Characterisation of Bone Loss and Development of an Electrostimulation Intervention to Improve Bone Health in Adults with Spinal Cord Injury

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Declaration

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Abstract

Bone loss following spinal cord injury (SCI) is typically more rapid compared to that associated with aging or postmenopause, leading to high susceptibility to fragility fractures, subsequent health complications and reduced quality of life. Characteristics of this complex form of bone loss have not been fully studied or understood. Furthermore, most of the available rehabilitation approaches are limited by different factors ranging from the low muscle forces that are achievable to safety considerations. Therefore, the aims of this thesis were to develop a more detailed characterisation of bone loss in the paralysed limbs following SCI, and to assess the feasibility and effectiveness of a novel approach of Recruiting Antagonistic Muscle Pairs using Electrical Stimulation (RAMP-ES) to maximise the muscle forces produced and its potential to achieve bone stimulation.

In this thesis, longitudinal bone loss in the fibula and the regional variation in bone loss across tibia cross-sections over twelve months following SCI were studied for the first time. Furthermore, RAMP-ES protocols were developed and tested in able-bodied participants before assessing the effectiveness of the novel approach on muscle and bone health in people with chronic SCI.

Longitudinal loss in BMC and BMD in the fibula was found to be smaller compared to the tibia (-6.9±5.1% and -6.6± 6.0% vs -14.8 ±12.4% and -14.4±12.4%, p=0.02 and p=0.03, respectively), in line with previous cross-sectional reports. In the tibia, bone loss was found to vary regionally in the diaphysis, with total loss at four months being a strong predictor of total loss after twelve months postinjury (r=0.84 and r=0.82 for 4% and 66% tibial sites, respectively, both $P < 0.001$). The novel RAMP-ES protocol was developed and tested in able-bodied participants and was found to be practical. A four-month RAMP-ES training schedule improved muscle size (7.3-19.8%) and strength in people with chronic SCI, but it had no clear effect on bone density.

These findings contribute a better characterisation of bone loss and showed that the RAMP-ES intervention can improve muscle health following SCI. More studies

should be done to further assess or improve the effectiveness of RAMP-ES for bone health applications.

Table of Contents

List of Tables

List of Figures

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Publications

Manuscripts

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Abbreviations

aBMD - areal bone mineral density

ASIA - American Spinal Injury Association

ASIS - anterior superior iliac spine

BMC - bone mineral content

BMD - bone mineral density

CSA - cross-sectional area

CTx - C-telopeptide

DAC - data acquisition card

DF - distal femur

DP - deoxypyridinoline

DT - distal tibia

DXA - dual-energy x-ray absorptiometry

FES - functional electrical stimulation

HR-pQCT - high resolution pQCT

iPTH - intact parathyroid hormone

IQR - interquartile range

MVC-maximum voluntary contraction

pQCT- peripheral quantitative computed tomography

PT - proximal tibia

RAMP-ES - recruitment of antagonistic muscle pairs using electrical stimulation

ROI - region of interest

SD - standard deviation

SCI - spinal cord injury

ToA - total area

TrD - trabecular density

vBMD - volumetric bone mineral density

Chapter 1: Introduction

1.1 Bone and mechano-adaptation

Bone consists of bone cells and extracellular minerals and organic matrix (which includes collagen and other non-collagenous components), which all contribute to the important mechanical, mineral homeostasis and endocrinal roles that bone plays in the human body[1]. Long bones consist of a medullary cavity and two types of bone; cortical (compact) and trabecular (spongy) bone, which are different in porosity, function and location as shown in Figure 1.1. This figure also shows the anatomical parts of long bones, which consist of a tubular shaft (diaphysis) and two extended rounded ends (epiphyses) which are connected by the metaphysis[2].

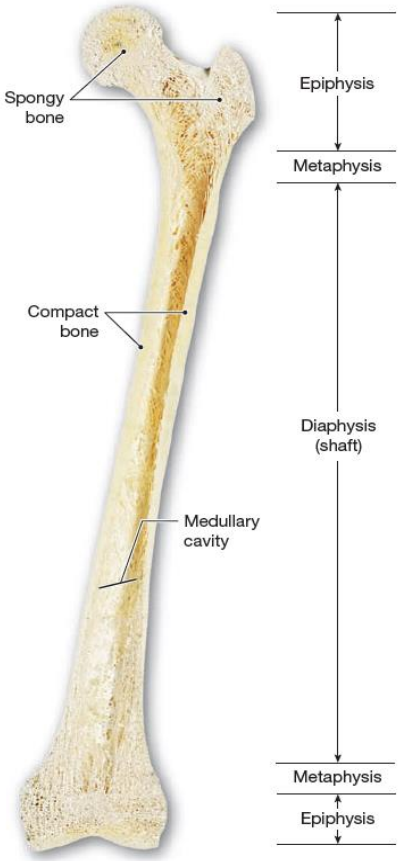

Figure 1.1: Structure of a long bone (femur) that shows the trabecular (spongy) and cortical (compact) bones and the medullary cavity. Bone diaphysis and epiphysis are connected by the metaphysis [2]

Bone tissue consists of 3 types of cells: osteoblasts, osteocytes and osteoclasts, which communicate with and respond to each other[3]. The osteoblasts and osteoclasts are responsible for bone formation and resorption, respectively[3]. The process of continuous renewal of bone tissue through formation and resorption in adulthood is called bone remodelling, which is essential to maintain bone health[4](Figure 1.2). Osteocytes plays a vital role in bone remodelling by regulating osteoblast and osteoclast function[5]. They work as mechano-sensors that detect mechanical strains, through different mechanisms, and produce factors that act on bone cells to induce either bone formation or resorption[5]. The Mechanostat theory describes how bone adapts its structure to different mechanical loading conditions[6](Figure 1.3). In the case of increased mechanical loading such as that following an increase in body size or after beginning to exercise, bone formation is induced resulting in increased bone mass and strength. For example, bone mass is up to 40% [7] and 30%[8] larger at the racket arm of tennis players and at tibias of sprinters compared to controls, respectively. However, it is essential for the load amplitude to exceed a certain threshold in order to have an anabolic effect on bone mass[6].

On the other hand, when mechanical loading decreases (unloading), bone resorption is induced, leading to bone loss, which is referred to as disuse osteoporosis. In disuse osteoporosis, bone loss is caused primarily by the sudden loss of mechanical loading such as in paralysis following SCI.

