, S., Luo, J. and Lee, R., 2014. An accelerometry-based approach to assess loading intensity of physical activity on bone. *Research quarterly for exercise and sport*, *85*(2), pp.245-250.

Kemmler, W. and von Stengel, S., 2013. Exercise frequency, health risk factors, and diseases of the elderly. *Archives of physical medicine and rehabilitation*, *94*(11), pp.2046-2053.

Kemmler, W. and Von Stengel, S., 2014. Dose–response effect of exercise frequency on bone mineral density in post-menopausal, osteopenic women. *Scandinavian Journal of Medicine & Science in Sports*, *24*(3), pp.526-534.

Kenkre, J.S. and Bassett, J.H.D., 2018. The bone remodelling cycle. *Annals of clinical biochemistry*, *55*(3), pp.308-327.

Kersh, M.E., Martelli, S., Zebaze, R., Seeman, E. and Pandy, M.G., 2018. Mechanical loading of the femoral neck in human locomotion. *Journal of Bone and Mineral Research*, *33*(11), pp.1999-2006.

Keylock, L., Alway, P., Felton, P., McCaig, S., Brooke-Wavell, K., King, M. and Peirce, N., 2022. Lumbar bone stress injuries and risk factors in adolescent cricket fast bowlers. *Journal of Sports Sciences*, *40*(12), pp.1336-1342.

Kim, S., Baker, B.S., Sharma-Ghimire, P., Bemben, D.A. and Bemben, M.G., 2018. Association between bone-specific physical activity scores and pQCT-derived measures of bone strength and geometry in healthy young and middle-aged premenopausal women. *Archives of Osteoporosis*, *13*, pp.1-9.

Kim, S.W., Seo, M.W., Jung, H.C. and Song, J.K., 2021. Effects of high-impact weight-bearing exercise on bone mineral density and bone metabolism in middle-aged premenopausal women: a randomized controlled trial. *Applied Sciences*, *11*(2), p.846.

Kirby, T.J., McBride, J.M., Haines, T.L. and Dayne, A.M., 2011. Relative net vertical impulse determines jumping performance. *Journal of applied biomechanics*, *27*(3), pp.207-214.

Kish, K., Mezil, Y., Ward, W.E., Klentrou, P. and Falk, B., 2015. Effects of plyometric exercise session on markers of bone turnover in boys and young men. *European journal of applied physiology*, *115*, pp.2115-2124.

Kistler-Fischbacher, M., Weeks, B.K. and Beck, B.R., 2021a. The effect of exercise intensity on bone in postmenopausal women (part 1): a systematic review. *Bone*, *143*, p.115696.

Kistler-Fischbacher, M., Weeks, B.K. and Beck, B.R., 2021b. The effect of exercise intensity on bone in postmenopausal women (part 2): a meta-analysis. *Bone*, *143*, p.115697.

Klein-Nulend, J., Bacabac, R.G. and Bakker, A.D., 2012. Mechanical loading and how it affects bone cells: the role of the osteocyte cytoskeleton in maintaining our skeleton. *Eur Cell Mater*, *24*(2), pp.279-291.

Klein-Nulend, J., van Oers, R.F., Bakker, A.D. and Bacabac, R.G., 2015. Bone cell mechanosensitivity, estrogen deficiency, and osteoporosis. *Journal of biomechanics*, *48*(5), pp.855-865.

Kleissen, R.F.M., Buurke, J.H., Harlaar, J. and Zilvold, G., 1998. Electromyography in the biomechanical analysis of human movement and its clinical application. *Gait & posture*, *8*(2), pp.143-158.

Kohrt, W.M., Ehsani, A.A. and Birge JR, S.J., 1997. Effects of exercise involving predominantly either joint-reaction or ground-reaction forces on bone mineral density in older women. *Journal of Bone and Mineral Research*, *12*(8), pp.1253-1261.

Kontulainen, S., Sievänen, H., Kannus, P., Pasanen, M. and Vuori, I., 2002. Effect of long-term impact-loading on mass, size, and estimated strength of humerus and radius of female racquet-sports players: a peripheral quantitative computed tomography study between young and old starters and controls. *Journal of Bone and Mineral Research*, *18*(2), pp.352-359.

Kontulainen, S.A., Johnston, J.D., Liu, D., Leung, C., Oxland, T.R. and McKay, H.A., 2008. Strength indices from pQCT imaging predict up to 85% of variance in bone failure properties at tibial epiphysis and diaphysis. *J Musculoskelet Neuronal Interact*, *8*(4), pp.401-9.

Korhonen, M.T., Suominen, H., Viitasalo, J.T., Liikavainio, T., Alen, M. and Mero, A.A., 2010. Variability and symmetry of force platform variables in maximum-speed running in young and older athletes. *Journal of Applied Biomechanics*, *26*(3), pp.357-366.

Kotha, S.P. and Guzelsu, N., 2003. Tensile damage and its effects on cortical bone. *Journal of Biomechanics*, *36*(11), pp.1683-1689.

Kouvelioti, R., Kurgan, N., Falk, B., Ward, W.E., Josse, A.R. and Klentrou, P., 2018. Response of sclerostin and bone turnover markers to high intensity interval exercise in young women: does impact matter?. *BioMed research international*, *2018*.

Krahl, H., Michaelis, U., Pieper, H.G., Quack, G. and Montag, M., 1994. Stimulation of bone growth through sports: A radiologic investigation of the upper extremities in professional tennis players. *The American Journal of Sports Medicine*, *22*(6), pp.751-57.

Lafortune, M.A. and Hennig, E.M., 1991. Contribution of angular motion and gravity to tibial acceleration. *Medicine and science in sports and exercise*, *23*(3), pp.360-363.

Lalayiannis, A.D., Fewtrell, M., Biassoni, L., Silva, S., Goodman, N., Shroff, R. and Crabtree, N.J., 2021. Studying bone mineral density in young people: The complexity of choosing a pQCT reference database. *Bone*, *143*, p.115713.

Lambert, C., Beck, B.R., Harding, A.T., Watson, S.L. and Weeks, B.K., 2020. Regional changes in indices of bone strength of upper and lower limbs in response to high-intensity impact loading or high-intensity resistance training. *Bone*, *132*, p.115192.

LaMothe, J.M. and Zernicke, R.F., 2004. Rest insertion combined with high-frequency loading enhances osteogenesis. *Journal of Applied Physiology*, *96*(5), pp.1788-1793.

Laughton, CA., Davis, IM., Hamill, J., 2003. Effect of strike pattern and orthotic intervention on tibial shock during running. *J Applied Biomech*, 19(2):153-168.

Leardini, A., Benedetti, M.G., Berti, L., Bettinelli, D., Nativo, R. and Giannini, S., 2007. Rear-foot, mid-foot and fore-foot motion during the stance phase of gait. *Gait & posture*, *25*(3), pp.453-462.

Lees, M.J., Beggs, C.B., Barlow, M.J., Rutherford, Z.H., Bansil, K., Gannon, L. and Hind, K., 2018. Bone density and cross-sectional geometry of the proximal femur are bilaterally elevated in elite cricket fast bowlers. *Journal of Clinical Densitometry. 21*(3), pp.399-405.

Lehmann, L.A., Alvarez, R.E., Macovski, A., Brody, W.R., Pelc, N.J., Riederer, S.J. and Hall, A.L., 1981. Generalized image combinations in dual KVP digital radiography. *Medical physics*, *8*(5), pp.659-667.

Li, S., Demirci, E. and Silberschmidt, V.V., 2013. Variability and anisotropy of mechanical behavior of cortical bone in tension and compression. *Journal of the mechanical behavior of biomedical materials*, *21*, pp.109-120.

Lim, Y.Y., Sterzing, T., Teo, C.J., Alonzo, R., Pan, J.W., Teng, P.S. and Kong, P.W., 2020. Between-limb asymmetry in kinetic and temporal characteristics during bilateral plyometric drop jumps from different heights. *Journal of Sports Sciences*, *38*(14), pp.1605-1614.

Lin, J.T. and Lane, J.M., 2004. Osteoporosis: a review. *Clinical Orthopaedics and Related Research*, *425*, pp.126-134.

Lin, C.F., Huang, T.H., Tu, K.C., Lin, L.L., Tu, Y.H. and Yang, R.S., 2012. Acute effects of plyometric jumping and intermittent running on serum bone markers in young males. *European journal of applied physiology*, *112*, pp.1475-1484.

List, R., Gülay, T., Stoop, M. and Lorenzetti, S., 2013. Kinematics of the trunk and the lower extremities during restricted and unrestricted squats. *The Journal of Strength & Conditioning Research*, *27*(6), pp.1529-1538.

Liu, L., Maruno, R., Mashimo, T., Sanka, K., Higuchi, T., Hayashi, K., Shirasaki, Y., Mukai, N., Saitoh, S. and Tokuyama, K., 2003. Effects of physical training on cortical bone at midtibia assessed by peripheral QCT. *Journal of Applied Physiology*, *95*(1), pp.219-224.

Lodder, M., Lems, W., Ader, H., Marthinsen, A., Van Coeverden, S., Lips, P., Netelenbos, J., Dijkmans, B. and Roos, J., 2004. Reproducibility of bone mineral density measurement in daily practice. *Annals of the rheumatic diseases*, *63*(3), pp.285-289.

Lombard W, Starling L, Wewege L, Lambert M., 2020. Changes in countermovement jump performance and subjective readiness-to-train scores following a simulated soccer match. *Euro J Sport Sci*, 21(5):647-55.

Lombard, W., Reid, S., Pearson, K. and Lambert, M., 2017. Reliability of metrics associated with a counter-movement jump performed on a force plate. *Measurement in Physical Education and Exercise Science*, *21*(4), pp.235-243.

López-Carrizosa, M.C., Samper-Ots, P.M. and Pérez, A.R., 2010. Serum C-telopeptide levels predict the incidence of skeletal-related events in cancer patients with secondary bone metastases. *Clinical and Translational Oncology*, *12*, pp.568-573.

Löppönen, A., Karavirta, L., Portegijs, E., Koivunen, K., Rantanen, T., Finni, T., Delecluse, C., Roie, E.V. and Rantalainen, T., 2021. Day-to-Day Variability and Year-to-Year Reproducibility of Accelerometer-Measured Free-Living Sit-to-Stand Transitions Volume and Intensity among Community-Dwelling Older Adults. *Sensors*, *21*(18), p.6068.

Loundagin LL, Schmidt TA, Edwards WB., 2018. Mechanical fatigue of bovine cortical bone using ground reaction force waveforms in running. *J Biomech Eng, 140*(3):031003.

Lovell, R. and Abt, G., 2013. Individualization of time–motion analysis: a case-cohort example. *International journal of sports physiology and performance*, *8*(4), pp.456-458.

Lucas-Cuevas, A.G., Encarnación-Martínez, A., Camacho-García, A., Llana-Belloch, S. and Pérez-Soriano, P., 2017. The location of the tibial accelerometer does influence impact acceleration parameters during running. *Journal of sports sciences*, *35*(17), pp.1734-1738.

Macdonald, H., Kontulainen, S., Petit, M., Janssen, P., McKay, H., 2006. Bone strength and its determinants in pre-and early pubertal boys and girls. *Bone*, 39(3):598-608.

Macdonald, H.M., Kontulainen, S.A., Khan, K.M. and McKay, H.A., 2007. Is a school-based physical activity intervention effective for increasing tibial bone strength in boys and girls?. *Journal of Bone and Mineral Research*, *22*(3), pp.434-446.

MacKelvie, K.J., Khan, K.M. and McKay, H.A., 2002. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. *British journal of sports medicine*, *36*(4), pp.250-257.

MacKelvie, K.J., McKay, H.A., Petit, M.A., Moran, O. and Khan, K.M., 2002. Bone mineral response to a 7-month randomized controlled, school-based jumping intervention in 121 prepubertal boys: associations with ethnicity and body mass index. *Journal of Bone and Mineral Research*, *17*(5), pp.834-844.

Mackie, E.J., 2003. Osteoblasts: novel roles in orchestration of skeletal architecture. *The international journal of biochemistry & cell biology*, *35*(9), pp.1301-1305.

Maïmoun, L., Coste, O., Philibert, P., Briot, K., Mura, T., Galtier, F., Mariano-Goulart, D., Paris, F. and Sultan, C., 2013. Peripubertal female athletes in high-impact sports show improved bone mass acquisition and bone geometry. *Metabolism*, *62*(8), pp.1088-1098.

Makaruk, H. and Sacewicz, T., 2011. The effect of drop height and body mass on drop jump intensity. *Biology of Sport*, *28*(1), p.63.

Malisoux, L., Gette, P., Urhausen, A., Bomfim, J. and Theisen, D., 2017. Influence of sports flooring and shoes on impact forces and performance during jump tasks. *PloS one*, *12*(10), p.0186297.

Malone, JJ., Lovell, R., Varley, MC., Coutts, AJ., 2017. Unpacking the black box: applications and considerations for using GPS devices in sport. *Int J of Sports Phys & Perform*, 12(2):S2-18.

Marin-Puyalto, J., Mäestu, J., Gómez-Cabello, A., Lätt, E., Remmel, L., Purge, P., Vicente-Rodríguez, G. and Jürimäe, J., 2019. Frequency and duration of vigorous physical activity bouts are associated with adolescent boys' bone mineral status: A cross-sectional study. *Bone*, *120*, pp.141-147.

Martelli, S., Beck, B., Saxby, D., Lloyd, D., Pivonka, P. and Taylor, M., 2020. Modelling human locomotion to inform exercise prescription for osteoporosis. *Current Osteoporosis Reports*, *18*, pp.301-311.

Matijevich, E.S., Scott, L.R., Volgyesi, P., Derry, K.H. and Zelik, K.E., 2020. Combining wearable sensor signals, machine learning and biomechanics to estimate tibial bone force and damage during running. *Human Movement Science*, *74*, p.102690.

Matijevich, ES., Branscombe, LM., Scott, LR., and Zelik, KE., 2019. Ground reaction force metrics are not strongly correlated with tibial bone load when running across speeds and slopes: Implications for science, sport and wearable tech. *PloS one*, 14(1):0210000.

McGrath, J., Neville, J., Stewart, T., Clinning, H. and Cronin, J., 2021. Can an inertial measurement unit (IMU) in combination with machine learning measure fast bowling speed and perceived intensity in cricket?. *Journal of Sports Sciences*, *39*(12), pp.1402-09.

McKay, H.A., MacLean, L., Petit, M., MacKelvie-O'Brien, K., Janssen, P., Beck, T. and Khan, K.M., 2005. "Bounce at the Bell": a novel program of short bouts of exercise improves proximal femur bone mass in early pubertal children. *British journal of sports medicine*, *39*(8), pp.521-526.

McNerny, E.M., Gardinier, J.D. and Kohn, D.H., 2015. Exercise increases pyridinoline cross-linking and counters the mechanical effects of concurrent lathyrogenic treatment. *Bone*, *81*, pp.327-337.

Meakin, L.B., Sugiyama, T., Galea, G.L., Browne, W.J., Lanyon, L.E. and Price, J.S., 2013. Male mice housed in groups engage in frequent fighting and show a lower response to additional bone loading than females or individually housed males that do not fight. *Bone*, *54*(1), pp.113-117.

Meardon, SA., Derrick, TR., Willson, JD., Baggaley, M., Steinbaker, CR., Marshall, M., and Willy, RW., 2021. Peak and Per-Step Tibial Bone Stress During Walking and Running in Female and Male Recreational Runners. *American J Sports Med*, 49(8):2227-37.

Meijer, K., Dethmers, J., Savelberg, H.H., Willems, P. and Wijers, B., 2006. The influence of third generation artificial soccer turf characteristics on ground reaction forces during running. In *ISBS-Conference Proceedings Archive*.

Mentiplay, B.F. and Clark, R.A., 2018. Modified conventional gait model versus cluster tracking: Test-retest reliability, agreement and impact of inverse kinematics with joint constraints on kinematic and kinetic data. *Gait & posture*, *64*, pp.75-83.

Mercer, J.A., Bates, B.T., Dufek, J.S. and Hreljac, A., 2003. Characteristics of shock attenuation during fatigued running. *Journal of Sports Science*, *21*(11), pp.911-919.

Meslier, Q.A. and Shefelbine, S.J., 2023. Using Finite Element Modeling in Bone Mechanoadaptation. *Current Osteoporosis Reports*, *21*(2), pp.105-116.

Messina, C., Albano, D., Gitto, S., Tofanelli, L., Bazzocchi, A., Ulivieri, F.M., Guglielmi, G. and Sconfienza, L.M., 2020. Body composition with dual energy X-ray absorptiometry: from basics to new tools. *Quantitative Imaging in Medicine and Surgery*, *10*(8), p.1687.

Milanović, Z., Čović, N., Helge, E.W., Krustrup, P. and Mohr, M., 2022. Recreational football and bone health: A systematic review and meta-analysis. *Sports Medicine*, *52*(12), pp.3021-3037.

Milgrom, C., Voloshin, A., Novack, L., Milgrom, Y., Ekenman, I. and Finestone, A.S., 2022. In vivo strains at the middle and distal thirds of the tibia during exertional activities. *Bone Reports*, *16*, p.101170.

Milner, CE., Ferber, R., Pollard, CD., Hamill, J., and Davis, IS., 2006. Biomechanical factors associated with tibial stress fracture in female runners. *Med & Sci in Sport & Ex*, 38(2):323.