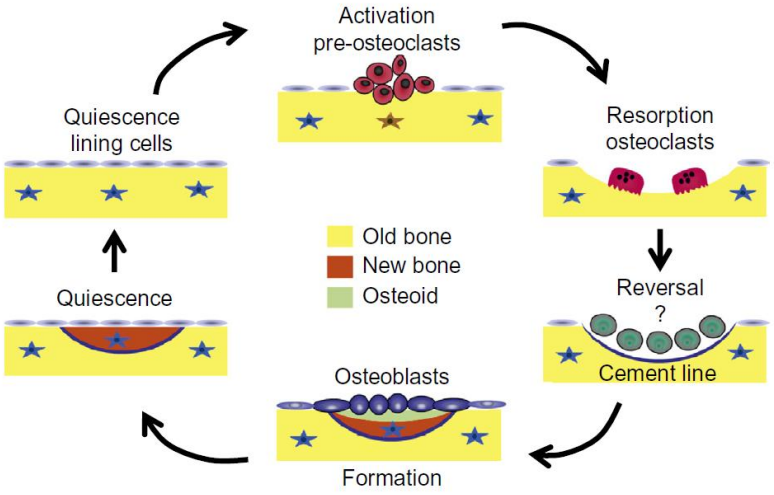

Figure 1.2: Cycle of bone remodelling: following signals produced by osteocytes apoptosis, preosteoclasts are activated on bone surface before differentiating into osteoclasts which resorb bone. Then osteoblasts deposit matrix proteins and minerals to form new bone. After bone formation, quiescent surfaces are covered by bone lining cells (flattened osteoblasts) [9]

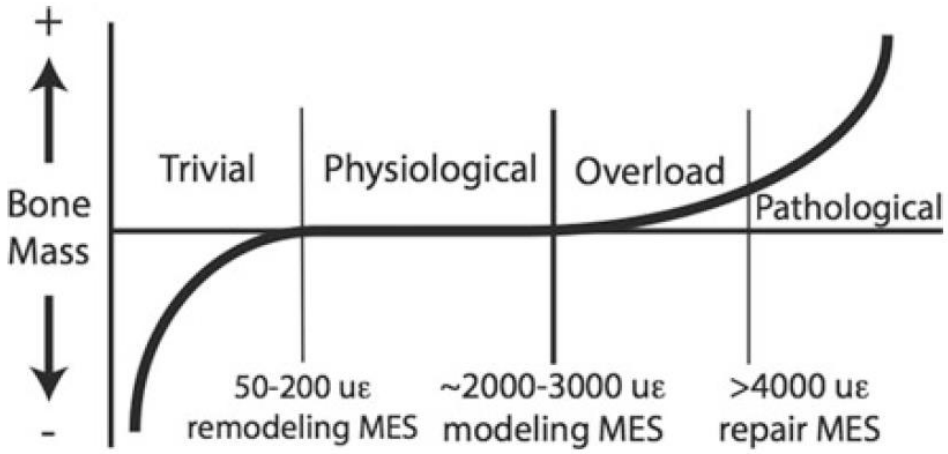

Figure 1.3: Windows and thresholds of mechanical usage proposed by Frost's mechanostat theory[10]. (MES is minimum effective strain and is measured here in microstrain - με)

1.2 Bone Strength and Osteoporosis

Bone strength is commonly estimated by bone mineral density (BMD)[11], which is defined as the bone mineral content (BMC, measured in mg), divided by the projected bone area (mg/cm^2)[12]. Areal bone mineral density (aBMD) is most commonly measured clinically using DXA and is also used as predictor of bone fracture, with one standard deviation decrease in femoral neck BMD, associated with more than 50% higher risk of sustaining non-vertebral fractures [13]. However, BMD cannot be used solely to assess bone strength without measuring bone structural properties (size, shape and architecture) which highly influence its mechanical strength[14]. For example, bone cross-sectional area (CSA) influences compressive and tensile strength, whereas increases in outer and inner cortex diameters (larger CSA and thinner cortex), with no changes in bone mass, lead to dramatic increase in bone strength against bending and torsion loads (measured by cross-sectional moment of inertia)[14][15]. Bone strength is also influenced by cortical and trabecular structure and microarchitecture[11][16]. Cortical bone material properties such as the quality and orientation of collagen fibres[17] and porosity[18][19] influence bone tensile strength and elastic properties (toughness), respectively, which are important to endure bending moments [17]. Microcracks/microdamage, which develops in cortical bone material (usually along bone cement lines) during everyday loading activities and can accumulate throughout the years (Figure 1.4) [17], have also been found to negatively affect bone toughness and stiffness (deformation resistance)[20]. Increased trabecular connectivity reduces the unsupported space within trabecular rods, leading to better strain resistance and improved strength as shown in Figure 1.5 [16]. Trabecular microarchitecture properties (connectivity, trabecular number and separation) were shown to be a major predictor of vertebral fractures in men that are independent of BMD[21].

Osteoporosis is characterised by the loss in bone macro- and micro-structure and geometry, alongside the diminishing bone strength, which puts individuals at a higher risk of sustaining fragility fractures, which are commonly caused by aging and post-menopause[22]. Individuals are diagnosed with osteoporosis if BMD of the femoral neck is 2.5 SDs below the mean of a healthy population, and with osteopenia if BMD value is greater than 1 SD and less than 2.5 SDs lower than the mean[23].

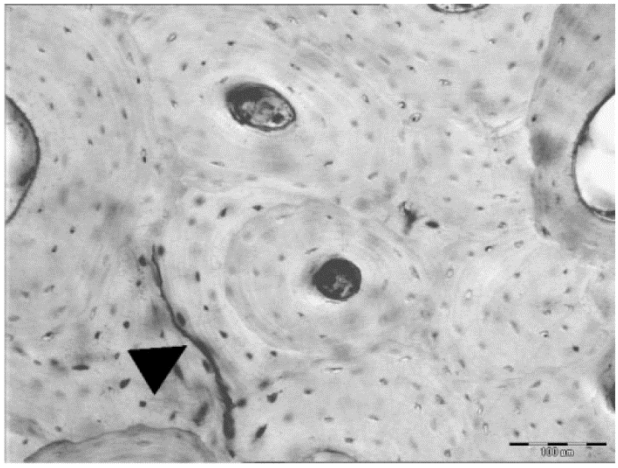

Figure 1.4: Black arrow showing a microcrack developing along a cortical bone cement line[17]

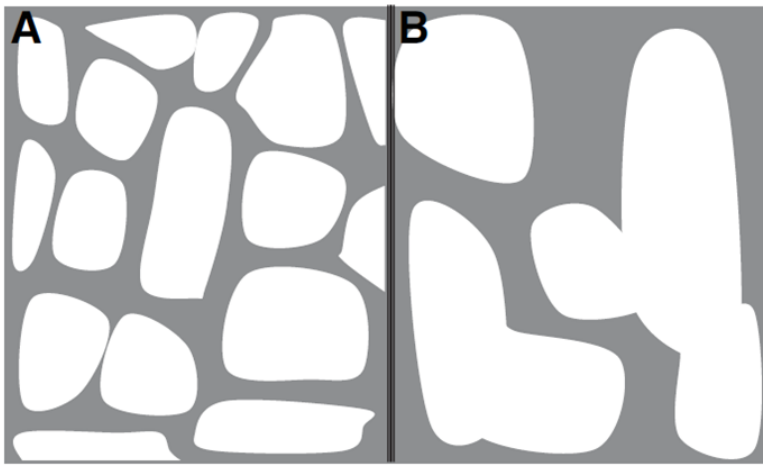

Figure 1.5: Both A and B have the same bone mass, but the many, more connected trabeculae in A are mechanically stronger that the fewer more distanced ones in B[16]

1.3 Spinal cord injury (SCI)

Spinal cord injury (SCI) affects the transmission of sensory, motor and autonomic signals on the sites below the level of the injury[24]. The global incidence rate ranges between 52-54 cases per 1 million between 1993-2012[25]. SCI is more prevalent in younger individuals and among males than females, and is mostly caused by accidents and falls[26].

SCI severity is generally classified into being complete or incomplete injury. Complete SCI is characterised by the loss of any sensory and motor function, while in the incomplete SCI some sensory or/and motor function is preserved below the neurological level[24]. SCI severity is further classified on the American Spinal Injury Association (ASIA) Impairment Scale (AIS) as:

A: Complete

B: Sensory Incomplete. Only sensory function is preserved

C: Motor Incomplete. Preserved motor function with less than half of main muscle functions have a muscle grade ≥ 3.

D: Motor Incomplete with half or more than half of main muscle functions have a muscle grade ≥ 3.

E: Normal

People with SCI experience significant physical and mental consequences following the injury, putting them at elevated risk of mortality[27] and medical complications such as pulmonary[28], cardiovascular[29], musculoskeletal[30] and psychological[31] morbidities. In this thesis, the focus will be on the effects of SCI on bone health.

In people with complete SCI, who lose motor and sensory function, bone loss is severe, rapid and site-specific, affecting sites that are below the lesion level[32]. Bone at the knee and ankle joints is more prone to fragility fractures in SCI[33], unlike the general population who predominantly experience fractures in the proximal femur and vertebrae[34]. People with SCI are at 23-fold higher risk of experiencing femur fractures compared to the uninjured population[35]. About 50% of these fractures are associated with clinical complications[36] [37] in people with SCI, such as infections and pressure ulcers[38][36]. Therefore, bone loss and fractures are important secondary clinical consequence of SCI.

Different interventions have been developed and studied to mitigate bone loss following SCI. The most-commonly used interventions are those that use electrical stimulation to induce muscle contractions and increase bone loading to try to stimulate bone formation. A more detailed description of bone loss following SCI, including its aetiology, diagnosis, temporal and site-specific variation and the results of interventional trials is provided in the next chapter.

1.4 Muscle function

Muscle contraction is classified into concentric, eccentric or isometric, depending on whether and how muscle changes in length[39]. In concentric contraction, the tension exceeds the load and the muscle shortens, whilst the muscle length increases in eccentric contractions and remains the same length in isometric contractions[2]. The magnitude of the produced muscle force/torque depends on several factors such as the length of the sarcomere (functional unit of the muscle fibre Figure 1.6), the joint moment arm and the rate of length change of muscle fibres.

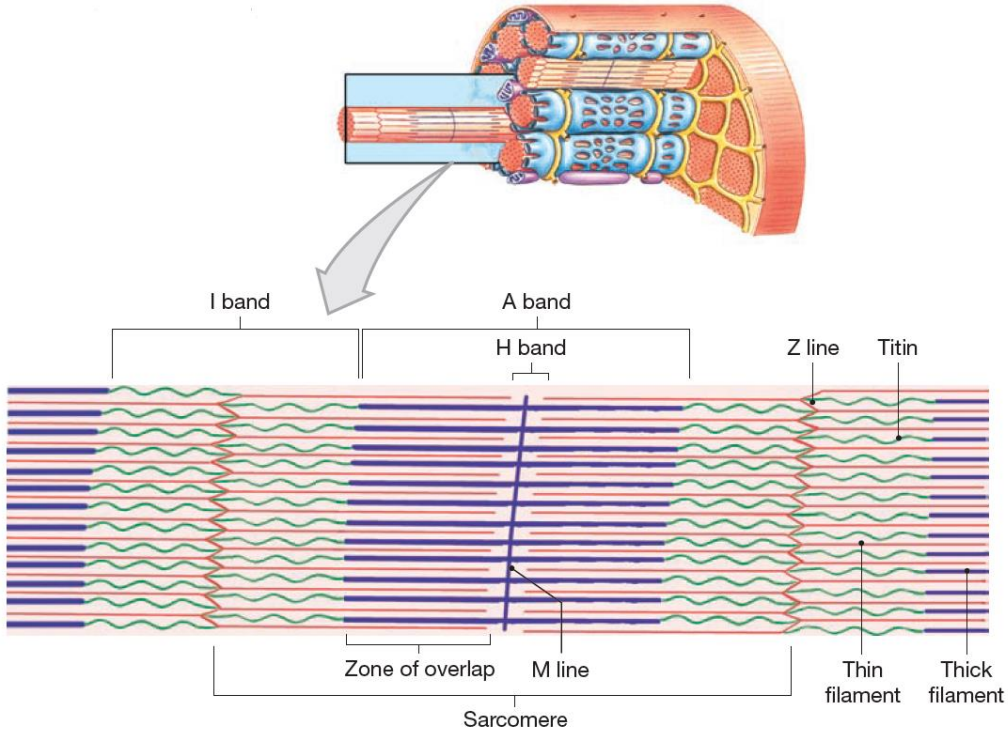

Figure 1.6: Sarcomere is the smallest functional unit of muscle fibre. Muscle contraction occurs when thin and thick filaments interact and form cross-bridges at the "Zone of overlap"

Muscle force is highly dependent on sarcomere length. Muscle should be stimulated with the sarcomere resting length being within an optimal length range, in order to produce maximal force. If the muscle is stimulated while the sarcomere resting length is too short (muscle is shortened) or too lengthened (muscle is stretched), the

muscle force produced is reduced[2]. At the joints, muscle force also depends on the length of the muscle moment arm, which is the perpendicular distance between the muscle/tendon line of force and the centre of rotation of the joint[40] (Figure 1.7). A longer moment arm length results in a greater joint torques but reduced angular velocity. The length of the muscle moment arm (and sarcomere length as well) changes at different degrees of joint angle (Figure 1.8), which is, therefore, an important determinant of muscle force/torque.

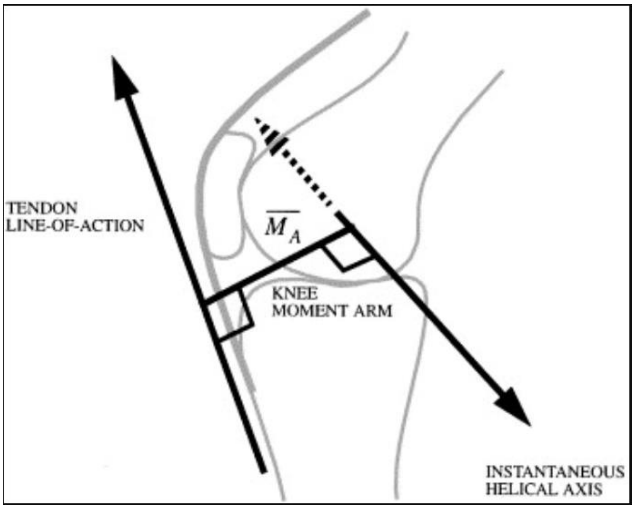

Figure 1.7: perpendicular distance between the muscle/tendon line of force and the centre of rotation of the joint[41]