Mittag, U., Kriechbaumer, A. and Rittweger, J., 2018. Torsion-an underestimated form shaping entity in bone adaptation?. *Journal of Musculoskeletal & Neuronal Interactions*, *18*(4), p.407.

Moir, G.L., 2008. Three different methods of calculating vertical jump height from force platform data in men and women. *Measurement in Physical Education and Exercise Science*, *12*(4), pp.207-218.

Moisio, K.C., Hurwitz, D.E. and Sumner, D.R., 2004. Dynamic loads are determinants of peak bone mass. *Journal of Orthopaedic Research*, *22*(2), pp.339-345.

Montgomery, G., Abt, G., Dobson, C., Smith, T., Evans, W. and Ditroilo, M., 2019. The mechanical loading and muscle activation of four common exercises used in osteoporosis prevention for early postmenopausal women. *Journal of Electromyography and Kinesiology*, *44*, pp.124-131.

Mooney, M., O'Brien, B., Cormack, S., Coutts, A., Berry, J., and Young, W., 2011. The relationship between physical capacity and match performance in elite Australian football: a mediation approach. *J Science and Med in Sport*, 14(5):447-52.

Moore, I.S. and Willy, R.W., 2019. Use of wearables: tracking and retraining in endurance runners. *Current Sports Medicine Reports*, *18*(12), pp.437-444.

Moreira, C.A. and Bilezikian, J.P., 2017. Stress fractures: concepts and therapeutics. *The Journal of Clinical Endocrinology & Metabolism*, *102*(2), pp.525-534.

Morel, J., Combe, B., Francisco, J. and Bernard, J., 2001. Bone mineral density of 704 amateur sportsmen involved in different physical activities. *Osteoporosis International*, *12*, pp.152-157.

Morgan, S.L. and Prater, G.L., 2017. Quality in dual-energy X-ray absorptiometry scans. *Bone*, *104*, pp.13-28.

Mosti MP, Carlse T, Aas E, Hoff J, Stunes AK, Syversen U., 2014 Maximal strength training improves bone mineral density and neuromuscular performance in young adult women. *J Strength & Con Research,* 28(10):2935-45.

Murray, D.W. and Rushton, N., 1990. The effect of strain on bone cell prostaglandin E 2 release: a new experimental method. *Calcified tissue international*, *47*, pp.35-39.

Narisawa, S., Fröhlander, N. and Millán, J.L., 1997. Inactivation of two mouse alkaline phosphatase genes and establishment of a model of infantile hypophosphatasia. *Developmental dynamics: an official publication of the American Association of Anatomists*, *208*(3), pp.432-446.

Nedergaard, N.J., Robinson, M.A., Eusterwiemann, E., Drust, B., Lisboa, P.J. and Vanrenterghem, J., 2017. The relationship between whole-body external loading and body-worn accelerometry during team-sport movements. *International journal of sports physiology and performance*, *12*(1), pp.18-26.

Newton, MD., Owen, AD., and Baker, JS., 2019. Monitoring External and Internal Training Loads: Relationships with Injury Risk in Professional Soccer: A Review. *EC Orthopaedics,* 10(8):686-97.

Nichols, J.F., Rauh, M.J., Barrack, M.T. and Barkai, H.S., 2007. Bone mineral density in female high school athletes: interactions of menstrual function and type of mechanical loading. *Bone*, *41*(3), pp.371-377.

Niinimäki, S., Narra, N., Härkönen, L., Abe, S., Nikander, R., Hyttinen, J., Knüsel, C. and Sievänen, H., 2017. The relationship between loading history and proximal femoral diaphysis cross-sectional geometry. *American Journal of Human Biology*, *29*(4), p.22965.

Nikander, R., Sievänen, H., Heinonen, A., Daly, R.M., Uusi-Rasi, K. and Kannus, P., 2010. Targeted exercise against osteoporosis: a systematic review and meta-analysis for optimising bone strength throughout life. *BMC medicine*, *8*(1), p.47.

Nikander, R., Sievanen, H., Ojala, K., Kellokumpu-Lehtinen, P.L., Palva, T., Blomqvist, C., Luoto, R. and Saarto, T., 2012. Effect of exercise on bone structural traits, physical performance and body composition in breast cancer patients--a 12-month RCT. *J Musculoskelet Neuronal Interact*, *12*(3), pp.127-135.

Nikander, R., Sievänen, H., Uusi-Rasi, K., Heinonen, A. and Kannus, P., 2006. Loading modalities and bone structures at nonweight-bearing upper extremity and weight-bearing lower extremity: a pQCT study of adult female athletes. *Bone*, *39*(4), pp.886-894.

Nilsson, M., Ohlsson, C., Mellström, D. and Lorentzon, M., 2013. Sport-specific association between exercise loading and the density, geometry, and microstructure of weight-bearing bone in young adult men. *Osteoporosis International*, *24*, pp.1613-1622.

Nogueira, R.C., Weeks, B.K. and Beck, B.R., 2014. An in-school exercise intervention to enhance bone and reduce fat in girls: The CAPO Kids trial. *Bone*, *68*, pp.92-99.

O'Brien, C.A., Plotkin, L.I., Galli, C., Goellner, J.J., Gortazar, A.R., Allen, M.R., Robling, A.G., Bouxsein, M., Schipani, E., Turner, C.H. and Jilka, R.L., 2008. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. *PloS one*, *3*(8), p.e2942.

Orchard, J.W., James, T., Portus, M., Kountouris, A. and Dennis, R., 2009. Fast bowlers in cricket demonstrate up to 3-to 4-week delay between high workloads and increased risk of injury. *The American journal of sports medicine*, *37*(6), pp.1186-1192.

Orr, A.W., Helmke, B.P., Blackman, B.R. and Schwartz, M.A., 2006. Mechanisms of mechanotransduction. *Developmental cell*, *10*(1), pp.11-20.

Ottewell, P.D., 2016. The role of osteoblasts in bone metastasis. *Journal of bone oncology*, *5*(3), pp.124-127.

Park, S.G., Jeong, S.U., Lee, J.H., Ryu, S.H., Jeong, H.J., Sim, Y.J., Kim, D.K. and Kim, G.C., 2018. The changes of CTX, DPD, osteocalcin, and bone mineral density during the postmenopausal period. *Annals of rehabilitation medicine*, *42*(3), pp.441-448.

Parry, D.A., 1988. The molecular fibrillar structure of collagen and its relationship to the mechanical properties of connective tissue. *Biophysical chemistry*, *29*(1-2), pp.195-209.

Patel, R., Blake, G.M., Rymer, J. and Fogelman, I., 2000. Long-term precision of DXA scanning assessed over seven years in forty postmenopausal women. *Osteoporosis international*, *11*, pp.68-75.

Pead, M.J. and Lanyon, L.E., 1990. Adaptive remodeling in bone: Torsion versus compression. *Trans. Orthopaed. Res. Soc*, *15*, p.104.

Peng, H.T., 2011. Changes in biomechanical properties during drop jumps of incremental height. *The Journal of Strength & Conditioning Research*, *25*(9), pp.2510-2518.

Pennington, Z., Ehresman, J., Lubelski, D., Cottrill, E., Schilling, A., Ahmed, A.K., Feghali, J., Witham, T.F. and Sciubba, D.M., 2021. Assessing underlying bone quality in spine surgery patients: a narrative review of dual-energy X-ray absorptiometry (DXA) and alternatives. *The Spine Journal*, *21*(2), pp.321-331.

Pereira, A.F., Javaheri, B., Pitsillides, A.A. and Shefelbine, S.J., 2015. Predicting cortical bone adaptation to axial loading in the mouse tibia. *Journal of the Royal Society Interface*, *12*(110), p.20150590.

Pinho, J.P., Forner-Cordero, A., Pereira, R.M.R., Hernandez, A.J., Dórea, E.L., Mezêncio, B., Takayama, L., Alvarenga, J.C., Serrão, J.C. and Amadio, A.C., 2020. A High-intensity

exercise intervention improves older women lumbar spine and distal tibia bone microstructure and function: a 20-week randomized controlled trial. *IEEE Journal of Translational Engineering in Health and Medicine*, *8*, pp.1-8.

Pino-Ortega, J., Rojas-Valverde, D., Gómez-Carmona, CD., Bastida-Castillo, A., Hernández-Belmonte, A., García-Rubio, J., Nakamura, FY., and Ibáñez, SJ., 2019. Impact of contextual factors on external load during a congested-fixture tournament in elite U'18 basketball players. *Frontiers Psych*, 10:1100.

Plunkett, N.A., Partap, S. and O'Brien, F.J., 2010. Osteoblast response to rest periods during bioreactor culture of collagen–glycosaminoglycan scaffolds. *Tissue Engineering Part A*, *16*(3), pp.943-951.

Prawiradilaga, R., Madsen, A., Jørgensen, N. and Helge, E., 2020. Acute response of biochemical bone turnover markers and the associated ground reaction forces to high-impact exercise in postmenopausal women. *Biology of sport*, *37*(1), pp.41-48.

Quarles, L.D., Yohay, D.A., Lever, L.W., Caton, R. and Wenstrup, R.J., 1992. Distinct proliferative and differentiated stages of murine MC3T3-E1 cells in culture: An in vitro model of osteoblast development. *Journal of Bone and Mineral Research*, *7*(6), pp.683-692.
Raab-Cullen, D.M., Thiede, M.A., Petersen, D.N., Kimmel, D.B. and Recker, R.R., 1994. Mechanical loading stimulates rapid changes in periosteal gene expression. *Calcified tissue international*, *55*, pp.473-478.

Rantalainen, T., Nikander, R., Daly, R.M., Heinonen, A. and Sievänen, H., 2011. Exercise loading and cortical bone distribution at the tibial shaft. *Bone*, *48*(4), pp.786-791.

Rantalainen T, Heinonen A, Komi PV, Linnamo V., 2008. Neuromuscular performance and bone structural characteristics in young healthy men and women. *Euro J Applied Phys*, 102(2):215-22.

Rantalainen T, Nikander R, Heinonen A, Multanen J, Häkkinen A, Jämsä T, Kiviranta I, Linnamo V, Komi PV, Sievänen H., 2010. Neuromuscular performance and body mass as indices of bone loading in premenopausal and postmenopausal women. *Bone*,46(4):964-69.

Rantalainen, T., Heinonen, A., Linnamo, V., Komi, P.V., Takala, T.E. and Kainulainen, H., 2009. Short-term bone biochemical response to a single bout of high-impact exercise. *Journal of sports science & medicine*, *8*(4), p.553.

Raper, D.P., Witchalls, J., Philips, E.J., Knight, E., Drew, M.K. and Waddington, G., 2018. Use of a tibial accelerometer to measure ground reaction force in running: A reliability and validity comparison with force plates. *Journal of science and medicine in sport*, *21*(1), pp.84-88.

Ribeiro, N.F. and Santos, C.P., 2017. Inertial measurement units: A brief state of the art on gait analysis. In *2017 IEEE 5th Portuguese Meeting on Bioengineering.* pp. 1-4.

Rinaldi, G., Wisniewski, C.A., Setty, N.G. and LeBoff, M.S., 2011. Peripheral quantitative computed tomography: optimization of reproducibility measures of bone density, geometry, and strength at the radius and tibia. *Journal of Clinical Densitometry*, *14*(3), pp.367-373.

Rittweger, J., Simunic, B., Bilancio, G., De Santo, N.G., Cirillo, M., Biolo, G., Pisot, R., Eiken, O., Mekjavic, I.B. and Narici, M., 2009. Bone loss in the lower leg during 35 days of bed rest is predominantly from the cortical compartment. *Bone*, *44*(4), pp.612-618.

Robertson, D.G.E. and Dowling, J.J., 2003. Design and responses of Butterworth and critically damped digital filters. *Journal of Electromyography and Kinesiology*, *13*(6), pp.569-573.

Robling, A.G., Burr, D.B. and Turner, C.H., 2001. Recovery periods restore mechanosensitivity to dynamically loaded bone. *Journal of Experimental Biology*, *204*(19), pp.3389-3399.

Robling, A.G., Castillo, A.B. and Turner, C.H., 2006. Biomechanical and molecular regulation of bone remodeling. *Annu. Rev. Biomed. Eng.*, *8*, pp.455-498.

Robling, A.G., Daly, R., Fuchs, R.K. and Burr, D.B., 2019. Mechanical adaptation. In *Basic and applied bone biology,* pp. 203-233.

Robling, A.G., Hinant, F.M., Burr, D.B. and Turner, C.H., 2002. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *Journal of Bone and Mineral Research*, *17*(8), pp.1545-1554.

Rodríguez-Rosell, D., Pareja-Blanco, F., Aagaard, P. and González-Badillo, J.J., 2018. Physiological and methodological aspects of rate of force development assessment in human skeletal muscle. *Clinical physiology and functional imaging*, *38*(5), pp.743-762.

Rogers, RS., and Hinton, PS., 2010. Bone loading during young adulthood predicts bone mineral density in physically active, middle-aged men. *The Physician and Sportsmedicine*, 38(2):146-55.

Rojas-Valverde, D., Gómez-Carmona, CD., Gutiérrez-Vargas, R., and Pino-Ortega, J., 2019. From big data mining to technical sport reports: The case of inertial measurement units. *BMJ Open Sport & Ex Med*, 5(1):000565.

Rosen, H.N., Tollin, S., Balena, R., Middlebrooks, V.L., Beamer, W.G., Donohue, L.R., Rosen, C., Turner, A., Holick, M. and Greenspan, S.L., 1995. Differentiating between orchiectomized rats and controls using measurements of trabecular bone density: a comparison among DXA, histomorphometry, and peripheral quantitative computerized tomography. *Calcified tissue international*, *57*, pp.35-39.

Rowlands, A.V., Edwardson, C.L., Dawkins, N.P., Maylor, B.D., Metcalf, K.M. and Janz, K.F., 2020. Physical activity for bone health: how much and/or how hard?. *Medicine & Science in Sports & Exercise*, *52*(11), pp.2331-2341.

Rubin, C., Gross, T., Qin, Y.X., Fritton, S., Guilak, F. and McLeod, K., 1996. Differentiation of the bone-tissue remodeling response to axial and torsional loading in the turkey ulna. *JBJS*, *78*(10), pp.1523-33.

Rubin, C.T. and Lanyon, L.E., 1985. Regulation of bone mass by mechanical strain magnitude. *Calcified tissue international*, *37*, pp.411-417.

Ruff C., 2003. Growth in bone strength, body size, and muscle size in a juvenile longitudinal sample. *Bone*, 33(3):317-29.

Ryan, A.S. and Elahi, D., 1998. Loss of bone mineral density in women athletes during aging. *Calcified tissue international*, *63*, pp.287-292.

Salari, N., Ghasemi, H., Mohammadi, L., Behzadi, M.H., Rabieenia, E., Shohaimi, S. and Mohammadi, M., 2021. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. *Journal of orthopaedic surgery and research*, *16*, pp.1-20.

Salazar, V.S., Gamer, L.W. and Rosen, V., 2016. BMP signalling in skeletal development, disease and repair. *Nature Reviews Endocrinology*, *12*(4), pp.203-221.

Santos, A., Bakker, A.D. and Klein-Nulend, J., 2009. The role of osteocytes in bone mechanotransduction. *Osteoporosis international*, *20*, pp.1027-1031.

Santos, L., Elliott-Sale, K.J. and Sale, C., 2017. Exercise and bone health across the lifespan. *Biogerontology*, *18*, pp.931-946.

Sasaki, J.E., da Silva, K.S., da Costa, B.G.G. and John, D., 2016. Measurement of physical activity using accelerometers. In *Computer-assisted and web-based innovations in psychology, special education, and health*, pp. 33-60.

Sayers, A., Mattocks, C., Deere, K., Ness, A., Riddoch, C. and Tobias, J.H., 2011. Habitual levels of vigorous, but not moderate or light, physical activity is positively related to cortical bone mass in adolescents. *The Journal of Clinical Endocrinology & Metabolism*, *96*(5), pp.E793-E802.

Schaffler, M.B., Cheung, W.Y., Majeska, R. and Kennedy, O., 2014. Osteocytes: master orchestrators of bone. *Calcified tissue international*, *94*, pp.5-24.

Schelling X, Torres L., 2016. Accelerometer load profiles for basketball-specific drills in elite players. *J Sports Sci & Med*, 15(4):585.

Schmidt, M., Rheinländer, C., Nolte, K.F., Wille, S., Wehn, N. and Jaitner, T., 2016. IMU-based determination of stance duration during sprinting. *Procedia engineering*, *147*, pp.747-52.

Schoenau E., 2005. From mechanostat theory to development of the" Functional Muscle-Bone-Unit". *J Musculoskeletal & Neuronal Interactions*, 5(3):232.

Scott, J.P., Sale, C., Greeves, J.P., Casey, A., Dutton, J. and Fraser, W.D., 2011. The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise. *Journal of Applied Physiology*, *110*(2), pp.423-432.

Seabra, A., Fernandes, R.J., Marques, E., Moura, M., Ubago-Guisado, E., Hernando, E. and Gallardo, L., 2017. Impact of futsal and swimming participation on bone health in young athletes. *Journal of human kinetics*, *60*(1), pp.85-91.

Senington, B., Lee, R.Y. and Williams, J.M., 2020. Biomechanical risk factors of lower back pain in cricket fast bowlers using inertial measurement units: a prospective and retrospective investigation. *BMJ open sport & exercise medicine*, *6*(1), p.000818.