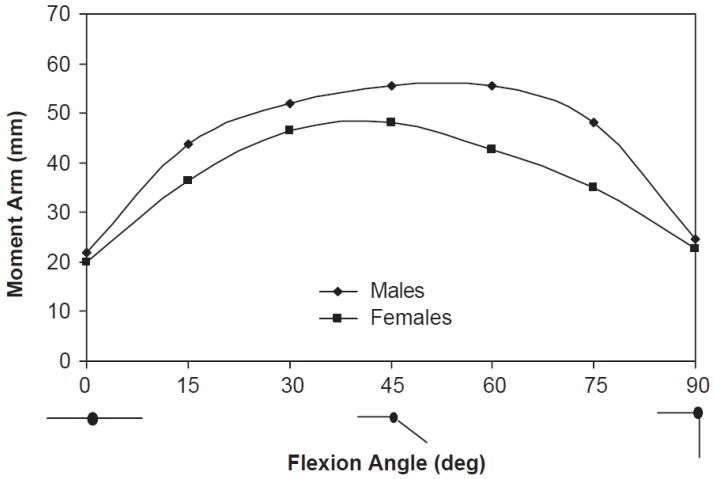

Figure 1.8: Moment arm length of patellar tendon in human knee measured at different flexion angles in males and females[42]

Muscle force is also dependent on the velocity of the sarcomere/muscle fibre contraction. Contracting at slower velocities gives enough time for more cross-bridges to form, which increases muscle efficiency. At higher shortening velocities, during concentric contractions the generated force is lower than that under isometric contractions (when the velocity is zero). Force is the greatest when the muscle stretches during eccentric contractions[43] as shown in Figure 1.9.

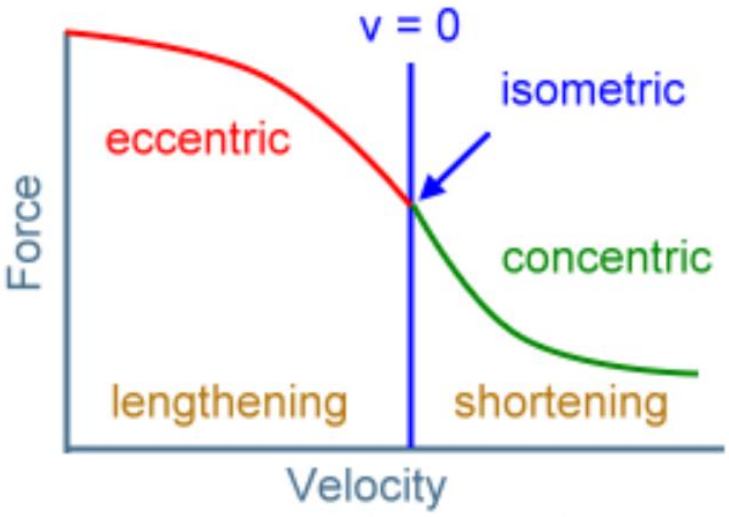

Figure 1.9: Muscle force-velocity relationship[44]

Muscle function is generally assessed during either voluntary or involuntary (electrically elicited) contractions[45]. Voluntary contraction assessments are used with able-bodied individuals who have intact neuromuscular function. Tests based on involuntary contraction (stimulated) allows assessment of those with paralysis or limited mobility [45]. Muscle strength can be assessed during eccentric, concentric and isometric conditions[46], with the latter being the most preferred one due to the safety and ease of use associated with it[45].Isokinetic dynamometry is widely used to evaluate muscle strength as well as improve muscle strength following sport injuries[47] (Figure 1.10). It reliably assesses dynamic muscle function by providing a constant velocity within a range of motion of a limb joint, as well as assessing isometric muscle strength under a constant joint angle[48].

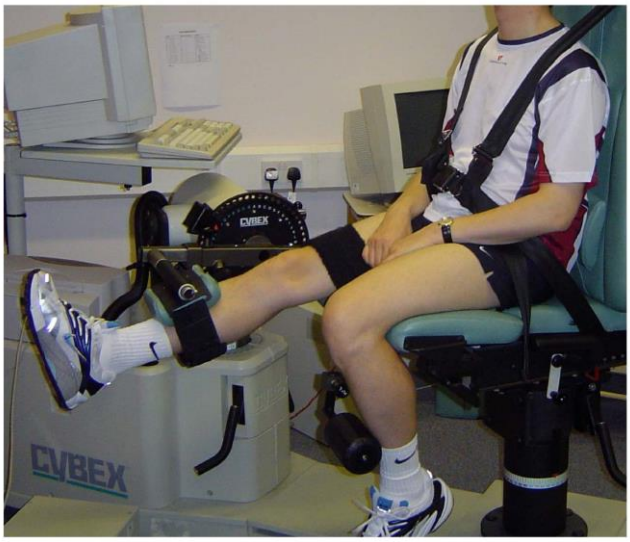

Figure 1.10: Isokinetic dynamometer used for muscle strength assessments[49]

1.5 Muscle and bone interaction

The interaction between muscle and bone occurs at different mechanical, molecular and biochemical levels, with the former thought to be the most important stimulus and consequently the area where most is known about its influence[50]. Muscle contraction is initiated by a series of processes which start by the arrival of electrical impulses (action potentials) through motor neurons at the neuromuscular junction, and leads to the release of calcium ions from the muscle fibres, which in turn start contracting[2]. When muscles contract, they shorten and pull on the tendon they are attached to, causing them to stretch. Tendons then apply this force to the bone which leads to joint torques being produced[2] (Figure 1.11).

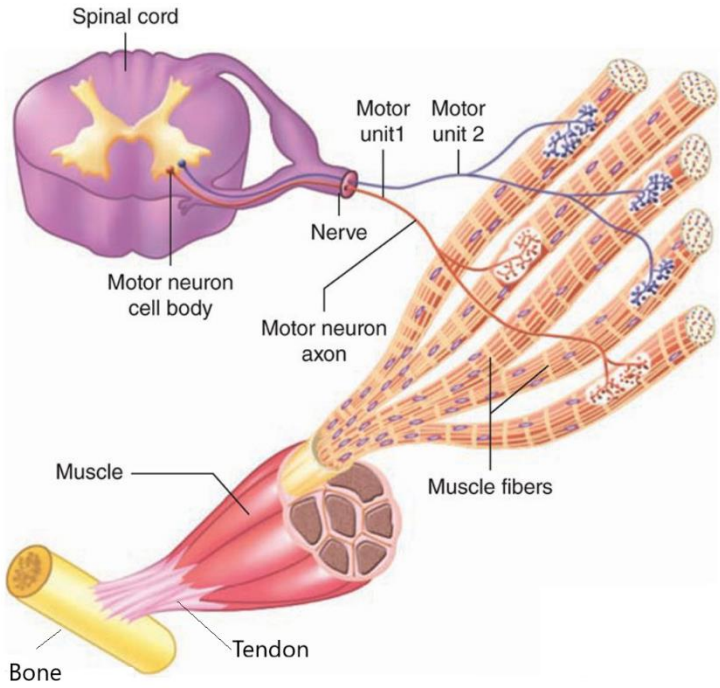

Figure 1.11: Initiation of skeletal muscle contraction by electrical impulses propagating through motor neurons to muscle fibres. Contraction of muscle fibres causes tendons to pull and move attached bones[51]

However, when the spinal cord is injured, the electrical impulses produced by the nervous system, that are supposed to induce muscle contractions, can no longer be transmitted to the motor units (neuromuscular junction). Functional electrical stimulation (FES) is used as a rehabilitation technique that produces external electrical current and delivers it to the motor neurons within peripheral nerves via surface electrodes attached to the skin[52]. This allows the paralysed muscles to contract (if the lower motor neurons or neuromuscular junction are not damaged [52]) and apply a load to the bone. Electrically-induced muscle forces are influenced by a number of factors or stimulation settings, such as electrode placement, stimulation waveform and joint angle[53][54][55]. Sustained training may act as a stimulus for muscle hypertrophy and increases in strength, in addition to improvements in bone health.

Studies in individuals without SCI showed that greater muscle forces lead to greater stimulus on bone and subsequently larger bone adaptations. This has been evidenced by greater bone mass, geometry and strength reported in sprinters compared to

endurance athletes and less active controls due to the higher muscle forces produced by high-speed eccentric contractions that occur during these sports[8][56]. In addition, from animal studies where magnitude of applied force or strain is associated with the magnitude of bone adaptation[57]. Most current ES interventions aimed to improve bone health in people with SCI produce lower muscle forces compared to those produced during daily activities[58]. For example, the compressive forces applied on the knees of uninjured individuals during walking and stair descending are 101% and 124% of body weight, respectively[59]. Furthermore, 50 Nm knee extensor torque is required to achieve standing and walking using electrical stimulation[60], which is greater than what can be produced from muscles of paralysed people (up to 3 Nm in one study)[61]. The low muscle forces induced by ES might partly be because these approaches stimulate a single muscle group which might not be sufficient to stimulate bone formation, due to the subsequent atrophy and weakness caused by the muscle paralysis. Stimulating multiple muscles simultaneously might help increase bone stimulation and induce bone formation, but this approach has not been tried before. As part of this thesis, a novel approach that recruits antagonistic muscle pairs simultaneously will be developed and tested in able-bodied individuals (Chapter 5) and in people with SCI (Chapter 6). This approach will aim at maximising muscle forces and potentially, enhancing bone strain to improve bone health in people with SCI. Indeed, the effectiveness of different interventions that uses ES to improve bone health in people with SCI is not very clear. Therefore, a thorough review of the literature was conducted in Chapter 2, to describe different aspects of osteoporosis following SCI and to assess the effectiveness of currently available interventions for people with SCI.

Moreover, developing a more detailed characterisation of bone loss in people with SCI would help improve our understanding of this complex form of bone loss, and that probably, could yield insights into developing more targeted and effective interventions to improve bone health. It could also inform current medical practice and help develop a more consistent approach for medical professionals on the detection and management of SCI-induced bone loss to prevent further bone loss and, probably, prevent fractures in this patient group.

1.6 Aims and Objectives

One of the aims of this thesis is to develop a more detailed characterisation of localised bone loss in the paralysed limbs following SCI. Another aim is to assess the feasibility of a novel approach of Recruiting Antagonistic Muscle Pairs using Electrical Stimulation and to investigate its effectiveness as physical intervention to maximise bone stimulation (RAMP-ES).

The objectives of this thesis were to:

- Characterise and compare longitudinal changes in the fibula and tibia bone following SCI.

- Characterise regional variations in bone loss following SCI, and their utility as an early predictor of the rate of subsequent bone loss.

- Develop and test the RAMP-ES protocols on able-bodied participants after considering the limitations of current available treatments.

Apply RAMP-ES intervention in people with SCI and assess its effectiveness in influencing muscle size and function, fat content and bone health

1.7 Thesis Outline

Chapter 1 - Introduction: This chapter provides an overview of the relevant topics in bone, and muscle such as bone mechano-adaptation, osteoporosis, muscle and bone interaction. This chapter ends by stating the aims and objectives of the thesis.

Chapter 2 – Literature Review: This chapter describes different aspects of osteoporosis in people with spinal cord injury, starting with its aetiology and effects throughout the years following the injury. This it reviews all the available treatments/interventions for osteoporosis in SCI.

Chapter 3 – Fibula response to disuse: A longitudinal analysis in people with spinal cord injury

This chapter is one of two chapters investigating the characterisation of bone loss in people with spinal cord injury. It describes longitudinal changes in the fibula bone in response to disuse during the first 12 months of SCI and compares these changes to those in the tibia.

Chapter 4 - Regional and temporal variation in bone loss during the first year following spinal cord injury

This is the second chapter (alongside Chapter 3) characterising bone loss following SCI. It investigates whether bone losses following SCI vary regionally within the tibia. It also assesses whether total and regional bone loss at the four-months postinjury are associated with total loss at twelve months postinjury.

Chapter 5 – Able-bodied Study

This chapter describes the effect of simultaneous stimulation of antagonistic muscle pairs on thigh muscles output, which was assessed in seven adult participants without SCI.

Chapter 6: Clinical Study of RAMP-ES stimulation

This chapter introduces in detail the methods and results of the clinical study that was conducted at Queen Elizabeth Hospital in Glasgow. This study assessed the effect of four months RAMP-ES training on muscle size, muscle strength, fat fraction and bone health of people with chronic SCI.

Chapter 7 Discussion and Conclusion: A summary of the main conclusions of this thesis are provided here.

Chapter 2: Literature Review

Osteoporosis after spinal cord injury: aetiology, effects and therapeutic approaches

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Author Disclosures

Shima Abdelrahman, Sylvie Coupaud, Alex Ireland, Elizabeth Winter and Mariel Purcell declare that they have no conflict of interest.

Abstract

Osteoporosis is a long-term consequence of spinal cord injury (SCI) that leads to a high risk of fragility fractures. The fracture rate in people with SCI is twice that of the general population. At least 50% of these fractures are associated with clinical complications such as infections.

This review article presents key features of osteoporosis after SCI, starting with its aetiology, a description of temporal and spatial changes in the long bones and the subsequent fragility fractures. It then describes the physical and pharmacological approaches that have been used to attenuate the bone loss.

Bone loss after SCI has been found to be highly site-specific and characterised by large inter-variability and site-specific changes. The assessment of the available interventions is limited by the quality of the studies and the lack of information on their effect on fractures, but this evaluation suggests that current approaches do not appear to be effective.

More studies are required to identify factors influencing rate and magnitude of bone loss following SCI. In addition, it is important to test these interventions at the sites that are most prone to fracture, using detailed imaging techniques, and to associate bone changes with fracture risk.

In summary, bone loss following SCI presents a substantial clinical problem. Identification of at-risk individuals and development of more effective interventions are urgently required to reduce this burden.

Keywords: spinal cord injury, BMD, disuse osteoporosis, electrical stimulation, bisphosphonates

2.1 Introduction

Spinal cord injury (SCI) is a life changing event that has a substantial impact on the individual's physical and mental health. A global annual incidence of 8.0 to 246.0 cases per million inhabitants has been reported[62] with an increase in the percentage of cases of tetraplegia and complete lesions over a 20 years period(1994-

2013)[63][64]. People with SCI experience secondary medical complications such as those affecting their body composition[65][66]. They experience extensive declines in bone density and strength that put them at high risk of fragility fractures and associated morbidity and mortality[67].

This review article summarises different aspects of bone loss and osteoporosis after SCI.

It discusses the factors that have been shown to contribute to the SCI-induced bone loss and describes in detail how bone loss develops in the acute and chronic phases of the injury until it reaches its steady state. Factors influencing the large inter-site and inter-individual patterns of bone loss observed in individuals with SCI are also described, in addition to clinical consequences e.g. fractures of SCI-related bone loss and associated complications. It also reviews different physical and pharmacological interventions that have been tested in patients with SCI, to investigate their effectiveness in reversing or attenuating bone loss in acute and chronic phases respectively.