Serguienko, A., Wang, M.Y. and Myklebost, O., 2018. Real-time vital mineralization detection and quantification during in vitro osteoblast differentiation. *Biological procedures online*, *20*, pp.1-5.

Sheerin, K., Besier, T., Reid, D. and Hume, P., 2016. The reliability and variability of three-dimensional tibial acceleration during running.

Sheerin, K.R., Reid, D. and Besier, T.F., 2019. The measurement of tibial acceleration in runners—A review of the factors that can affect tibial acceleration during running and evidence-based guidelines for its use. *Gait & posture*, *67*, pp.12-24.

Shipov, A., Sharir, A., Zelzer, E., Milgram, J., Monsonego-Ornan, E. and Shahar, R., 2010. The influence of severe prolonged exercise restriction on the mechanical and structural properties of bone in an avian model. *The Veterinary Journal*, *183*(2), pp.153-160.

Sievänen, H., Koskue, V., Rauhio, A., Kannus, P., Heinonen, A. and Vuori, I., 1998. Peripheral quantitative computed tomography in human long bones: evaluation of in vitro and in vivo precision. *Journal of bone and mineral research*, *13*(5), pp.871-882.

Simoes, D., Craveiro, V., Santos, M.P., Camões, M., Pires, B. and Ramos, E., 2021. The effect of impact exercise on bone mineral density: A longitudinal study on non-athlete adolescents. *Bone*, *153*, p.116151.

Sirard, J.R., Forsyth, A., Oakes, J.M. and Schmitz, K.H., 2011. Accelerometer test-retest reliability by data processing algorithms: results from the Twin Cities Walking Study. *Journal of Physical Activity and Health*, *8*(5), pp.668-674.

Sittichokechaiwut, A., Edwards, J.H., Scutt, A.M. and Reilly, G.C., 2010. Short bouts of mechanical loading are as effective as dexamethasone at inducing matrix production by human bone marrow mesenchymal stem cell. *Eur Cell Mater*, *20*(4).

Söderman, K., Bergström, E., Lorentzon, R. and Alfredson, H., 2000. Bone mass and muscle strength in young female soccer players. *Calcified tissue international*, *67*, pp.297-303.

Srinivasan, S., Agans, S.C., King, K.A., Moy, N.Y., Poliachik, S.L. and Gross, T.S., 2003. Enabling bone formation in the aged skeleton via rest-inserted mechanical loading. *Bone*, *33*(6), pp.946-955.

Srinivasan, S., Ausk, B.J., Poliachik, S.L., Warner, S.E., Richardson, T.S. and Gross, T.S., 2007. Rest-inserted loading rapidly amplifies the response of bone to small increases in strain and load cycles. *Journal of applied physiology*, *102*(5), pp.1945-1952.

Srinivasan, S., Weimer, D.A., Agans, S.C., Bain, S.D. and Gross, T.S., 2002. Low-magnitude mechanical loading becomes osteogenic when rest is inserted between each load cycle. *Journal of Bone and Mineral Research*, *17*(9), pp.1613-1620.

Stagi, S., Cavalli, L., Cavalli, T., de Martino, M. and Brandi, M.L., 2016. Peripheral quantitative computed tomography (pQCT) for the assessment of bone strength in most of bone affecting conditions in developmental age: a review. *Italian journal of pediatrics*, *42*, pp.1-20.

Stief, F., Böhm, H., Michel, K., Schwirtz, A. and Döderlein, L., 2013. Reliability and accuracy in three-dimensional gait analysis: a comparison of two lower body protocols. *Journal of applied biomechanics*, *29*(1), pp.105-111.

Stiles, V.H., Griew, P.J. and Rowlands, A.V., 2013. Use of accelerometry to classify activity beneficial to bone in premenopausal women. *Medicine and science in sports and exercise*, *45*(12), pp.2353-2361.

Strope, M.A., Nigh, P., Carter, M.I., Lin, N., Jiang, J. and Hinton, P.S., 2015. Physical activity–associated bone loading during adolescence and young adulthood is positively

associated with adult bone mineral density in men. *American journal of men's health*, *9*(6), pp.442-450.

Sugiyama, T., Yamaguchi, A. and Kawai, S., 2002. Effects of skeletal loading on bone mass and compensation mechanism in bone: a new insight into the “mechanostat” theory. *Journal of bone and mineral metabolism*, *20*, pp.196-200.

Siu, W.S., Qin, L. and Leung, K.S., 2003. pQCT bone strength index may serve as a better predictor than bone mineral density for long bone breaking strength. *Journal of bone and mineral metabolism*, *21*, pp.316-322.

Sundh, D., Nilsson, M., Zoulakis, M., Pasco, C., Yilmaz, M., Kazakia, G.J., Hellgren, M. and Lorentzon, M., 2018. High-impact mechanical loading increases bone material strength in postmenopausal women—a 3-month intervention study. *Journal of Bone and Mineral Research*, *33*(7), pp.1242-1251.

Szabo, K.A., Webber, C.E., Gordon, C., Adachi, J.D., Tozer, R. and Papaioannou, A., 2011. Reproducibility of peripheral quantitative computed tomography measurements at the radius and tibia in healthy pre-and postmenopausal women. *Canadian Association of Radiologists Journal*, *62*(3), pp.183-189.

Szulc, P., 2018. Bone turnover: Biology and assessment tools. *Best Practice & Research Clinical Endocrinology & Metabolism*, *32*(5), pp.725-738.

Szulc, P., Naylor, K., Hoyle, N.R., Eastell, R., Leary, E.T., 2017. Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. *Osteoporosis International*, *28*, pp.2541-2556.

Tagliaferri, C., Wittrant, Y., Davicco, M.J., Walrand, S. and Coxam, V., 2015. Muscle and bone, two interconnected tissues. *Ageing research reviews*, *21*, pp.55-70.

Takayanagi, H., Kim, S., Koga, T., Nishina, H., Isshiki, M., Yoshida, H., Saiura, A., Isobe, M., Yokochi, T., Inoue, J.I. and Wagner, E.F., 2002. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Developmental cell*, *3*(6), pp.889-901.

Tan, T., Chiasson, DP., Hu, H., and Shull, PB., 2019. Influence of IMU position and orientation placement errors on ground reaction force estimation. *J Biomech*, 97:109416.

Teitelbaum, S.L., 2007. Osteoclasts: what do they do and how do they do it?. *The American journal of pathology*, *170*(2), pp.427-435.

Tenforde, A.S., Carlson, J.L., Sainani, K.L., Chang, A.O., Kim, J.H., Golden, N.H. and Fredericson, M., 2018. Sport and triad risk factors influence bone mineral density in collegiate athletes. *Med Sci Sports Exerc*, *50*(12), pp.2536-43.

Tervo, T., Nordstrom, P., Neovius, M. and Nordstrom, A., 2008. Constant adaptation of bone to current physical activity level in men: a 12-year longitudinal study. *The Journal of Clinical Endocrinology & Metabolism*, *93*(12), pp.4873-4879.

Theodoropoulos, JS., Bettle, J., and Kosy, JD., 2020. The use of GPS and inertial devices for player monitoring in team sports: A review of current and future applications. *Ortho reviews*, 12(1):7863.

Thomas, C., Comfort, P., Chiang, C.Y. and Jones, P.A., 2015. Relationship between isometric mid-thigh pull variables and sprint and change of direction performance in collegiate athletes. *Journal of trainology*, *4*(1), pp.6-10.

Tobias, J.H., Steer, C.D., Mattocks, C.G., Riddoch, C. and Ness, A.R., 2007. Habitual levels of physical activity influence bone mass in 11-year-old children from the United Kingdom: findings from a large population-based cohort. *Journal of Bone and Mineral Research*, *22*(1), pp.101-109.

Tolar, J., Teitelbaum, S.L. and Orchard, P.J., 2004. Osteopetrosis. *New England Journal of Medicine*, *351*(27), pp.2839-2849.

Toombs, R.J., Ducher, G., Shepherd, J.A. and De Souza, M.J., 2012. The impact of recent technological advances on the trueness and precision of DXA to assess body composition. *Obesity*, *20*(1), pp.30-39.

Torres-Costoso, A., López-Muñoz, P., Martínez-Vizcaíno, V., Álvarez-Bueno, C. and Cavero-Redondo, I., 2020. Association between muscular strength and bone health from children to young adults: a systematic review and meta-analysis. *Sports Medicine*, *50*, pp.1163-1190.

Tran, J., Netto, K., Aisbett, B. and Gastin, P., 2010. Validation of accelerometer data for measuring impacts during jumping and landing tasks.

Troy, K.L., Edwards, W.B., Bhatia, V.A. and Bareither, M.L., 2013. In vivo loading model to examine bone adaptation in humans: a pilot study. *Journal of orthopaedic research*, *31*(9), pp.1406-1413.

Troy, K.L., Mancuso, M.E., Johnson, J.E., Wu, Z., Schnitzer, T.J. and Butler, T.A., 2020. Bone adaptation in adult women is related to loading dose: a 12-month randomized controlled trial. *Journal of Bone and Mineral Research*, *35*(7), pp.1300-1312.

Turner, C.H. and Robling, A.G., 2005. Mechanisms by which exercise improves bone strength. *Journal of bone and mineral metabolism*, *23*, pp.16-22.

Turner, C.H. and Robling, A.G., 2003. Designing exercise regimens to increase bone strength. *Exercise and sport sciences reviews*, *31*(1), pp.45-50.

Tveit, M., Rosengren, B.E., Nyquist, F., Nilsson, J.Å. and Karlsson, M.K., 2013. Former male elite athletes have lower incidence of fragility fractures than expected. *Medicine and science in sports and exercise*, *45*(3), pp.405-410.

Ubago-Guisado, E., Gómez-Cabello, A., Sánchez-Sánchez, J., García-Unanue, J. and Gallardo, L., 2015. Influence of different sports on bone mass in growing girls. *Journal of sports sciences*, *33*(16), pp.1710-1718.

Ueberschär, O., Fleckenstein, D., Warschun, F., Kränzler, S., Walter, N. and Hoppe, M.W., 2019. Measuring biomechanical loads and asymmetries in junior elite long-distance runners through triaxial inertial sensors. *Sports Orthopaedics and Traumatology*, *35*(3), pp.296-308.

Udagawa, N., Takahashi, N., Akatsu, T., Tanaka, H., Sasaki, T., Nishihara, T., Koga, T., Martin, TJ., and Suda, T., 1990. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proc Natl Acad Sci USA*, 87:7260-7264.

Umemura, Y., Ishiko, T., Yamauchi, T., Kurono, M. and Mashiko, S., 1997. Five jumps per day increase bone mass and breaking force in rats. *Journal of Bone and Mineral Research*, *12*(9), pp.1480-1485.

Vainionpää, A., Korpelainen, R., Leppäluoto, J. and Jämsä, T., 2005. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. *Osteoporosis international*, *16*, pp.191-197.

Vainionpää, A., Korpelainen, R., Väänänen, H.K., Haapalahti, J., Jämsä, T. and Leppäluoto, J., 2009. Effect of impact exercise on bone metabolism. *Osteoporosis international*, *20*, pp.1725-1733.

Vainionpää, A., Korpelainen, R., Vihriälä, E., Rinta–Paavola, A., Leppäluoto, J. and Jämsä, T., 2006. Intensity of exercise is associated with bone density change in premenopausal women. *Osteoporosis International*, *17*, pp.455-463.

Vanrenterghem, J., Nedergaard, N.J., Robinson, M.A. and Drust, B., 2017. Training load monitoring in team sports: a novel framework separating physiological and biomechanical load-adaptation pathways. *Sports medicine*, *47*, pp.2135-2142.

Varley, I., Greeves, J.P. and Sale, C., 2019. Seasonal difference in bone characteristics and body composition of elite speed skaters. *International Journal of Sports Medicine*, *40*(01), pp.9-15.

Varley, I., Hughes, D.C., Greeves, J.P., Fraser, W.D. and Sale, C., 2017. Increased training volume improves bone density and cortical area in adolescent football players. *International journal of sports medicine*, *38*(05), pp.341-346.

Varley, I., Sale, C., Greeves, J.P., Morris, J.G., Sunderland, C. and Saward, C., 2023. Relationship between football-specific training characteristics and tibial bone adaptation in male academy football players. *Sports*, *11*(4), p.86.

Varley, I., Ward, M., Thorpe, C., Beardsley, N., Greeves, J., Sale, C. and Saward, C., 2023. External training load is associated with adaptation in bone and body composition over the course of a season in elite male footballers. *Bone Reports*, *18*, p.101643.

Varley, I., Ward, M., Thorpe, C., Beardsley, N., Greeves, J., Sale, C. and Saward, C., 2022. Modelling changes in bone and body composition over a season in elite male footballers. *International Journal of Sports Medicine*, *43*(08), pp.729-739.

Vashishth, D., Tanner, K.E. and Bonfield, W., 2001. Fatigue of cortical bone under combined axial-torsional loading. *Journal of Orthopaedic Research*, *19*(3), pp.414-420.

Vasikaran, S., Eastell, R., Bruyère, O., Foldes, A.J., Garnero, P., Griesmacher, A., McClung, M., Morris, H.A., Silverman, S., Trenti, T. and Wahl, D.A., 2011. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporosis international*, *22*, pp.391-420.

Vatsa, A., Mizuno, D., Smit, T.H., Schmidt, C.F., MacKintosh, F.C. and Klein-Nulend, J., 2006. Bio imaging of intracellular NO production in single bone cells after mechanical stimulation. *Journal of Bone and Mineral Research*, *21*(11), pp.1722-1728.

Vazquez, M., Evans, B.A., Riccardi, D., Evans, S.L., Ralphs, J.R., Dillingham, C.M. and Mason, D.J., 2014. A new method to investigate how mechanical loading of osteocytes controls osteoblasts. *Frontiers in endocrinology*, *5*, p.208.

Verheul, J., Nedergaard, N.J., Vanrenterghem, J. and Robinson, M.A., 2020. Measuring biomechanical loads in team sports–from lab to field. *Science and Medicine in Football*, *4*(3), pp.246-252.

Vicente-Rodriguez, G., Ara, I., Perez-Gomez, J., Serrano-Sanchez, JA., Dorado Cecilia., and Calbet, JA., 2004. High femoral bone mineral density accretion in prepubertal soccer players. *Med & Sci in Sports & Ex*, 36(10):1789-95.

Vicente-Rodriguez, G., Dorado, C., Ara, I., Perez-Gomez, J., Olmedillas, H., Delgado-Guerra, S. and Calbet, J.A.L., 2007. Artistic versus rhythmic gymnastics: effects on bone and muscle mass in young girls. *International journal of sports medicine*, *28*(05), pp.386-393.

Vimalraj, S., 2020. Alkaline phosphatase: Structure, expression and its function in bone mineralization. *Gene*, *754*, p.144855.

Vlachopoulos, D., Barker, A.R., Ubago-Guisado, E., Fatouros, I.G., Knapp, K.M., Williams, C.A. and Gracia-Marco, L., 2017. Longitudinal adaptations of bone mass, geometry, and

metabolism in adolescent male athletes: The PRO-BONE Study. *Journal of Bone and Mineral Research*, *32*(11), pp.2269-2277.

Vlachopoulos, D., Barker, A.R., Ubago-Guisado, E., Williams, C.A. and Gracia-Marco, L., 2018. The effect of a high-impact jumping intervention on bone mass, bone stiffness and fitness parameters in adolescent athletes. *Archives of Osteoporosis*, *13*, pp.1-12.

Walsh, M.S., Ford, K.R., Bangen, K.J., Myer, G.D. and Hewett, T.E., 2006. The validation of a portable force plate for measuring force-time data during jumping and landing tasks. *The Journal of Strength & Conditioning Research*, *20*(4), pp.730-734.

Wang, H. and Dueball, S., 2018. Subject-specific musculoskeletal model for studying bone strain during dynamic motion. *Journal of Visualized Experiments*, (134), p.e56759.

Warden, S.J., 2006. Breaking the rules for bone adaptation to mechanical loading. *Journal of Applied Physiology*, *100*(5), pp.1441-1442.

Warden, S.J., Bogenschutz, E.D., Smith, H.D. and Gutierrez, A.R., 2009. Throwing induces substantial torsional adaptation within the midshaft humerus of male baseball players. *Bone*, *45*(5), pp.931-41.

Warden, S.J., Carballido-Gamio, J., Avin, K.G., Kersh, M.E., Fuchs, R.K., Krug, R. and Bice, R.J., 2019. Adaptation of the proximal humerus to physical activity: A within-subject controlled study in baseball players. *Bone*, *121*, pp.107-15.

Warden, S.J., Fuchs, R.K. and Turner, C.H., 2004. Steps for targeting exercise towards the skeleton to increase bone strength. *Europa medicophysica*, *40*, pp.223-232.

Warden, SJ., Edwards, WB., and Willy, RW., 2021. Preventing Bone Stress Injuries in Runners with Optimal Workload. *Current Osteoporosis Reports*, 19(3):298-307.

Warden, S.J., Mantila Roosa, S.M., Kersh, M.E., Hurd, A.L., Fleisig, G.S., Pandy, M.G. and Fuchs, R.K., 2014. Physical activity when young provides lifelong benefits to cortical bone size and strength in men. *Proceedings of the National Academy of Sciences*, *111*(14), pp.5337-5342.

Weatherholt, A.M. and Warden, S.J., 2016. Tibial bone strength is enhanced in the jump leg of collegiate-level jumping athletes: a within-subject controlled cross-sectional study. *Calcified Tissue International*, *98*, pp.129-139.

Wee, H. and Voloshin, A., 2013. Transmission of vertical vibration to the human foot and ankle. *Annals of biomedical engineering*, *41*, pp.1172-1180.

Weeks, B.K. and Beck, B.R., 2008. The BPAQ: a bone-specific physical activity assessment instrument. *Osteoporosis international*, *19*, pp.1567-1577.

Weeks, B.K., Young, C.M. and Beck, B.R., 2008. Eight months of regular in-school jumping improves indices of bone strength in adolescent boys and girls: the POWER PE study. *Journal of bone and mineral research*, *23*(7), pp.1002-1011.

Weinbaum, S., Cowin, S.C. and Zeng, Y., 1994. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. *Journal of biomechanics*, *27*(3), pp.339-360.

Wen, H.J., Huang, T.H., Li, T.L., Chong, P.N. and Ang, B.S., 2017. Effects of short-term step aerobics exercise on bone metabolism and functional fitness in postmenopausal women with low bone mass. *Osteoporosis International*, *28*, pp.539-547.

West, SW., Clubb, J., Torres-Ronda, L., Howells, D., Leng, E., Vescovi, JD., Carmody, S., Posthumus, M., Dalen-Lorentsen, T., and Windt, J., 2020. More than a Metric: How Training Load is Used in Elite Sport for Athlete Management. *Int J Sports Med,* 42(04):300-06.

Wiebe, P.N., Blimkie, C.J., Farpour-Lambert, N., Briody, J., Marsh, D., Kemp, A., Cowell, C. and Howman-Giles, R., 2008. Effects of single-leg drop-landing exercise from different heights on skeletal adaptations in prepubertal girls: a randomized controlled study. *Pediatric exercise science*, *20*(2), pp.211-228.

Wiig, H., Raastad, T., Luteberget, LS., Ims, I., and Spencer, M., 2019. External load variables affect recovery markers up to 72 h after semiprofessional football matches. *Frontiers Phys*, 10:689.

Wilks, D.C., Winwood, K., Gilliver, S.F., Kwiet, A., Chatfield, M., Michaelis, I., Sun, L.W., Ferretti, J.L., Sargeant, A.J., Felsenberg, D. and Rittweger, J., 2009. Bone mass and geometry of the tibia and the radius of master sprinters, middle and long distance runners, race-walkers and sedentary control participants: a pQCT study. *Bone*, *45*(1), pp.91-97.

Willy, R.W., 2018. Innovations and pitfalls in the use of wearable devices in the prevention and rehabilitation of running related injuries. *Physical Therapy in Sport*, *29*, pp.26-33.

Wilsgaard, T., Emaus, N., Ahmed, L.A., Grimnes, G., Joakimsen, R.M., Omsland, T.K. and Berntsen, G.R., 2009. Lifestyle impact on lifetime bone loss in women and men: the Tromsø Study. *American journal of epidemiology*, *169*(7), pp.877-886.

Winters-Stone, K.M. and Snow, C.M., 2006. Site-specific response of bone to exercise in premenopausal women. *Bone*, *39*(6), pp.1203-1209.

Winters-Stone, K.M. and Snow, C.M., 2003. Musculoskeletal response to exercise is greatest in women with low initial values. *Medicine and science in sports and exercise*, *35*(10), pp.1691-1696.

Wolff J. 1892. Das Gesetz der Transformation der Knochen. Berlin: A Hirschwald (Springer-Verlag published an excellent English translation of this monograph in 1986).

Worthen, L.C., Kim, C.M., Kautz, S.A., Lew, H.L., Kiratli, B.J. and Beaupre, G.S., 2005. Key characteristics of walking correlate with bone density in individuals with chronic stroke. *Journal of Rehabilitation Research & Development*, *42*(6).

Worthington, P., King, M. and Ranson, C., 2013. The influence of cricket fast bowlers' front leg technique on peak ground reaction forces. *Journal of Sports Sciences*, *31*(4), pp.434-441.

Wu, J., Ishizaki, S., Kato, Y., Kuroda, Y. and Fukashiro, S., 1998. The side-to-side differences of bone mass at proximal femur in female rhythmic sports gymnasts. *J Bone & Min Research*, 13(5):900-06.

Wu, X.T., Sun, L.W., Qi, H.Y., Shi, H. and Fan, Y.B., 2016. The bio-response of osteocytes and its regulation on osteoblasts under vibration. *Cell biology international*, *40*(4), pp.397-406.

Yan, C., Bice, R.J., Frame, J.W., Warden, S.J. and Kersh, M.E., 2022. Multidirectional basketball activities load different regions of the tibia: a subject-specific muscle-driven finite element study. *Bone*, *159*, p.116392.

Yang, PF., Bruggemann, GP., and Rittweger, J., 2011. What do we currently know from in vivo bone strain measurements in humans. *J Musculoskelet Neuronal Interact*, 11(1):8-20.

Yavropoulou, M.P. and Yovos, J.G., 2016. The molecular basis of bone mechanotransduction. *Journal of musculoskeletal & neuronal interactions*, *16*(3), p.221.

Yingling, VR., Davies, S., and Silva, MJ., 2001. The effects of repetitive physiologic loading on bone turnover and mechanical properties in adult female and male rats. *Calc Tissue Int*, 68(4):235-39.

Yu, P.A., Hsu, W.H., Hsu, W.B., Kuo, L.T., Lin, Z.R., Shen, W.J. and Hsu, R.W.W., 2019. The effects of high impact exercise intervention on bone mineral density, physical fitness,

and quality of life in postmenopausal women with osteopenia: A retrospective cohort study. *Medicine*, *98*(11).

Yu, W., Qu, H., Hu, G., Zhang, Q., Song, K., Guan, H., Liu, T. and Qin, J., 2014. A microfluidic-based multi-shear device for investigating the effects of low fluid-induced stresses on osteoblasts. *PloS one*, *9*(2), p.e89966.

Yuan, Y., Chen, X., Zhang, L., Wu, J., Guo, J., Zou, D., Chen, B., Sun, Z., Shen, C. and Zou, J., 2016. The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. *Progress in Biophysics and Molecular Biology*, *122*(2), pp.122-130.

Yusni, Y. and Rahman, S., 2019. The effect of chronic exercise on a biomarker of bone resorption in healthy adults. *Family Medicine & Primary Care Review*, (3), pp.270-276.

Zernicke, R., MacKay, C. and Lorincz, C., 2006. Mechanisms of bone remodeling during weight-bearing exercise. *Applied physiology, nutrition, and metabolism*, *31*(6), pp.655-660.

Zerwekh, J.E., Ruml, L.A., Gottschalk, F. and Pak, C.Y., 1998. The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. *Journal of Bone and Mineral Research*, *13*(10), pp.1594-1601.

Zhang, Y., Paul, E.M., Sathyendra, V., Davison, A., Sharkey, N., Bronson, S., Srinivasan, S., Gross, T.S. and Donahue, H.J., 2011. Enhanced osteoclastic resorption and responsiveness to mechanical load in gap junction deficient bone. *PloS one*, *6*(8), p.e23516.

Zhao, C., Irie, N., Takada, Y., Shimoda, K., Miyamoto, T., Nishiwaki, T., Suda, T. and Matsuo, K., 2006. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell metabolism*, *4*(2), pp.111-121.

Zhao, R., Zhao, M. and Zhang, L., 2014. Efficiency of jumping exercise in improving bone mineral density among premenopausal women: a meta-analysis. *Sports medicine*, *44*, pp.1393-1402.

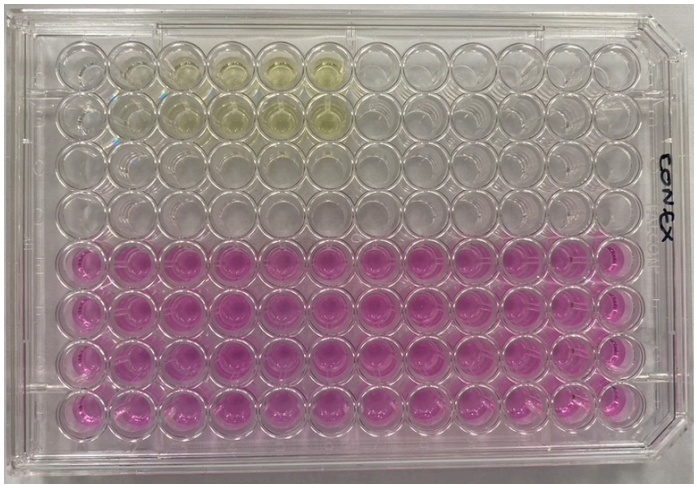
Zhao, S., Arnold, M., Ma, S., Abel, R.L., Cobb, J.P., Hansen, U. and Boughton, O., 2018. Standardizing compression testing for measuring the stiffness of human bone. *Bone & joint research*, *7*(8), pp.524-538.

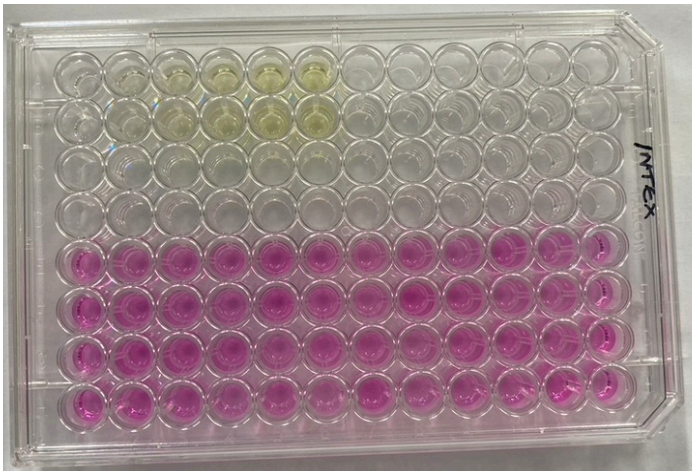
Zhong, Z., Zeng, X.L., Ni, J.H. and Huang, X.F., 2013. Comparison of the biological response of osteoblasts after tension and compression. *The European Journal of Orthodontics*, *35*(1), pp.59-65.

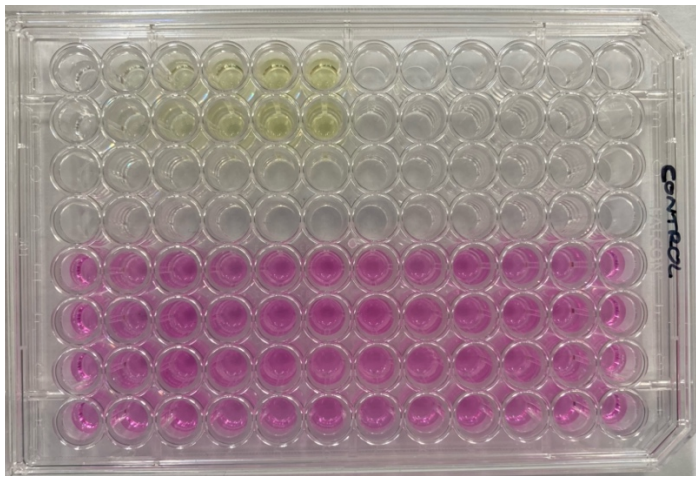
Zouch, M., Zribi, A., Alexandre, C., Chaari, H., Frere, D., Tabka, Z. and Vico, L., 2015. Soccer increases bone mass in prepubescent boys during growth: a 3-yr longitudinal study. *Journal of Clinical Densitometry*, *18*(2), pp.179-186.

11 Appendices

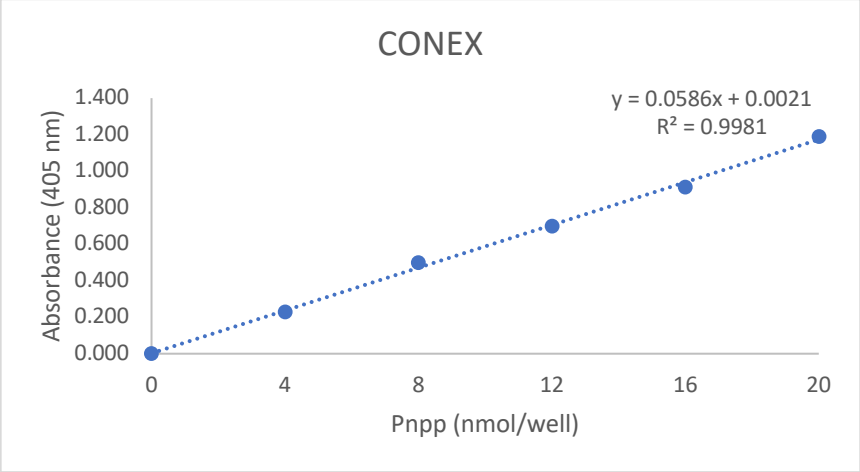
Appendix I. Images of ALP Alisa (Chapter 4).

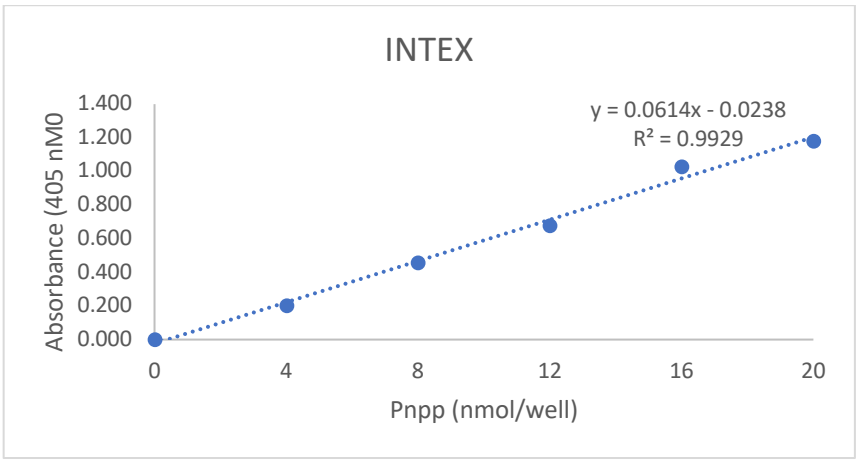


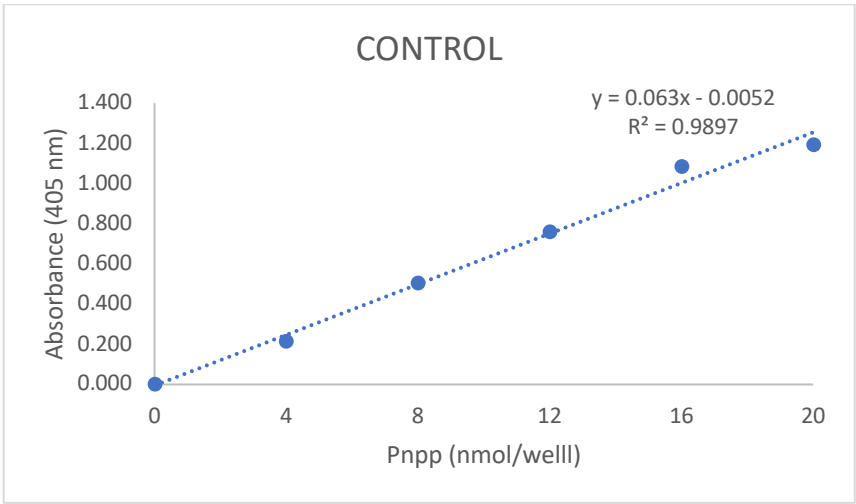




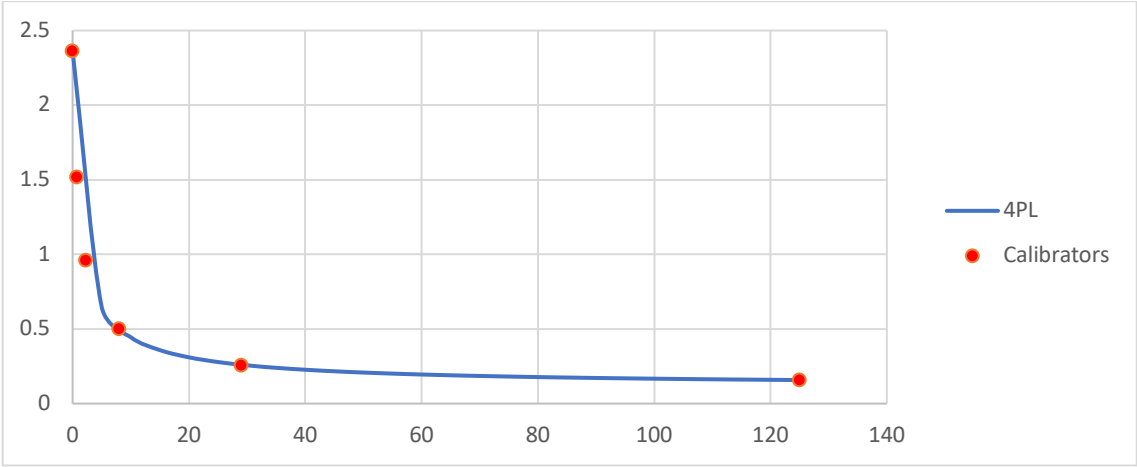
Appendix II. Standard curves for ALP processing (Chapter 4).