2.2 Aetiology of bone loss after SCI:

The type of osteoporosis that develops after spinal cord injury (SCI) has been reported to be induced by a combination of factors. The main causal factor is understood to be from mechanical unloading[68], that has also been evidenced by the reported bone loss following space flights and bed rest[69][70]. In addition, neuronal and hormonal changes have been found to contribute to its pathogenesis[68].

2.2.1 Unloading and the bone formation-resorption imbalance

SCI causes immediate disuse and a subsequent loss of biomechanical stress on bones, which is a substantial stimulus for the bone remodelling process controlled by osteocytes[68][71][69]. This absence of mechanical loading leads to an adaptive response involving inhibition of osteoblastic bone formation and increases in osteoclastic bone resorption resulting in demineralisation. In some cases, the imbalance between bone formation and resorption is so great and sustained that it leads to severe bone loss.

This has been widely documented in both acute and chronic SCI. Bone resorption biochemical markers in blood and urine, such as total deoxypyridinoline(DPD) ,N-telopeptide (NTx), serum and urinary type I collagen C-telopeptide (CTx) and hydroxyproline have been found to be significantly increased in acute[72][73][74][75] and chronic SCI, but significantly lower in chronic than in acute SCI[75]. Nonetheless, elevated levels of DPD were evident in 30% of patients 10 years or more after injury[76]. This significant rise in bone resorption rate after SCI has been found to be associated with an increase in osteoblastic bone formation activity, evidenced by minor increases in serum osteocalcin(one month after injury)[74] and total alkaline phosphatase. However, there is no consensus on the significance of these small increases in osteoblastic activity, which were found to be minor in some studies[72] but substantial in others[77][73].

The increase in bone resorption and the associated calcium efflux from bones after SCI leads to abnormally high concentrations of calcium in blood (hypercalcemia)[78]and urine (hypercalciuria)[79][80].

2.2.2 Neurovascular changes

Bone also undergoes neurovascular changes caused by the neurological lesion and the subsequent disturbance of bone tissue innervation[68]. Whilst it is clear that the loss of motor function and the associated reduction in bone loading caused by neural damage contribute substantially to bone loss following SCI, the contribution of other neurally-mediated mechanisms of bone loss is less clear[81]. Some of the neuronal changes are directly caused by the significant reduction in sensory and autonomic nerve fibres and nerve-derived factors (neuropeptides) which have been documented to regulate and modulate bone metabolism[82][68][83].

The autonomic nervous system has been documented to regulate skeletal metabolism through different pathways[84][85]. Increased activity of the sympathetic nervous system is known to suppress bone formation and favour bone resorption[84]. Subsequently, one would expect that the attenuated sympathetic activity after SCI should increase bone mass instead. It is clear that the extensive bone loss that occurs after SCI cannot be explained directly by the attenuated sympathetic activity[86]. Furthermore, interruption to the sympathetic system and the subsequent vasomotor

irregularity can better (and in part) explain the loss in bone mass[87]. The interrupted sympathetic nerves (which have their processes distributed along bone vessels[88]), lead to vascular modification in the sub-lesional areas[68][89]. Changes in bone blood flow after SCI have been evidenced by high intramedullary pressure and arteriovenous shunting in the legs leading to venous stasis and adverse consequences for bone metabolism[90][77]. The effect of the reduced parasympathetic activity after SCI[91] is an area of active research, and our understanding of its role in regulating bone metabolism is currently limited[86][84], which further emphasises the multi-factorial and complex aetiology of bone loss after SCI.

2.2.3 Hormonal changes:

Hypercalciuria is prevalent in the acute phase of SCI as a result of the abnormally high ionised calcium levels which are found to get back to normal during the chronic phase[92]. These changes (in the acute phase) are followed by changes in calcium regulatory hormone levels. Serum intact parathyroid hormone (iPTH) level was found to be suppressed in acute and sub-acute SCI as expected for this negative feedback loop (1-4 months)[73][72][74][93]. It increases in the chronic phase compared to the acute phase but it stays within or below the lower reference ranges[92]. Only one study reported a decreased level of PTH in the chronic phase, which was associated with normal ionised calcium levels[79]. This decline in PTH has been suggested in this study to be driven by “low-grade increased calcium release”, which in turn indicates persistently elevated bone resorption even after years of injury[79], a hypothesis that is also supported by the high levels of bone resorption markers reported during the chronic phase[92].

Reported changes in vitamin D levels in acute SCI, show reduced levels of 1,25(OH)2 vitamin D (the biologically active form of vitamin D), as a result of bone resorption and the suppression of PTH[74][93]. However, different results were reported for 25-hydroxyvitamin D (25(OH)D) in the acute phase: with one study reporting normal[93], and others reporting low levels[94][95]. Using different reference values to define normal levels of vitamin D level and other factors such as ethnicity and season might have contributed to this variability in their results. In

people with chronic SCI, low levels of 25-hydroxyvitamin D (25(OH)D) have been found to be prevalent[96][97][95], which might be accompanied by mild secondary hyperthyroidism[98]. This could be due to limited exposure to sunlight, prescription of medications that increase vitamin D metabolism and, perhaps, restricted dairy intake[97]. Only one study reported normal levels of 25(OH)D in the chronic phase, which was explained by the majority of medically stable and active participants included in this study[79].

SCI is associated with severe muscle atrophy affecting the sublesional areas[99][100] within the first few days after injury[101][102]. About one third reduction in thigh muscle cross sectional area (CSA) has been found to occur within only 6 to 24 weeks postinjury[101] [103]. The denervation and atrophy of these muscles (alongside other factors such as reduced physical activity) are believed to be one of the determinants of insulin resistance[66], which is also associated with increased intra-muscular fat in people with SCI[104].

Sex hormones also play a major role in regulating bone metabolism. Oestrogen has been shown to prevent osteocyte apoptosis[105], and both oestrogen and androgen inhibit bone resorption and promote bone formation via many mechanisms[106][107]. SCI causes the inhibition of sex hormone production and secretion[68]. In people with acute SCI, testosterone levels were significantly lower than in the uninjured control group, with no further change after week 16 post injury[74].

When investigating hypothalamus-pituitary-ovary and hypothalamus- pituitary-thyroid axes in women with SCI, approximately 80% women were found to have at least one axis abnormality[108]. These findings suggest that bone loss can be linked to impaired endocrine function in people with SCI.

2.3 Structural and geometric changes in bone after SCI:

2.3.1 Densitometric assessment of bone parameters after SCI:

Most of the studies on bone loss in the early acute phase and throughout the chronic phase following SCI, have used medical imaging technologies such as dual-energy x-

ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT).

DXA enables scanning of sites that are generally the most susceptible to osteoporotic fractures in the general population such as the vertebrae and proximal femur (hips) and wrist[34], aiding in detection of osteoporosis and reducing the risk of such clinically and economically costly fractures[109].

Furthermore, its low radiation dose, low price, wide availability and ease of use have all made it the predominantly used technique to diagnose osteoporosis[110][109] and assess susceptibility to fractures clinically[111]. For similar reasons, it has been the default densitometric technique for measuring bone density in clinical trials[110] to evaluate the effectiveness of interventions.