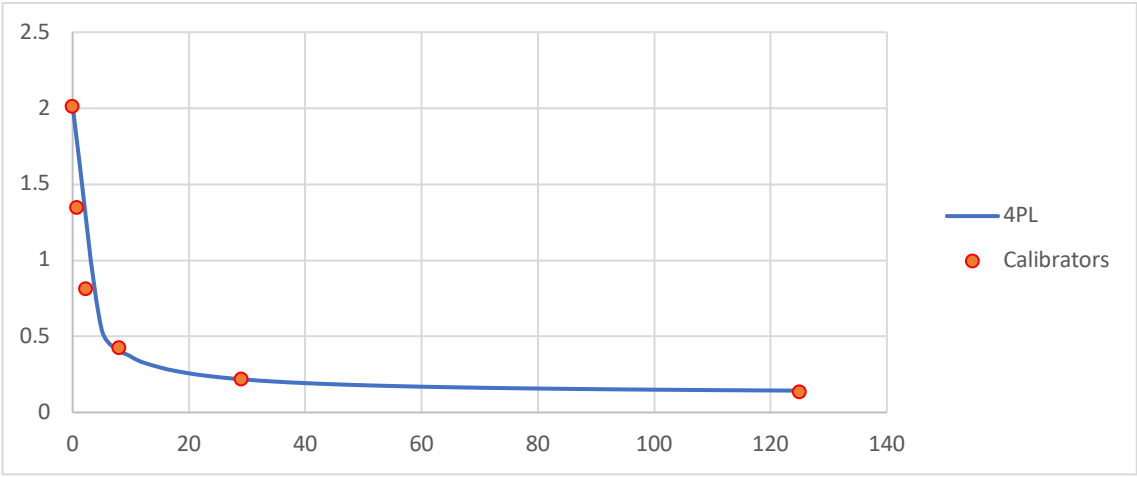






Appendix III. Standard curves for PINP processing (Chapter 4).





Appendix IV. External load in the field: Online questionnaire (Chapter 5)

Participant Information Sheet

External load can be defined as the work completed by an athlete independent of their internal characteristics i.e., acceleration, force, etc. This is not to be confused with internal load which is the biological stresses imposed on an individual (Bourdon et al., 2017) i.e., heart rate, blood lactate, etc. External load monitoring in an applied setting is an area of interest in research due to its association to injury and performance (Halson, 2014). We are interested in finding how current practitioners monitor external load within their field and whether they relate this load to bone in any way.

This questionnaire is designed to gather information on athlete external load monitoring practices within an applied sport and exercise environment. Insight into the monitoring methods used in elite sport will provide a more unified and accurate understanding of how external load is monitored. To take part you should be over 18 and be employed in the field of sport and exercise. You are relevant to this study no matter what level of sport you work in (amateur or professional).

Completion of this questionnaire will take approximately 5-10 minutes. Your participation is voluntary, all the data that you provide will remain anonymous and you will not be identifiable in the dissemination of this research. Your data will be stored securely on a security protected hard drive. If you wish to withdraw your consent after data collection, you have up to two weeks after completion to do so. You can do this by contacting the researchers via email, details supplied at the end of the questionnaire. If you have any questions prior to completing the questionnaire, please contact the researcher below before commencing.

Contact

Researcher
Reece Scott: reece.scott@ntu.ac.uk

Supervisor
Dr Ian Varley: ian.varley@ntu.ac.uk

Consent form

If you agree to participate in the project outlined above, please read the following statements before continuing to the questionnaire.

I understand how to complete the questionnaire and agree to do so as honestly as I can.

I have had the opportunity to ask any questions, communicate and discuss any concerns and queries associated with the study.

I understand that my participation is voluntary, and I have the right to withdraw or discontinue participation at any time with no obligation to provide reasons behind the decision.

I understand that my responses in the questionnaire will be recorded and analysed for content.

I understand that all information which I have provided will be treated as private and confidential and communicated to others with my identity concealed.

I understand that I can withdraw my data using my unique identifier after I've completed the survey up until 2 weeks after completing the survey.

I confirm I am aged 18 or over.

I can confirm that I meet the participant criteria.

- Yes
- No

Please create a unique identifier code; this should be a random 5 letters and/or numbers which you will need to keep in case you wish to withdraw from the study i.e., DI8HV. **Please make a note of your ID code for your records. Please do not include numbers/letters which we may link you to i.e., Name of your club.**

Demographics

The following questions are necessary to the research and will be used to create an anonymous profile of you, as a practitioner. This information will not be used to identify you or reveal your identity.

1. Which sport(s) do you work in? *Select all that are applicable.*

- Football/Soccer
- Cricket
- Basketball
- Hockey
- Tennis
- Volleyball
- Rugby
- American football
- Baseball
- Athletics
- Other (please specify): _____

2. What gender do you **primarily** work with? *Please select one.*

- Male
- Female
- Other

3. Which Continent do you work in? *Please select one.*

- Africa
- Asia
- Australia
- Europe – UK
- Europe – Rest of Europe
- North America
- South America

4. What role are you primarily employed as within the sport? *Please select one.*

- Physiotherapist
- Strength and Conditioning
- Sport Scientist
- Doctor
- Coach
- Other (please specify): _____

5. What age group of athletes do you work with? *Select all that are applicable.*

- Under 16
- 16 - 18
- 19 - 21
- 22 +

6. What level of sport do you currently work in? *Please select one.*

- International
- National
- University/Collegiate
- Regional
- Other (please specify): _____

External Load

External load can be defined as the **work completed by an athlete independent of their internal characteristics i.e., acceleration, force, etc.** This is not to be confused with internal load which is the biological stresses imposed on an individual (Bourdon et al., 2017) i.e., heart rate, blood lactate, etc.

7. Does your club/organisation monitor external load in your athletes?

- Yes
- No

If NO, goes to question 16.

Load Monitoring

8. How do you primarily use external load data? *Select all that are applicable.*

- Monitor Rehabilitation
- Inform on susceptibility to injury
- Inform performance
- Track performance
- Monitor the effectiveness of a training programme
- Other (please specify): _____

9. What systems do you use to monitor external load? *Select all that are applicable.*

- Global Positioning System (GPS)
- Inertial Measuring Unit (IMU)
- Motion analysis
- Force plates
- Surface Electromyography (sEMG)
- Other (please specify): _____

10. What are the main outputs you use? *Select all that are applicable.*

- GPS – PlayerLoad
- GPS – Total distance covered
- GPS – High speed distance
- IMU – Impact Load
- IMU – Step Count
- IMU – Peak positive acceleration
- Motion Analysis – Torque
- Motion Analysis – Moment
- Motion Analysis – Stiffness
- Force Plates – Peak ground reaction force
- Force Plates – Rate of force development
- Force Plates – Impulse
- sEMG – Amplitude
- Other (please specify): _____

11. When do you monitor external load? *Please select one.*

- Continuously (during training/competition)
- Intermittently (for testing purposes)
- When recovering from injury
- Other (please specify): _____

12. Have overuse stress related bone injuries occurred within your club/organisation?

- Yes
- No
- Unsure

13. Do you use any of the external load metrics attained to estimate load on bone?

- Yes
- No
- Unsure

If NO, go to question 17.

Bone Monitoring

By **bone monitoring we are referring to using external load methods and relating them to bone in any form**. It is important to know whether these methods have been translated into applied settings to understand if they are replicable and relevant to practitioners.

14. What are the **main outputs** you use specifically in relation to bone? *Select all that are applicable.*

- GPS – PlayerLoad
- GPS – Total distance covered
- GPS – High speed distance
- IMU – Bone Stimulus
- IMU – Impact Load
- IMU – Step Count
- Motion Analysis – Torque
- Motion Analysis – Moment
- Motion Analysis – Stiffness
- Force Plates – Peak ground reaction force
- Force Plates – Load rate
- Force Plates – Impulse
- sEMG – Amplitude
- Other (please specify): _____

15. When do you collect data? *Please select one.*

- Training
- Competition
- Training and Competition
- Other (please specify): _____

16. What is the primary reason you don't monitor external load?

- Lack of time
- Lack of equipment
- Lack of knowledge
- Don't feel it is needed
- Other (please specify): _____

Only answered as result of NO from Question 7.

17. What is the primary reason you don't relate external load to bone?

- Lack of time
- Lack of equipment
- Lack of Knowledge
- Don't feel it is needed
- Don't believe external load can be related to bone
- Other (please specify): _____

Only answered as result of NO from Question 13.

Sorry, you are unable to complete this questionnaire due to not meeting the minimum criteria (age and consent).

Only shown as result of NO from Consent form.

Final Page – Thank you

Thank you for taking the time to complete this questionnaire. If you have any questions regarding your participation in this research or wish to withdraw your data, please contact the research team.

If you are interested in the current studies related to external load and bone at NTU then please email the researcher below with your details.

Researcher
Reece Scott: reece.scott@ntu.ac.uk

Supervisor
Dr Ian Varley: ian.varley@ntu.ac.uk

Appendix V. Participant information sheet (Chapter 7).

Participant Information Sheet

Study Title: Unilateral bone characteristics within athletes

We would like to invite you to volunteer for our research study that is part of a student's PhD project. Before you decide we would like you to understand why the research is being done and what it would involve from you. **Reece Scott/Ian Varley will go through the information sheet with you and answer any questions you may have.** Please feel free to talk to others about the study if you wish. You may take as much time as you require to decide whether you would like to participate. This information sheet tells you the purpose of the study and what will happen to you if you take part and gives you a more detailed description about the conduct of the study. **Please ask if anything is unclear.**

Part 1

Study description: To observe bone characteristics within the dominant and non-dominant side within habitual, unilaterally loaded movements. To observe if there is an association between non-invasive bone loading measures and bone characteristics.

What is the purpose of the study?

The study is looking to establish if higher bone load observed in the dominant side shows different bone parameters than the non-dominant (less loaded) side.

Why have I been invited?

As an athlete you have taken part in a high volume of training in a sport which is known to have habitually unilateral actions OR you are bilaterally trained in a habitual manner.

Do I have to take part?

It is up to you to decide to join the study. We will describe the study to you and your club staff and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

One visit will involve having a bone scan (this will be in the form of peripheral quantitative computed tomography or pQCT and dual-energy X-ray absorptiometry or DEXA which are used for taking measurements of bone mass and size), for each scan you will be required to sit stationary in a chair with each leg inside the scanner for ~10 minutes each (pQCT) and lay motionless on a DEXA platform for approximately 7 minutes.

Expenses and Payments

You will not receive any payments for your participation in the study.

What will I have to do?

We will ask you to have three bone scans (2 pQCT and 1 DEXA) in one day. These will take place at Nottingham Trent University; each retrospective scan takes approximately 10 mins and is completely pain free. For the DEXA scan, you will be asked to remove your shoes and any metal objects and lay on your back on the bed of the scanner. An x-ray

beam will then pass slowly over your whole body for approximately 10 minutes. You will not feel any sensation from this beam. For the pQCT measurement, you will be asked to place your legs (individually) into the scanner and sit motionless while four sites of your tibia are assessed.

What are the possible disadvantages and risks of taking part?

DEXA and pQCT procedures use ionising radiation to form images of your body and provide the team with other clinical information. However, the amount of radiation used is extremely small, being less than you receive in a few days from natural sources of radiation in the environment.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study will help advance knowledge related to changes in bone strength and size in habitual functional movements. The results may also lead to changing in practice to maximise bone strength and the prevention of bone injury.

What happens when the research study stops?

The information from the study will be fedback to your club medical staff who will explain the results and maybe use the data to inform future practice.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

If new information becomes available that is applicable to the safety of the study, we will inform you of this information. If the study is stopped for any reason, you will be informed with regard to the reasons.

What will happen if I do not want to carry on with the study?

You are free to withdraw from the study at any point. If you withdraw from the study, identifiable data already collected with consent would be retained and used in the study. No further data would be collected, or any other research procedures carried out.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions, **tel: 07919407194/0115 8483452**. If you remain unhappy and wish to complain formally, you can do this by contacting Nottingham Trent University's technical manager, Mark Cosgrove tel: **0115 8486691**, who

is independent of the research program and will take you through the complaints procedure.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. All information will be coded and stored securely. Any information about you which leaves the University will have your name and address removed so that you cannot be recognised (i.e. in case of a publication). All data will be used for analysis in the present study. All data will be destroyed no later than 3 years post study completion.

Involvement of the club medical doctor?

If anything is flagged as a result of the bone scans, then the information will be passed on to your club medical doctor.

What will happen to any samples I give?

No samples are required in the present study.

Who is organising and funding the research?

The research is funded by Nottingham Trent University.

Further information

Who you should approach if you are unhappy with the study: if you wish to speak with someone outside of the research team, please contact Mark Cosgrove Tel: 0115 8486691, who is independent of the research program and will take you through the complaints procedure.
Given the current situation in the UK (and around the World) interactions between people from different households carries a risk of COVID19 infection. Other than when certain measurements are being made, the researcher will ensure they maintain a two-metre distance from participants. All facilities in which research is being conducted have been COVID19 risk assessed. To mitigate any risks when the need for particular measurements requires that a 2-m distance cannot be maintained, all participants will be provided with PPE (personal protective equipment – specifically a surgical mask and face shield). In addition, the researcher will also wear PPE.

Contact Details:

Reece Scott, MSc **Email:**
reece.scott@ntu.ac.uk
Academic Associate
School of Science and Technology,
Nottingham Trent University, Clifton Lane,
Nottingham, UK.
NG11 8NS.
Tel: 07919407194

Ian Varley, MRes, PhD **Email:**
Ian.Varley@ntu.ac.uk
Lecturer in Exercise Physiology
School of Science and Technology,
Nottingham Trent University, Clifton Lane,
Nottingham, UK.
NG11 8NS.
Tel: 0115 8483452

Appendix VI. Pre Scan consent form (Chapter 7).

Pre Scan Screening

Q1) Have you been subjected to any medical radiation exposures in the last 12 months? Y/N

If yes, please specify the number of scans, the type of scans and where they were performed.

_____

Q2) Have you been a volunteer for studies using the pQCT scanner at Nottingham Trent University in the last 12 months? Y/N

If yes, please specify the number of scans and the type of scans.

_____

Q3) Are you subjected to any other form of radiation exposure other than background (e.g. at work)? If yes, please provide details.

_____
_____

I understand that relevant sections of my medical notes and data collected may be looked at by individuals from Nottingham Trent University. I give permission for these individuals to have access to my records.

Participant:

Name _____
Date _____
Signature _____

Researcher taking consent:
Name _____
Date _____
Signature _____

Appendix VII. Consent form for (Chapter 7).

Participant Statement of Consent to Participate

Study Title: Unilateral bone characteristics within athletes

Please place your initials in the boxes provided in response to the following statements;

1) I, *(participant name)*…… agree to partake in the above study. ☐

2) I understand from the participant information sheet dated 18/03/2020, which I have read in full and from my discussion(s) with Reece Scott/Ian Varley that will involve me completing 2 visits to the laboratory producing high load, multi directional exercise on each occasion during the visits. Furthermore, I understand markers, sEMG and accelerometers will be stuck to my skin. ☐

3) It has also been explained to me by Reece Scott or Ian Varley that the risks and side effects that may result from my participation are as follows: tiredness and/or fatigue and the small risk of tripping/falling and musculoskeletal injuries during the trials and in the laboratory. ☐ Potential allergy to plaster. I also understand that although it is extremely unlikely, high intensity exercise has been known to reveal unsuspected heart or circulation problems and very rarely these can have serious or fatal consequences.

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction. ☐

5) I undertake to abide by University regulations and the advice of researchers regarding safety. ☐

6) I am aware that I can withdraw my consent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and that my personal data will be destroyed and that my medical care or legal rights will not be affected. ☐

7) I understand that any personal information, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous. ☐

8) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of questionnaire data, personal details and performance data will be handled in accordance with this policy. ☐

9) I understand the results of my tests will be available to our club medical staff who will then be able to feedback the information to myself. ☐

10) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research. ☐

11) I am aware the club medical staff may be asked for information on my training, dietary and ☐

medical records. This information will be approved by myself prior to it being received.

11) I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers. ☐

12) I understand that the scans within the study produce ionising radiation. This has been explained to me within the participant information sheet. ☐

13) I confirm that I am aware that I need to complete a COVID19 symptom questionnaire prior to every trial in the study / visit to the University's research facilities. ☐

14) I confirm that I recognise that my involvement with this research could result in an increased risk of me contracting COVID19, despite all the mitigation employed by the researchers. ☐

Participant signature: Date:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix VIII. COVID questionnaire for (Chapter 7).

COVID-19 Symptom Questionnaire

1. Study Title: _____

2. Participant Name: _____

3 Date: _____

4. Do you have:

A high temperature / fever	Yes ☐	No ☐
A sore throat	Yes ☐	No ☐
A new continuous cough*	Yes ☐	No ☐
Loss of, or change in, taste or smell	Yes ☐	No ☐

* A new, continuous cough means coughing for longer than an hour, or three or more coughing episodes in 24 hours.

5. Have you, or anyone you share a house with, been in close contact with anyone with a suspected or confirmed case of COVID-19 in the last two weeks? Yes ☐ No ☐

6. Have you travelled to a 'high-risk' region for COVID-19 in the last two weeks?

Yes ☐ No ☐

7. Please confirm that ALL of the questions 4-6 have been answered "NO" and that there are no reasons why you should not participate in the research study:

Yes – I can confirm that all of my responses to questions 4-6 above were "NO" ☐

No – I answered "Yes" to some or all of the questions 4-6 above. ☐

Appendix IX. Health screen form for (Chapter 7).

Health screen

Name or Number .....

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a)	on medication, prescribed or otherwise	Yes ☐	No ☐
(b)	attending your general practitioner	Yes ☐	No ☐
(c)	on a hospital waiting list	Yes ☐	No ☐

2. **In the past two years,** have you had any illness which require you to:

(a)	consult your GP	Yes ☐	No ☐
(b)	attend a hospital outpatient department	Yes ☐	No ☐
(c)	be admitted to hospital	Yes ☐	No ☐

3. **Have you ever** had any of the following?

(a)	Convulsions/epilepsy	Yes ☐	No ☐
(b)	Asthma	Yes ☐	No ☐
(c)	Eczema	Yes ☐	No ☐
(d)	Diabetes	Yes ☐	No ☐
(e)	A blood disorder	Yes ☐	No ☐
(f)	Head injury	Yes ☐	No ☐
(g)	Digestive problems	Yes ☐	No ☐
(h)	Heart problems	Yes ☐	No ☐
(i)	Problems with bones or joints	Yes ☐	No ☐
(j)	Disturbance of balance / coordination	Yes ☐	No ☐
(k)	Numbness in hands or feet	Yes ☐	No ☐
(l)	Disturbance of vision	Yes ☐	No ☐
(m)	Ear / hearing problems	Yes ☐	No ☐
(n)	Thyroid problems	Yes ☐	No ☐
(o)	Kidney or liver problems	Yes ☐	No ☐

(p) Allergy to nuts, alcohol etc. Yes ☐ No ☐

(q) Any problems affecting your nose e.g. recurrent nose bleeds Yes ☐ No ☐

(r) Any nasal fracture or deviated nasal septum Yes ☐ No ☐

4. **Has any,** otherwise healthy, member of your family under the age of 50 died suddenly during or soon after exercise? Yes ☐ No ☐

5. Are there any reasons why blood sampling may be difficult? Yes ☐ No ☐

6. Have you had a blood sample taken previously? Yes ☐ No ☐

7. Have you had a cold, flu or any flu like symptoms in the last Month? Yes ☐ No ☐

COVID19

8. Do you think you have had COVID-19? Yes ☐ No ☐

9 If YES, was this confirmed via a swab test? Yes ☐ No ☐

10. If YES, was this confirmed via an anti-body test? Yes ☐ No ☐

11. State the dates over which you had COVID-19 symptoms:
FROM _____ TO _____

NB Please note that in the 7-day period prior to any visit to the University to undertake a trial in a research study or to visit a University research facility YOU WILL NEED TO COMPLETE a COVID-19 symptom questionnaire. Please DO NOT come to the University if you have not completed this questionnaire and the member of research staff supervising the research study has not confirmed you should attend.

Women only (delete if not applicable)

Are you pregnant, trying to become pregnant or breastfeeding? Yes ☐ No ☐

If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled.)

.....
.....
.....

1 Appendix X. Anthropometric data (Chapter 7).

2 Table 7.3. Participants' Age, anthropometrics and body composition

Variables	**Fast bowler (Asymmetrical)**		***P***	**Footballer (Symmetrical)**		***P*** 3
Age (years)	24 ± 5			19* ± 1		.001
Height (cm)	190.9 ± 7.5			181.9* ± 4.7		.001
Body mass (kg)	90.9 ± 11.9			76.3* ± 5.4		.000
Overall participation (years)	12.9 ± 3.8			10.5 ± 2.8		
WBLM (kg)	70.27 ± 5.45			63.36* ± 4.67		.002
WBFM (%)	17.8 ± 6.9			11.8* ± 2.6		.007
	Left leg	*Right leg*		*Left leg*	*Right leg*	
Lean mass (kg)	12.34 ± 1.07	12.36 ± 1.23	.79	10.82 ± 1.06	10.98** ± 1.07	.03
Fat mass (%)	16.8 ± 5.6	16.8 ± 5.4	.76	12.2 ± 3.3	12.1 ± 3.4	.60

4 WBLM = Whole Body Lean Mass. WBFM = Whole Body Fat Mass. Values are represented mean (±1SD).
5 * depicts a significant difference between fast bowlers and footballers (P >0.05).
6 ** depicts a significant difference between left leg and right leg (P >0.05).

Appendix XI. External load data of cricket and football subjects (Chapter 7).

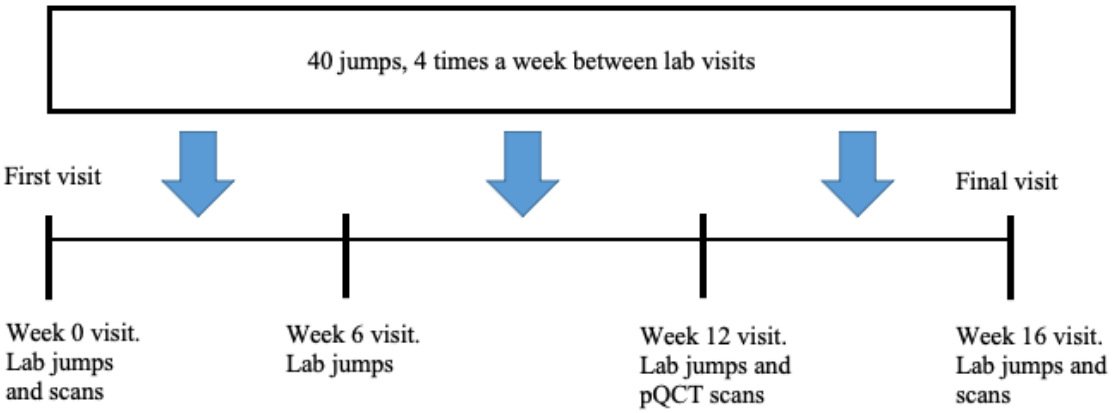
Table 7.4. Participants' external load metrics

Variables	**Fast Bowler (Asymmetrical)**				**Footballer (Symmetrical)**			
	Dominant leg	**Non-dominant leg**	**% Difference**	***P***	**Dominant leg**	**Non-dominant leg**	**% Difference**	***P***
Peak acceleration (g)	26.9* ± 7.9	17.6 ± 6.1	53%†	.00	14.7 ± 6.2	17.1 ± 8.5	14%	.19
PPA (g)	21.1* ± 6.3	15.5 ± 4.9	38%	.00	11.3 ± 4.3	12.4 ± 4.8	10%	.19
Cumulative load	94756 ± 40019	90609 ± 47036	5%	.58	59933 ± 44989	65631 ± 51108	9%	.52
Relative load	1038 ± 469	997 ± 556	4%	.62	749 ± 550	831 ± 639	10%	.49

Values are represented mean (±1SD). * depicts a significant difference between left leg and right leg (P >0.05).

Appendix XII. Overview of study requirements (Chapter 8).

Overview: We will follow the changes that may occur in bone from a home jumping intervention over a 16-week period. Scans will take place at the time points specified below to track changes in bone.



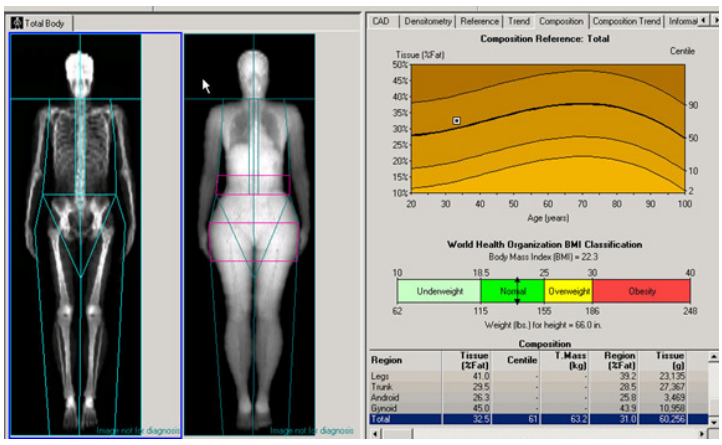
Inclusion criteria:

- 18 - 25 years old.
- Partake in physical activity no more than 2x per week.
- No regular participation (more than once per week) in exercise that influences bone e.g., gymnastics, court sports, ball sports or racquet sports.
- Have no current unresolved cardiovascular complaints to avoid any cardiovascular complications when performing activities of daily living.
- Access to travel.

Exclusion criteria:

- Prescribed medication that influences bone metabolism.
- Joint replacement or prostheses
- Currently or recently injured
- Medical conditions adversely affected by exposure to ionising radiation.
- History of high levels of ionising radiation exposure (*e.g.,* medical treatment).
- Breastfeeding women, pregnant women and women trying to become pregnant
- Females who are on any form of contraception that may influence changes in bone
- Females who have current or previous history of an endocrine disorder

Whole body DXA scans (right) allows bone mass, lean mass, body fat %, bone mineral density, etc to be retrieved. We can then see the changes between the initial scan and week 16 (post intervention) to understand the benefits of the exercise programme.



Appendix XIII. Participant information form (Chapter 8).

Participant Information Sheet

Study Title: Dose response effect of drop jumps on bone characteristics

You are invited to volunteer for our research study that is part of a student's PhD project. Before you decide to take part, we would like you to understand why the research is being performed and what it involves. **Reece Scott/Ian Varley will go through the information sheet with you and answer any questions you may have.** Please feel free to talk to others about the study if you wish. You may take as much time as you require to decide whether you would like to participate. This information sheet tells you the purpose of the study and what will happen to you if you take part and gives you a more detailed description of the study. **Please ask if anything is unclear.**

Part 1
Study description:
To observe if there is a positive effect on bone that can be identified from an exercise intervention involving jumping from various heights. We will follow the changes that may occur in bone from an exercise intervention over a 16-week period.

What is the purpose of the study?
To identify an optimal exercise intervention to benefit bone health.

Why have I been invited?
You have expressed interest and believe you meet the inclusion criteria (i.e. have low physical activity status (defined as partaking in physical training activities no more than 2x per week) and don't currently participate in exercise programmes known to influence bone.

Do I have to take part?
It is up to you to decide to join the study. We will describe the study and go through this information sheet with you. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time without giving a reason.

Expenses and Payments
You will not receive any payments for your participation in this study.

What will I have to do?
We will ask you to visit Nottingham Trent University on 4 occasions. These visits will involve jump testing using various non-invasive methods (motion analysis, leg mounted device to measure acceleration and force plates) and/or bone scanning (peripheral quantitative computed tomography, known as pQCT and dual-energy X-ray absorptiometry, known as DEXA). For each scan you will be required to sit in a chair with each leg inside the scanner for approximately 15 minutes (pQCT) and lay motionless on a bed for approximately 10 minutes. DEXA scans will be performed on 2 of 4 visits and pQCT on 3 of 4 visits. Every week you will be required to perform drop jumps at home or somewhere suitable. This will consist of 40 diagonal drop jumps (20 x each side), 4 days a week with a minimum of 24 hours rest between sessions. The intervention will take place for a minimum of 16 weeks. This means 64 unsupervised home exercise sessions will take place across the 16 weeks. Please note, you may be asked to act as a control participant in which you will only take part in the bone scans.

What are the potential disadvantages and risks of taking part?
If you are participating in this trial, you will be scanned on 3 occasions (DXA and pQCT) to estimate bone density and structure. These scans emit ionising radiation.
Excessive long-term exposure to ionising radiation has been related to cancer. In this trial you will receive radiation exposure that you wouldn't have otherwise received. This amounts to the

equivalent of around three days of UK background radiation. This means that the risk is small enough to be considered trivial.

What are the potential benefits of taking part?
We cannot promise the study will help you, but we hypothesise the exercise that you take part in will improve bone characteristics. The results may also lead to changes in practice to help bone strength and the prevention of bone injury in wider populations.

What happens when the study stops?
The information from the study will be fedback to you by an investigator who will explain the results and use the data to inform future practice.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2
What if new relevant information becomes available?
If new information becomes available that is applicable to the safety of the study, we will inform you. If the study is stopped for any reason, you will be informed of the reasoning.

What will happen if I do not want to carry on with the study?
You are free to withdraw from the study at any point. If you withdraw from the study, data already collected will retained and used in the study if you consent to this. No further data would be collected, or any more procedures carried out.

What if there is a problem?
If you have concerns about any aspect of the study, you should ask to speak to the researchers who will do their best to answer your questions, **tel: 07919407194/0115 8483452**. If you remain unhappy and wish to complain formally, you can do this by contacting Nottingham Trent University's technical manager, Mark Cosgrove tel: **0115 8486691**, who is independent of the research program and will take you through the complaint's procedure.

Will taking part in this study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. All information will be coded and stored securely. Any information that is made public will have your details removed so that you cannot be recognised (*e.g.,* publication, presentations). All data will be used for analysis in the present study. All data will be destroyed no later than 3 years post study completion.

What will happen to any samples I give?
No samples are required in the present study.

Who is organising and funding the research?
The research is funded by Nottingham Trent University.

Further information
If you are unhappy and wish to speak with someone outside of the research team, please contact Mark Cosgrove Tel: 0115 8486691, who is independent of the research program and will take you through the complaint's procedure.

In line with current university COVID guidelines the researcher will ensure they maintain a 2m distance from participants were possible. All facilities in which research is being conducted have been COVID19 risk assessed. To mitigate against risk, when the need for measurements requires researchers to be in proximity, all participants will be provided with PPE (personal protective

equipment – specifically a surgical mask and face shield). In addition, the researcher will also wear PPE. Please note, these procedures may change as university guidelines are continually updated.

Contact Details:

Reece Scott, MSc
Email: reece.scott@ntu.ac.uk
Academic Associate
School of Science and Technology,
Nottingham Trent University, Clifton Lane,
Nottingham, UK.
NG11 8NS.
Tel: 07919407194

Ian Varley, MRes, PhD
Email: Ian.Varley@ntu.ac.uk
Lecturer in Exercise Physiology
School of Science and Technology,
Nottingham Trent University, Clifton Lane,
Nottingham, UK.
NG11 8NS.
Tel: 0115 8483452

Appendix XIV. Consent form (Chapter 8).

Participant Statement of Consent to Participate

Study Title: Dose response effect of drop jumps on bone characteristics

Please place your initials in the boxes provided in response to the following statements;

1) I, *(participant name)*…… agree to partake in the above study. ☐

2) I understand from the participant information sheet dated 04/11/2021, which I have read in full and from my discussion(s) with Reece Scott/Ian Varley that will involve me completing 4 visits to the laboratory producing drop jump exercises on each occasion. Furthermore, I understand markers and IMU's will be stuck to my skin. ☐

3) I understand from the information supplied that I will partake in home exercise drop jumps 4 times a week (Mon, Wed, Fri, Sun) and the intervention will continue for a minimum of 16 weeks. ☐

4) It has also been explained to me by Reece Scott or Ian Varley that the risks and side effects that may result from my participation are as follows: tiredness and/or fatigue and the small risk of tripping/falling and musculoskeletal injuries during the trials and in the laboratory. ☐ Potential allergy to plaster. I also understand that although it is extremely unlikely, exercise has been known to reveal unsuspected heart or circulation problems and very rarely these can have serious or fatal consequences.

5) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction. ☐

6) I undertake to abide by University regulations and the advice of researchers regarding safety. ☐

7) I am aware that I can withdraw my consent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and that my personal data will be destroyed and that my medical care or legal rights will not be affected. ☐

8) I understand that any personal information, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous. ☐

9) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of questionnaire data, personal details and performance data will be handled in accordance with this policy. ☐

10) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise, that would prevent me from partaking in this research. ☐

11) I understand that the information collected about me will be used to support other ☐

research in the future and may be shared anonymously with other researchers.

12) I understand that the scans within the study produce ionising radiation. This has been explained to me within the participant information sheet. ☐

13) I confirm that I am aware that I need to complete a COVID19 symptom questionnaire prior to every trial in the study / visit to the University's research facilities. ☐

14) I confirm that I recognise that my involvement with this research could result in an increased risk of me contracting COVID19, despite all the mitigation employed by the researchers. ☐

Participant signature: Date:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix XV. BPAQ (Chapter 8).

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

1. Please list <u>any</u> sports or other physical activities you have participated in regularly. Please <u>tick</u> the boxes to indicate how oldyou were for each sport/activity and how many years you participated for.

Age: / Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

Bone-Specific Physical Activity Questionnaire (BPAQ)

SUBJECT ID:	DATE:

1. **Please list the sports or other physical activities (be as specific as possible) you participated in regularly during the last 12 months and indicate the average frequency (sessions per week).**

Activity:_____ Frequency (per week): _____

Activity:_____ Frequency (per week): _____

Activity:_____ Frequency (per week): _____

Activity:_____ Frequency (per week): _____

Activity:_____ Frequency (per week): _____

Activity:_____ Frequency (per week): _____

Activity:_____ Frequency (per week): _____

Appendix XVI. Sleep questionnaire (Chapter 8).

Pittsburgh Sleep Quality Index

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for most days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?