However, DXA measures areal BMD, which is highly affected by bone size, leading to larger bones appearing to have a greater density than smaller bones[109] resulting in both bone loss and fracture risk being underestimated[112]. DXA's inability to extract three-dimensional measurements of bone geometry limits its utility in describing detailed components of bone structure relevant to fracture risk[109][12]. Moreover, DXA does not distinguish between trabecular and cortical compartments[109]. This limitation of DXA is particularly relevant in disuse osteoporosis due to the differences in the extent and time-course of bone loss in these two bone compartments.

There is good evidence that, in people with SCI, trabecular bone has a more rapid response to disuse[76][113][114][115] and interventions[111] compared to cortical bone (when considering the percentage of the loss in bone). Therefore, it is recommended to obtain separate, detailed trabecular and cortical BMD measurements in order to assess the effectiveness of different bone interventions.

pQCT and high resolution pQCT (HR-pQCT) provide detailed volumetric parameters of trabecular and cortical compartments[33] making these techniques clinically relevant for this population. PQCT creates a two-dimensional map of attenuation values while the x-ray tube is rotating around the patient[116]. These attenuation values are expressed by Hounsfield units (HU) using the equation:

$$HU = 1000 * (\mu_T - \mu_w)/\mu_w$$

Where:

HU: the CT number in Hounsfield units,

μ_T: the linear attenuation coefficient of the tissue

μ_w: the linear attenuation coefficient of water (the CT number of water is 0 HU)[116]

A calibration phantom made of known concentrations of calcium hydroxyapatite (bone-equivalent) in water-equivalent plastic is used to convert the CT numbers to equivalent bone mineral density values[116].

Analysis of bone parameters using pQCT requires segmenting bone from the surrounding tissues using a thresholding criteria, so that values greater than the threshold are considered bone, while those less than the threshold are considered soft tissue[117]. Setting thresholds, especially for cortical bone is essential to accurately assess cortical geometry and density outcomes and create images that reflect the actual bone size[118]. If the threshold is too low, the segmented bone size will be too big and vice versa, setting a high threshold will result in missing parts of the bone and the segmented region will be too small[117]. Therefore, better thresholding techniques that give more accurate results have been developed and reported in the literature[117][119]. For example, the most accurate technique for calculating the optimal threshold for cortical thickness for a pQCT scanner requires scanning a standard bone-like phantom (such as the European Forearm Phantom) with a known wall thickness using a gradually increasing thresholds to create a cortical thickness-threshold curve, and the threshold that corresponds to the known wall thickness of the phantom will be the right threshold[119].

Image values are then averaged throughout the segmented bone region to calculate bone density, which give reasonable results when analysing total bone at distal sites[117]. However, when analysing cortical cortex, cortical density can be underestimated at the pixels that contain percentages of both soft and bone tissues, which is known as the partial volume effect[117]. This issue increases at lower image geometric resolution and when obtaining scans from patients with

osteoporosis and , specifically, thin cortical thickness[120]. The partial volume effect issue can be tackled by increasing the used threshold, which would exclude pixels with partial volume effect, which would have lower values compared to adjacent bone pixels[117].

pQCT and HR-pQCT assess volumetric density (vBMD) that considers bone depth (BMC/cm^3)[111] and quantify both hard and soft tissues within the region of interest[12]. pQCT also allows detailed, site-specific examination of regions such as the distal femur and proximal tibia[121]. These areas are particularly prone to fracture in individuals with SCI, who lose bone mass exclusively in sublesional sites[115] as shown in Figure 1. In contrast, the most commonly performed regional DXA scans focus on the proximal femur and the lumbar spine.

HR-pQCT acquires 3D images of bone microarchitecture which is of importance in assessing bone strength in individuals who are susceptible to fractures[121][109], especially people with SCI[33]. It provides trabecular and cortical parameters such as trabecular number, separation, anisotropy and trabecular and cortical thickness[109], shown to improve fracture risk prediction[122][123].

But in order to monitor bone changes accurately in these patients, the machine precision should be taken into consideration. The most commonly way of measuring the densitometer precision is by calculating the Least significant change (LSC), which indicates the smallest true change in a patient's bone density that can be measured by a densitometer with 95% confidence[116]. It is calculated according to the formula:

$$LSC = 1.96 * (\sqrt{2} * CV_{long})$$

Where CV_{long} is the long-term coefficient of variation of the scanner[116].

Despite their capabilities, these techniques impose certain practical constraints as imaging of patients with bilateral metal implants or spasticity that could cause movement artefacts is challenging[124]. Both pQCT and HR-pQCT are more sensitive to subjects motion and require them to stay still for longer times compared to DXA, which can make it difficult to achieve especially in children[125].

Movement artifacts reduce the quality of pQCT scans and can lead to missing data ot the need to rescanning[126]. HR-pQCT can only be used to scan peripheral sites such as distal radius and tibia due to its small field of view, which is compromised to achieve the optimum resolution[121]. Moreover, the 140mm gantry diameter makes it unusable for scanning obese patients or regions such as the thigh and proximal femur[124]. Nevertheless, these techniques offer detailed bone microarchitecture measurements for people with SCI, who are of higher risk of sustaining osteoporotic fractures mostly in their lower extremities, compared to the spine and upper extremities that are less prone to fracture[33][35]. Bone loss after SCI occurs at sublesional sites only, leading to upper limb bone health being preserved in people with paraplegia whilst people with tetraplegia are at risk of developing osteoporosis in both the upper and lower limbs[127][128].

Notwithstanding the substantial advantages that pQCT and HR-pQCT offer over DXA, they are not currently recommended to be used routinely by clinicians in the diagnosis of osteoporosis, fracture risk prediction or assessment of bone conditions treatments. This is due to the lack of international standards that regulate its clinical use with regards to aspects such as scanning and analysis protocols and anatomical sites, which were recommended recently by Cervinka et al[129][129].

The absence of standards for the clinical use of pQCT, combined with the ready availability of DXA machines in clinics has driven the development of protocols to make DXA scans more clinically relevant to people with SCI. Different DXA protocols and software have been developed and validated to acquire and analyse images at the knee region, which until now has not been a standard measurement site in DXA. Methods that extract sub-regions from total body DXA scan are not recommended due to their poor repeatability and image resolution[130]. A commercially available GE lunar software for the knee region has FDA approval and is being used for both clinical and research purposes[131][132][133]. Few studies investigated the accuracy of conventional DXA software designed for lumbar spine, proximal femur and forearm, in predicting BMD and fracture risk at the knee[134][135]. A DXA forearm software has been validated by McPherson et al.(2014) to be used to measure BMD at the knee in people with SCI[136]. It was found to be accurate in short-term assessments and has been proposed as a reliable

method to assess BMD at the distal femur and proximal tibia using DXA in clinical trials[135]. The International Society of Clinical Densitometry recommended a BMD analysis protocol based on lumbar spine software for the calculation of BMD at the knee[130][137],until knee-specific software is developed. There remains the need for valid manufacturer-adopted knee software to accurately measure BMD at the distal femur and proximal tibia for clinical use[130].