BED TIME _____

2. During the past month, how long does it usually take for you to fall asleep each night?

NUMBER OF MINUTES _____

3. During the past month, what time have you usually gotten up in the morning?

GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you . . .

a) Cannot get to sleep within 30 minutes

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

b) Wake up in the middle of the night or early morning

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

c) Have to get up to use the bathroom

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

d) Cannot breathe comfortably

- Not during the past month ☐

- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

e) Cough or snore loudly

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

f) Feel too cold

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

g) Feel too hot

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

h) Had bad dreams

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

i) Have pain

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

j) Other reason(s), please describe_____

How often during the past month have you had trouble sleeping because of this?

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

6. During the past month, how would you rate your sleep quality overall?

- Very good _____
- Fairly good _____
- Fairly bad _____

- Very bad _____

7. During the past month, how often have you taken medicine to help you sleep (prescribed or “over the counter")?

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

- No problem at all ☐
- Only a very slight problem ☐
- Somewhat of a problem ☐
- A very big problem ☐

10. Do you have a bed partner or roommate?

- No bed partner or room mate ☐
- Partner/roommate in other room ☐
- Partner in same room, but not same bed ☐
- Partner in same bed ☐

If you have a roommate or bed partner, ask him/her how often in the past month you have had . . .

a) Loud snoring

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

b) Long pauses between breaths while asleep

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

c) Legs twitching or jerking while you sleep

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

d) Episodes of disorientation or confusion during sleep

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

e) Other restlessness while you sleep; please describe _____

- Not during the past month ☐
- Less than once a week ☐
- Once or twice a week ☐
- Three or more time a week ☐

Thank you for completing this questionnaire

Appendix XVII. Food questionnaire form (Chapter 8).

Food Frequency Questionnaire King's College London

Subject name: ……

Subject number: ……

Date: ……

How to complete the food questionnaire

This questionnaire is accompanied by ***A Photographic Atlas of Food Portion Sizes***, which contains 78 pages of photographs for most of the foods mentioned. There are also supplementary photographs G1-G20 located at the back of the book.

A **B** **C** **D** **E**

FOOD EATEN	**FREQUENCY OF CONSUMPTION**									
	Nevereaten	Once per month or less	Onceper fort-night	Number of days perweek						
				1	2	3	4	5	6	7
BREAKFASTCEREALS (Cornflakes, Branflakes, All Bran, muesli etc)			Example		.....	.....				

Column A: lists the foods/food groups of interest.

Columns B to E: tick one box to show how often you eat the food.

Column F: write down the exact names of the foods you eat and describe the amounts using photographs or household measures. The page numbers [shown in brackets] refer to photographs in ***A photographic atlas of food portion sizes***. Write down the number of the photograph that represents the amount of food you eat. Where more than one page number is given, choose the page that most closely resembles the food in question. If you eat more than one serving a day, write down the number of the photograph for each serving that you have. There are no photographs available for some foods, so photographs of foods of a similar shape or texture have been suggested. In each case, choose the photograph that most closely represents the amount of food in question. Where there is no photograph, describe the amount using the household measures suggested.

Please describe your eating habits over the <u>PAST YEAR</u> by filling in the questionnaire on the followingpages

FOOD EATEN	FREQUENCY OF CONSUMPTION									
	Nevereaten	Once per month or less	Onceper fort-night	Number of days perweek						
				1	2	3	4	5	6	7
BREAKFAST CEREALS (Cornflakes, Branflakes, All Bran, muesli etc)										
BREAD *Toast*										
Sliced bread(ie in sandwiches)										
French bread,rolls										
CAKES (fruitcake, sponge cake, gateau, chocolate, ginger etc)										
TEA BREADS (scone, malt loaf etc)										
PASTRIES (doughnuts, custard tarts and										

other pastries and tarts)										

FOOD EATEN	**FREQUENCY OF CONSUMPTION**									
	Nevereaten	Once per month or less	Onceper fort-night	Number of days perweek						
				1	2	3	4	5	6	7
PUDDINGS *Rice pudding/ other milk puddings*										
Cheesecake										
Bread and butterpudding										
Fruit crumble/ pie/tart										
Sponge puddings										
BISCUITS										
MILK AND MILK PRODUCTS *Plain milk to drink/in coffee or tea/on cereals etc*										
Yoghurt/fromage frais										
CHEESE *Cream cheese/cheese spreads*										
Cottage cheese										

Cheddar cheese/other hard cheese										

FOOD EATEN	**FREQUENCY OF CONSUMPTION**									
	Never eaten	Once per month or less	Once per fort-night	Number of days per week						
				1	2	3	4	5	6	7
Brie cheeses/ other soft cheeses										
Other cheeses										
PLAIN RICE										
PIZZA AND PASTA *Pizza*										
Spaghetti/ tagliatelle etc										
Macaroni cheese/tinned spaghetti/other pasta										
Lasagne										
QUICHE AND SAVOURY FLANS										
MEAT/ CHICKEN *Roast or boiled*										
Steaks										
Beef burger										
Minced beef dishes (chilli con										

carne, shepherd's pie etc)										
Meat stew orcasserole										

FOOD EATEN	**FREQUENCY OF CONSUMPTION**									
	Never eaten	Once per month or less	Once per fort-night	Number of days per week						
				1	2	3	4	5	6	7
LIVER AND KIDNEY										
Steak and kidneypie										
POTATOES *Boiled*										
Chips										
Jacket potato										
Mashed potato										
Roast potato										
ROOT VEGETABLES *Carrots*										
Beetroot										
Parsnips										
BROCCOLI, CAULIFLOWER *Broccoli*										

Cauliflower										
GREEN LEAFY VEGETABLES *Cabbage*										
Brussels sprouts										

FOOD EATEN	**FREQUENCY OF CONSUMPTION**									
	Never eaten	Once per month or less	Once per fort-night	Number of days per week						
				1	2	3	4	5	6	7
Spinach, spring greens, kale, watercress, mustard and cress										
PEAS										
GREEN BEANS (runner beans, French beans, mange tout, sugar snaps etc)										
SALAD VEGETABLES *Lettuce*										
Cucumber										
Tomato										
Pepper										
OTHER VEGETABLES *Onions/spring onions*										
Leeks										

Courgettes										
Mushrooms										
MIXED VEGETABLES, COLESLAW										
BAKED BEANS			•							

FOOD EATEN	**FREQUENCY OF CONSUMPTION**									
	Nevereaten	Once per month or less	Onceper fort-night	Number of days perweek						
				1	2	3	4	5	6	7
DRIED BEANSAND PULSES (lentils, chickpeas, kidneybeans, dahl etc)										
CITRUS FRUIT *Oranges*										
Grapefruit										
Satsuma, clementine, mandarin, tangerine										
BANANAS										
BERRIES (raspberries, strawberries, blackberries etc)										
DRIED FRUIT (dates, sultanas,fruit mix)										

NUTS ANDSEEDS (sunflower,sesame etc)										
CRISPS ANDSAVOURY SNACKS										
LIVER PATE										
EGGS (boiled,poached, fried, scrambled etc)										
SOUP										

FOOD EATEN	FREQUENCY OF CONSUMPTION										
	Nevereaten	Once per month or less	Onceper fort-night	Number of days perweek							
				1	2	3	4	5	6	7	
FRUIT JUICE (orange juice,tomato juice, lemonade)											
SQUASH (orange, Ribena etc)											
BOVRIL ANDMARMITE											
OXO CUBES											
MILKY DRINKS (Complan, Horlicks, Ovaltine, Build-up)											
TEA											
SHANDY ANDCIDER											
BEERS ANDLAGERS											
WINE (white,red or rose)											
MARTINI, VERMOUTH, SHERRY, PORT, etc											
SPIRITS (vodka,whisky, gin, etc)											

Thank you for completing the questionnaire.

Appendix XVIII. Pre - Post changes in BMD (Chapter 8).

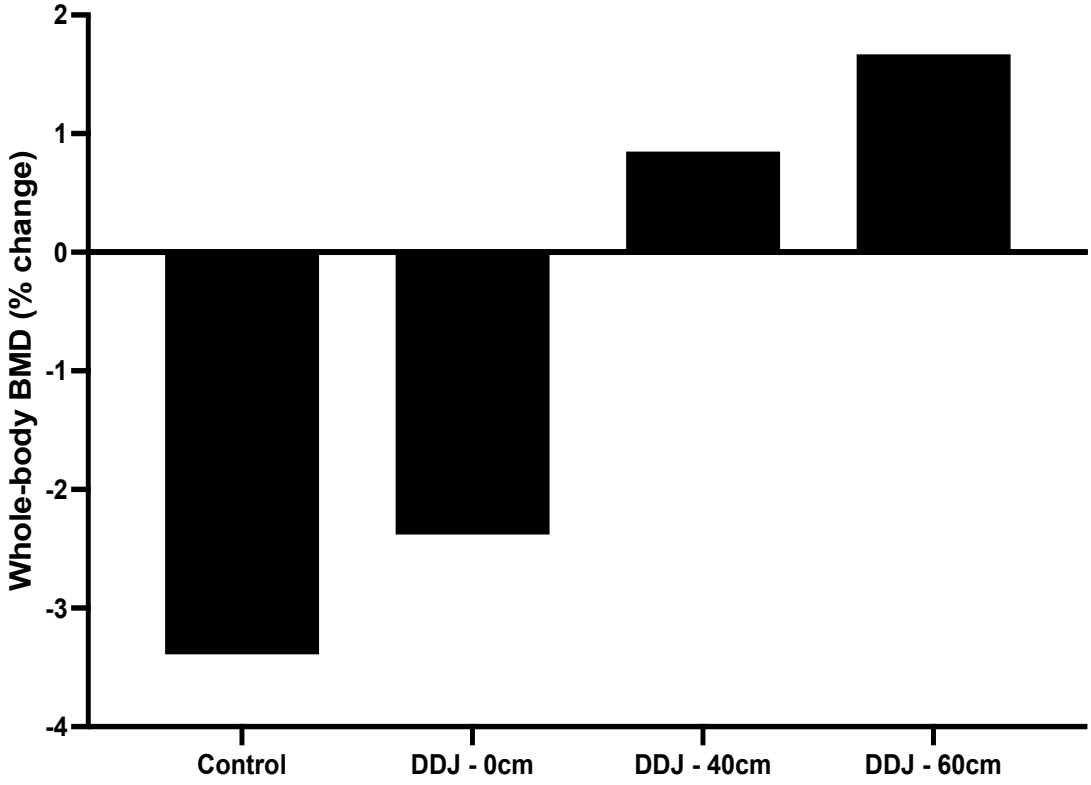


Figure 11.1. Percentage change of WB BMD between pre- and post-intervention.

Appendix XIX. Pre - Week 12 changes in Pqct metrics (Chapter 8).

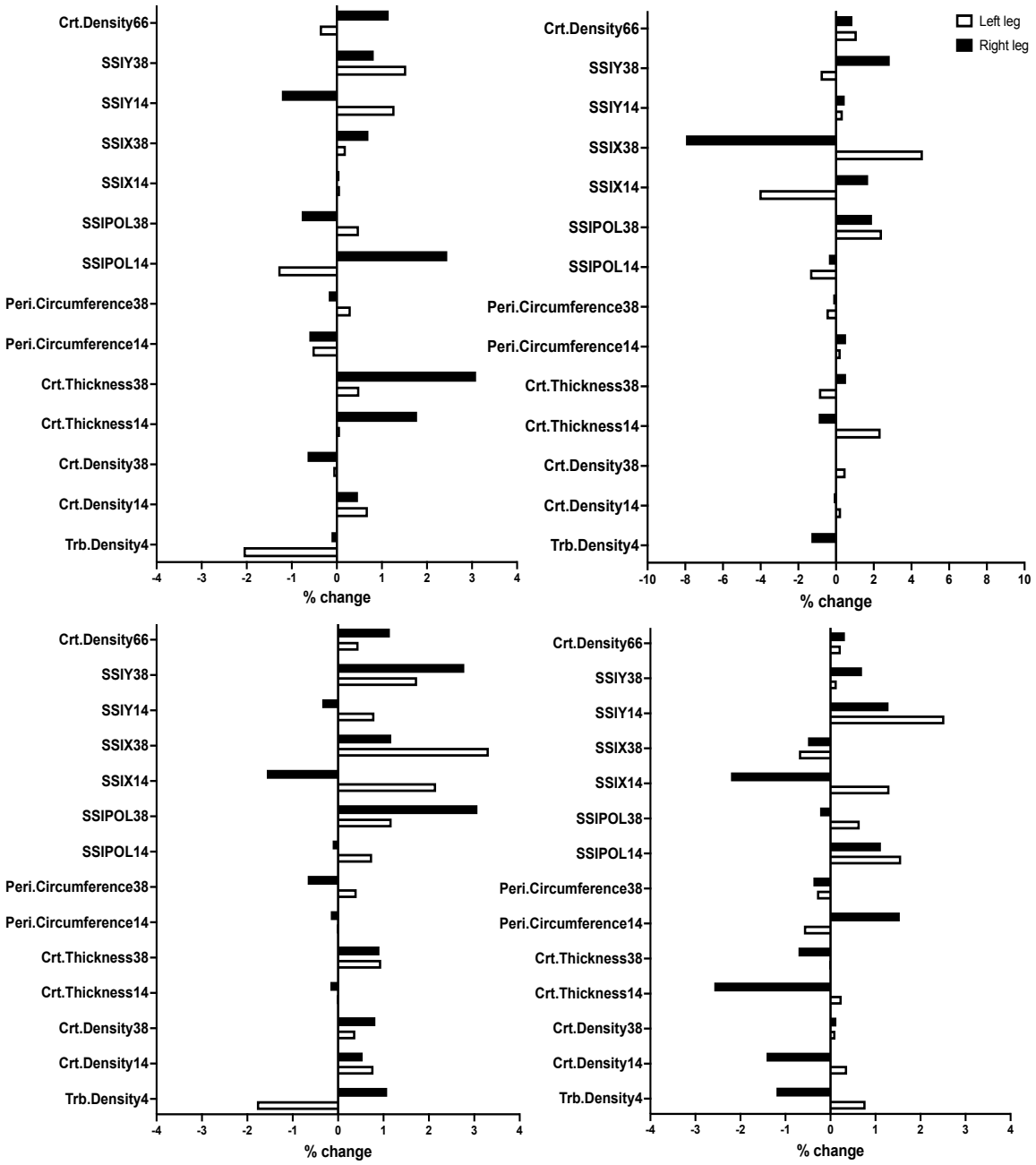


Figure 11.2. Percentage changes between pre-intervention and week 12 for pQCT variables. a) Control, b) 0cm, c) 40cm, d) 60cm.

Appendix XX. Pre - Post changes in Pqct metrics (Chapter 8).

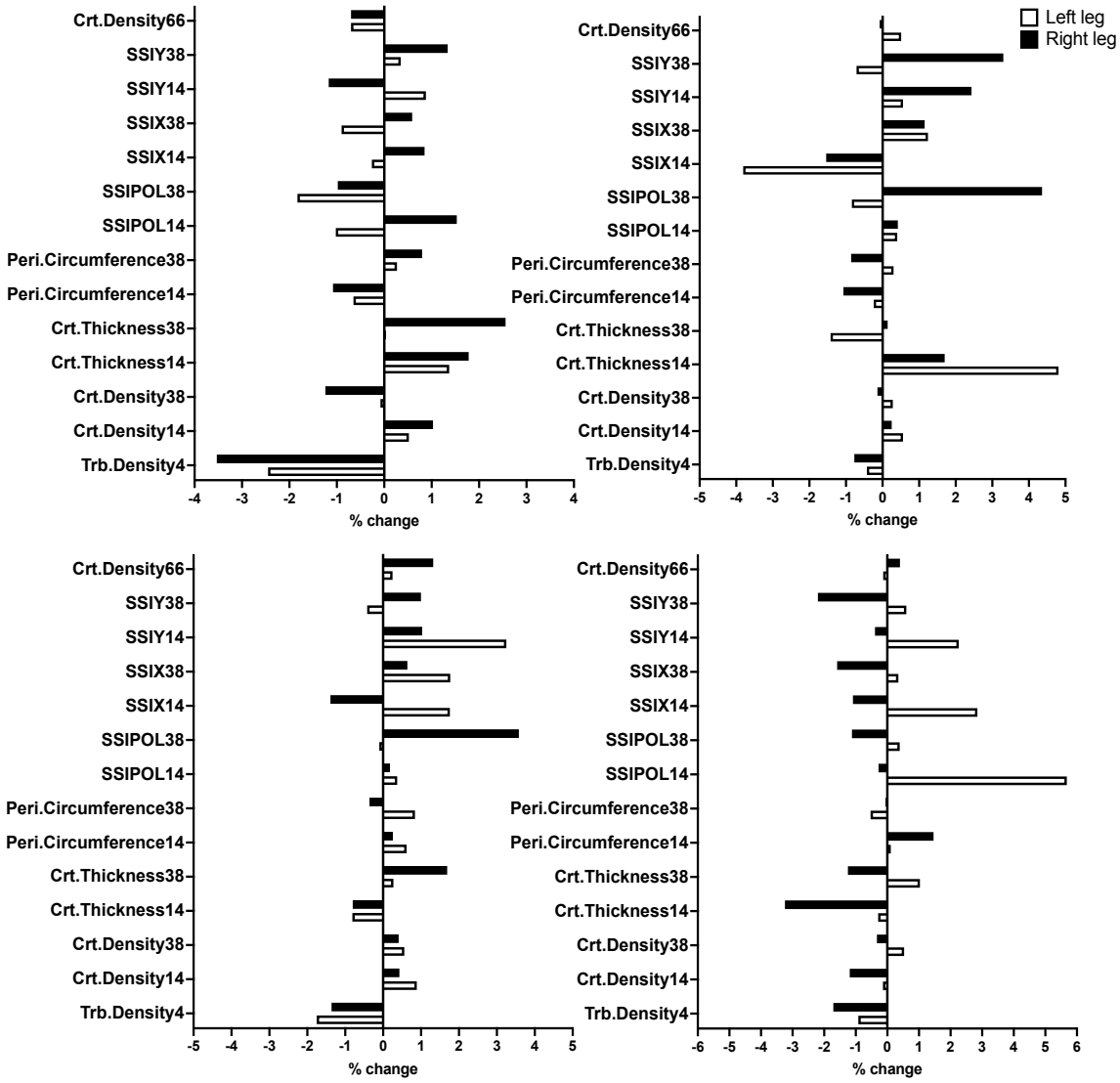


Figure 11.3. Percentage changes between pre-intervention and week 16 for pQCT variables. a) Control, b) 0cm, c) 40cm, d) 60cm.

Appendix XXI. 16-week adjusted gains Δ (95% CI) of bone characteristics measured via DXA (Chapter 8).

		Control			0cm		40cm
		0cm	*40cm*	*60cm*	*40cm*	*60cm*	*60cm*
WB BMD (g/cm^2)		-0.01 (-0.87, 0.59)	-0.05 (-0.12, 0.02)	-0.06 (-0.13, 0.01)	-0.03 (-0.10, 0.04)	-0.04 (-0.11, 0.02)	-0.01 (-0.08, 0.06)
WB BMC (g)		-151 (-474, 172)	-258 (-570, 55)	-236 (-542, 70)	-107 (-411, 198)	-85 (-383, 213)	22 (-266, 309)
WB Bone Area (cm^2)		-116 (-320, 88)	-134 (-333, 65)	-94 (-289, 101)	-18 (-211, 175)	21 (-168, 211)	40 (-144, 223)
Leg BMD (g/cm^2)	Right	0.01 (-0.10, 0.13)	-0.02 (-0.13, 0.89)	-0.05 (-0.16, 0.06)	-0.03 (-0.14, 0.07)	-0.06 (-0.17, 0.04)	-0.03 (-0.13, 0.07)
	Left	0.02 (-0.10, 0.13)	-0.02 (-0.14, 0.09)	-0.06 (-0.17, 0.05)	-0.04 (-0.15, 0.08)	-0.08 (-0.18, 0.03)	-0.04 (-0.14, 0.07)
Leg BMC (g)	Right	-34 (-108, 39)	-51 (-123, 21)	-53 (-123, 18)	-17 (-87, 53)	-18 (-86, 50)	1 (-65, 68)
	Left	-37 (-107, 33)	-50 (-118, 18)	-49 (-116, 19)	-13 (-80, 53)	-12 (-77, 53)	1 (-62,64)
Leg Bone Area (cm^2)	Right	-30 (-75, 15)	-29 (-74, 15)	-21 (-65, 22)	1 (-42, 43)	8 (-33, 50)	8 (-33, 48)
	Left	-34 (-76, 8)	-32 (-73, 9)	-18 (-58, 22)	2 (-38, 41)	16 (-23, 54)	14 (-24, 52)

Comparisons made between multiple groups. Control – 0cm, Control – 40cm, Control – 60cm, 0cm – 40cm, 0cm – 60cm, 40cm – 60cm.

Appendix XXII. 12-week adjusted gains Δ (95% CI) of bone characteristics measured via pQCT (Chapter 8).

	Control						0cm				40cm	
	0cm		*40cm*		*60cm*		*40cm*		*60cm*		*60cm*	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
4%												
Trb.density (g/cm^3)	-6 (-30, 18)	2 (-18, 22)	-3 (-26, 21)	-4 (-24, 17)	-9 (-33, 16)	3 (-19, 25)	4 (-19, 26)	-6 (-26, 15)	-3 (-25, 20)	1 (-19, 22)	-6 (-29, 16)	7 (-15, 29)
14%												
Crt.density (g/cm^3)	9 (-11, 29)	6 (-10, 22)	-1 (-20, 19)	-3 (-19, 13)	4 (-16, 24)	**18 (1, 34)***	-9 (-30, 10)	-9 (-26, 7)	-5 (-25, 15)	12 (-5, 29)	4 (-15, 24)	**21 (5, 37)***
Crt.thickness (mm)	-0.07 (-0.28, 0.14)	0.05 (-0.11, 0.20)	-0.01 (-0.22, 0.20)	0.03 (-0.11, 0.17)	-0.02 (-0.22, 0.19)	0.09 (-0.06, 0.24)	0.06 (-0.16, 0.27)	-0.01 (-0.16, 0.14)	0.05 (-0.16, 0.26)	0.05 (-0.10, 0.19)	0 (-0.22, 0.20)	0.06 (-0.09, 0.20)
Peri.circum (mm)	-0.66 (-3.68, 2.38)	-0.97 (-2.81, 0.86)	-0.46 (-3.50, 2.59)	-0.40 (-2.14, 1.34)	-0.03 (-3.09, 3.02)	-1.72 (-3.51, 0.06)	0.20 (-2.99, 3.39)	0.58 (-1.26, 2.41)	0.63 (-2.59, 3.84)	-0.75 (-2.62, 1.12)	0.43 (-2.80, 3.64)	-1.33 (-3.11, 0.45)
SSIX	33 (-24, 90)	-15 (-66, 35)	-18 (-74, 37)	15 (-35, 64)	-11 (-68, 46)	24 (-28, 76)	-51 (-105, 3)	15 (-35, 66)	-44 (-99, 12)	40 (-13, 93)	7 (-47, 68)	10 (-42, 62)
SSIY	7 (-48, 62)	-16 (-64, 31)	3 (-50, 57)	-7 (-54, 41)	-14 (-68, 41)	-25 (-74, 24)	-4 (-58, 51)	10 (-39, 59)	-20 (-76, 35)	-9 (-59, 41)	-17 (-71, 38)	-19 (-69, 31)
SSIPOL	20 (-85, 125)	45 (-51, 141)	-27 (-126, 72)	41 (-50, 132)	-41 (-146, 63)	14 (-85, 113)	-47 (-150, 56)	-4 (-97, 90)	-62 (-170, 47)	-31 (-132, 70)	-14 (-117, 88)	-27 (-124, 70)
38%												
Crt.density (g/cm^3)	9 (-11, 30)	-7 (-21, 7)	9 (-11, 29)	**-15 (-30, 0)***	2 (-20, 24)	-8 (-23, 7)	0 (-20, 20)	-8 (-22, 6)	-7 (-29, 15)	-1 (-16, 14)	-7 (-28, 15)	7 (-8, 22)
Crt.thickness (mm)	0.01 (-0.44, 0.47)	0.09 (-0.19, 0.36)	-0.05 (-0.49, 0.39)	0.13 (-0.13, 0.39)	0.00 (-0.43, 0.43)	0.18 (-0.09, 0.45)	-0.07 (-0.53, 0.40)	0.04 (-0.23, 0.31)	-0.01 (-0.47, 0.45)	0.09 (-0.18, 0.37)	0.05 (-0.40, 0.51)	0.05 (-0.21, 0.31)
Peri.circum (mm)	0.73 (-2.24, 3.69)	0.04 (-1.24, 1.33)	-0.06 (-3.03, 2.91)	0.36 (-0.89, 1.58)	0.45 (-2.42, 3.33)	0.16 (-1.09, 1.40)	-0.78 (-3.88, 2.32)	0.32 (-0.97, 1.60)	-0.27 (-3.30, 2.76)	0.11 (-1.20, 1.43)	0.51 (-2.52, 3.54)	-0.21 (-1.45, 1.04)
SSIX	-50 (-165, 65)	84 (-94, 262)	-39 (-149, 75)	-10 (-182, 163)	15 (-111, 142)	13 (-174, 201)	13 (-99, 126)	-94 (-272, 84)	65 (-62, 192)	-71 (-263, 122)	52 (-72, 176)	23 (-165, 211)
SSIY	25 (-42, 92)	-17 (-98, 65)	2 (-63, 67)	-18 (-98, 62)	14 (-53, 81)	1 (-83, 85)	-23 (-92, 42)	-1 (-83, 80)	-11 (-81, 59)	17 (-69, 104)	12 (-56, 81)	19 (-65, 103)
SSIPOL	-37 (-177, 104)	-33 (-185, 119)	-12 (-154, 129)	-62 (-210, 87)	-4 (-149, 140)	7 (-154, 167)	24 (-108, 157)	-28 (-172, 116)	32 (-104, 169)	40 (-116, 196)	8 (-128, 144)	68 (-85, 221)
66%												
Crt.density (g/cm^3)	-13 (-37, 10)	0 (-27, 27)	-7 (-30, 16)	-2 (-27, 24)	-5 (-28, 18)	4 (-22, 31)	7 (-18, 32)	-1 (-28, 26)	8 (-17, 33)	4 (-23, 32)	1 (-23, 25)	6 (-21, 32)

Trb = Trabecular. Crt = Cortical. Peri = Periosteal. *depicts a significant difference (P<0.05). Comparisons made between multiple groups. Control – 0cm, Control – 40cm, Control – 60cm, 0cm – 40cm, 0cm – 60cm, 40cm – 60cm.

Appendix XXIII. 16-week adjusted gains Δ (95% CI) of bone characteristics measured via pQCT (Chapter 8).

	Control						0cm				40cm	
	0cm		*40cm*		*60cm*		*40cm*		*60cm*		*60cm*	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
4%												
Trb.density (g/cm^3)	-10 (-42, 22)	-11 (-33, 11)	-6 (-38, 25)	-6 (-28, 17)	-10 (-43, 23)	-8 (-32, 16)	4 (-27, 34)	5 (-17, 27)	0 (-30, 30)	3 (-19, 25)	-3 (-34, 27)	-3 (-26, 21)
14%												
Crt.density (g/cm^3)	4 (-15, 22)	8 (-14, 30)	-4 (-22, 14)	4 (-18, 26)	8 (-10, 26)	21 (-1, 44)	-7 (-26, 11)	-4 (-26, 18)	4 (-15, 23)	13 (-10, 36)	12 (-7, 30)	17 (-5, 39)
Crt.thickness (mm)	-0.09 (-0.30, 0.11)	-0.11 (-0.28, 0.06)	0.07 (-0.14, 0.28)	0.02 (-0.14, 0.17)	0.04 (-0.16, 0.24)	0.05 (-0.11, 0.22)	0.16 (-0.05, 0.37)	0.13 (-0.04, 0.29)	0.13 (-0.07, 0.34)	0.16 (0.00, 0.33)	-0.03 (-0.23, 0.18)	0.04 (-0.12, 0.19)
Peri.circum (mm)	-0.23 (-2.41, 1.96)	0.08 (-2.57, 2.72)	-0.75 (-2.93, 1..43)	-1.04 (-3.55, 1.47)	-0.77 (-2.96, 1.43)	-1.97 (-4.55, 0.60)	-0.53 (-2.81, 1.76)	-1.12 (-3.76, 1.53)	-0.54 (-2.85, 1.77)	-2.05 (-4.75, 0.65)	-0.02 (-2.32, 2.29)	-0.93 (-3.50, 1.64)
SSIX	31 (-20, 82)	21 (-33, 74)	-15 (-64, 35)	20 (-32, 73)	-25 (-76, 26)	20 (-35, 76)	-45 (-94, 3)	0 (-54, 53)	**-56 (-105, -6)***	0 (-57, 56)	-10 (-58, 38)	0 (-55, 56)
SSIY	3 (-48, 54)	-35 (-89, 20)	-23 (-73, 26)	-21 (-75, 34)	-14 (-64, 37)	-5 (-62, 51)	-26 (-77, 24)	14 (-42, 70)	-17 (-68, 35)	29 (-28, 87)	10 (-41, 60)	15 (-42, 73)
SSIPOL	-20 (-89, 49)	24 (-91, 139)	-15 (-82, 51)	24 (-85, 133)	-13 (-82, 55)	39 (-81, 156)	5 (-65, 74)	1 (-112, 113)	7 (-65, 77)	14 (-107, 136)	2 (-66, 70)	14 (-102, 1300
38%												
Crt.density (g/cm^3)	0 (-22, 22)	-7 (-30, 15)	-2 (-24, 19)	-11 (-34, 11)	4 (-19, 27)	-4 (-28, 20)	-3 (-24, 19)	-4 (-26, 18)	4 (-19, 27)	3 (-20, 27)	6 (-16, 29)	7 (-16, 30)
Crt.thickness (mm)	0.06 (-0.32, 0.44)	0.04 (-0.34, 0.41)	-0.01 (-0.38, 0.35)	0.05 (-0.31, 0.40)	-0.07 (-0.42, 0.29)	0.14 (-0.23, 0.50)	-0.07 (-0.46, 0.32)	0.01 (-0.36, 0.38)	-0.13 (-0.50, 0.25)	0.10 (-0.27, 0.47)	-0.05 (-0.43, 0.32)	0.09 (-0.27, 0.45)
Peri.circum (mm)	0.06 (-1.39, 1.51)	1.60 (-3.16, 6.35)	-0.03 (-1.48, 1.42)	0.76 (-3.76, 5.27)	0.59 (-0.81, 2.00)	0.68 (-3.94, 5.31)	-0.06 (-1.39, 1.51)	-0.84 (-5.60, 3.92)	0.53 (-0.95, 2.01)	-0.91 (-5.78, 3.95)	0.62 (-0.86, 2.11)	-0.07 (-4.70, 4.56)
SSIX	-23 (-98, 51)	-12 (-139, 114)	-30 (-103, 42)	-1 (-124, 121)	-12 (-94, 70)	34 (-100, 167)	-7 (-79, 66)	11 (-115, 137)	11 (-71, 93)	46 (-90, 182)	18 (-62, 98)	35 (-98, 168)
SSIY	8 (-75, 92)	-16 (-108, 77)	12 (-69, 94)	5 (-86, 95)	-3 (-86, 81)	39 (-56, 134)	4 (-82, 90)	20 (-73, 113)	-11 (-99, 69)	54 (-43, 152)	-15 (-101, 71)	34 (-61, 130)
SSIPOL	-31 (-213, 150)	-93 (-278, 93)	-25 (-207, 158)	-82 (-262, 99)	-46 (-232, 141)	11 (-185, 207)	7 (-164, 178)	11 (-164, 186)	-14 (-190, 162)	104 (-87, 294)	-21 (-196, 1550	93 (-94, 279)
66%												
Crt.density (g/cm^3)	-11 (-33, 12)	-6 (-46, 34)	-10 (-32, 12)	-23 (-60, 15)	-4 (-26, 18)	-13 (-52, 26)	1 (-23, 24)	-16 (-56, 24)	6 (-23, 24)	-7 (-48, 34)	6 (-17, 29)	9 (-30, 48)

*depicts a significant difference (P<0.05) Comparisons made between multiple groups. Control – 0cm, Control – 40cm, Control – 60cm, 0cm – 40cm, 0cm – 60cm, 40cm – 60cm.

Appendix XXIV. Within person variance of load (CV%) at each timepoint within each intervention group (Chapter 8).

		0cm				40cm				60cm			
	Leg	Pre	Week 6	Week 12	Post	Pre	Week 6	Week 12	Post	Pre	Week 6	Week 12	Post
IP1	Left	20	25	38	26	15	16	19	16	10	23	27	29
	Right	14	31	18	22	20	10	18	31	16	17	23	24
IP2	Left	20	40	26	34	33	21	33	34	17	17	18	17
	Right	23	40	27	35	33	22	41	37	22	17	15	19
I1	Left	14	24	12	13	30	11	21	19	15	15	19	20
	Right	18	23	13	11	28	9	24	25	17	18	16	16
I2	Left	17	22	24	14	25	20	26	28	25	25	24	23
	Right	22	18	17	22	27	21	28	28	19	22	27	22
LR1	Left	67	94	70	107	19	33	32	31	21	22	27	32
	Right	54	67	79	69	25	25	28	43	22	31	22	29
LR2	Left	39	39	34	36	19	30	25	26	43	46	23	43
	Right	37	49	59	88	38	30	53	53	56	25	22	27
PA1	Left	140	84	78	83	54	61	61	48	40	61	49	50

	Right	127	77	65	77	35	53	46	59	43	82	25	82
PA2	Left	60	42	49	65	59	63	46	58	32	59	31	56
	Right	73	57	64	72	54	67	63	58	34	62	49	75
Ankle Moment 1	Left	30	32	21	44	21	36	24	49	40	42	32	35
	Right	27	36	31	45	34	33	33	27	23	18	27	14
Ankle Moment 2	Left	35	39	17	42	33	50	27	41	22	34	22	22
	Right	33	41	46	44	34	28	32	28	17	24	20	14

IP - Impact Peak. I - Impulse. LR - Load Rate. PA - Peak acceleration.

Appendix XXV. Exit form (Chapter 8).

Dose response exit survey.

How easy was the intervention to follow? Please highlight.

Very easy
Slightly easy
Slightly hard
Very hard

How many jumps do you believe you missed during the home intervention?
_____

What did you find most difficult about the intervention?

Do you believe this to be a realistic intervention for those who do not already partake in any activity? Please circle.

Yes
No

How?
_____
_____

What do you consider your dominant leg? Please circle.

Left leg
Right leg

Any further comments
_____
_____
_____