2.3.2 Time course of bone changes after SCI

Many longitudinal studies on people with SCI emphasise the substantial effect of time since injury on bone structural and geometric parameters during both the acute (5 weeks-12 months)[138] and the chronic phase[139]. Bone resorption markers have been shown to increase significantly to maximum levels within 10–16 weeks postinjury[72]. In the same study, BMD losses in the lower limbs were detected at follow-up (24^{th} week postinjury)[72]. Bone mass continues to decrease with time throughout the first 8 months[140], 12 months[138], 2 years[141] and even throughout the chronic phase (up to 19-25 years)[142][143] but at a slower rate in the later phases compared to the rapid loss during acute phase[113]. However, some studies found that bone loss reaches steady-state phase at 3-8 years postinjury depending on the bone parameter measured[114][139] with about 50% and 60% loss in bone mass in the femoral and tibial epiphyses, and 35% and 25% in the femoral and tibial shafts respectively[114] . Determining the time course of the adaptive modifications in bone geometry and structure is of clinical importance to assess the effectiveness of different rehabilitation interventions in reversing bone loss[139].

2.3.3 Patterns and time course of loss in cortical and trabecular compartments

Bone loss at epiphyseal sites (which are rich in trabecular bone) has been attributed to the decline in trabecular BMD with comparatively little loss from the outer cortical shell[144]. In contrast, bone loss at diaphyseal sites composed primarily of cortical bone is suggested to be characterised by a reduction in wall thickness via endocortical resorption in addition to smaller decreases in cortical BMD[76][12][145] as shown in Figure 2.

Exponential decreases in bone parameters with time postinjury (2 months to 50 years) have been described using pQCT, specifically in bone mass, total and trabecular BMD (BMDtot and BMDtrab, respectively) of the femoral and tibia epiphyses, as well as bone mass and cortical CSA of the diaphysis as shown in Figure 1[114]. In a longitudinal study using pQCT scans, significant decline in cortical BMD and cross sectional area (CSA) have been reported alongside the epiphyseal changes within the first 12 months postinjury[138]. In the acute phase, tibial and femoral cortical BMD losses make a substantial contribution to bone loss at these diaphyseal sites[138]. It has been suggested that acute cortical BMD losses may be transient, resulting from increased remodelling in the early stages post-injury[114]. Indeed, in the chronic phase cortical bone losses at these sites appear primarily attributable to decreased cortical bone cross-sectional area with little or no contribution of BMD losses reported[114]. Nevertheless, the loss in trabecular BMD seems to occur at a more rapid rate acutely and is of greater magnitude in the chronic phase than that in the cortical bone[114][146] as shown in Figure 2.1. Lower BMD in the proximal femur[67][143][147], femoral shaft and lumbar spine[147] have also been correlated with time since injury.

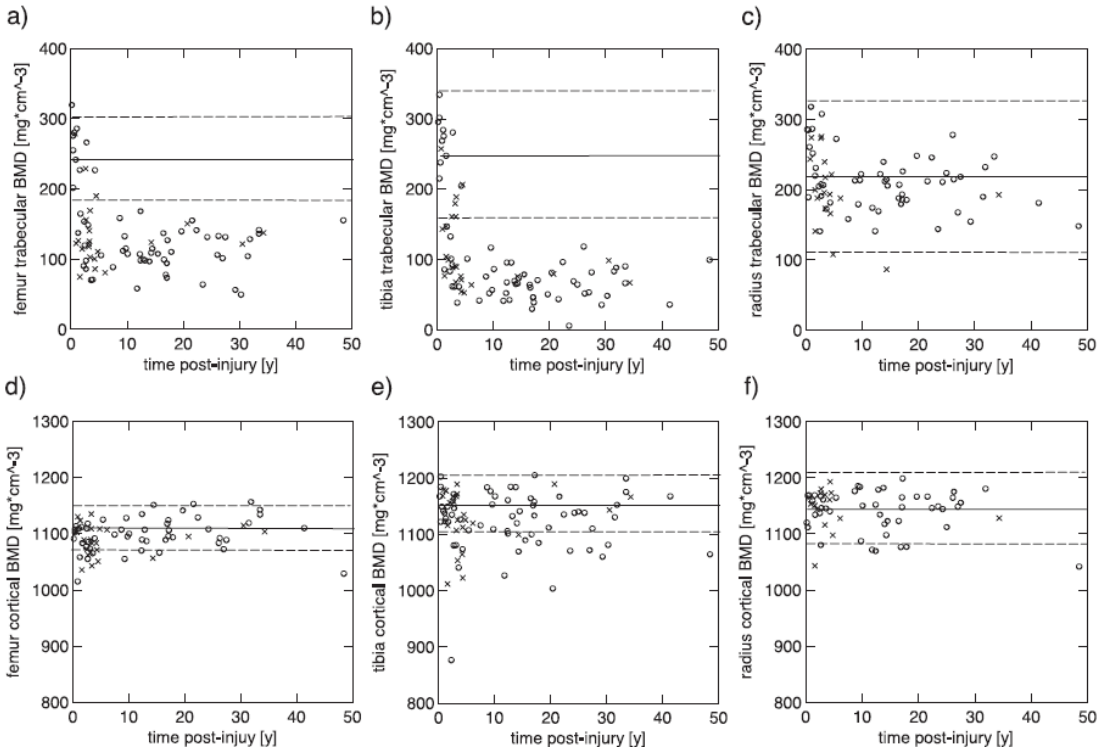

Figure 1.12: Differences in trabecular (upper row) and cortical (lower row) BMD with time (2 months to 50 years) postinjury in the femur (a,d), tibia (b,e) and radial (c,f) bones within a group of individuals with SCI-induced paraplegia (0) and tetraplegia (x)[114]. (Reproduced with permission)

2.4 Characteristic temporal and site-specific patterns

2.4.1 Acute SCI

During acute SCI (measured between week 8 and 12 months postinjury), DXA reveals significantly lower BMD in the lower limbs[74][72], total body, pelvis[74], proximal femur[75], midshaft and distal femur[148] with no difference in the hip (between week 8 and week 24 postinjury[72], although BMD loss might have been detected sooner than 24 weeks if the protocol had allowed it), lumbar spine or radius [72][74][75]. After only one year postinjury the distal femur and proximal tibia lost up to 52% and 70% of their BMD respectively[128].

pQCT scans during this phase showed a significant decline in tibial trabecular BMD(at 6 and 12 months) as well as in cortical BMD (only at 12 months)[138][149]. Other epiphyseal (BMC, total BMD) and diaphyseal (BMC, cortical CSA) parameters of the tibia and femur bones also decreased[138]. Lower trabecular BMD was reported in the radius and ulna(at 6 & 12months) and cortical BMD (at 12 months) in people with tetraplegia with no differences detected at these sites in those with paraplegia[149]. pQCT results in acute SCI further emphasise the rapid rate of trabecular loss compared to cortical loss.

2.4.2 Chronic SCI

Significant decreases in BMD and BMC have been documented at the distal femur[150][151] [152], proximal tibia[150][151][153] proximal femur[75][154], femoral neck[155][151][152][113][156], femoral shaft[151] and total femur[152]. This BMD loss seemingly occurs in the lumbar spine as well[112][157], but was previously undetectable by conventional DXA[75][158]. This was likely due to an overestimate of bone mass resulting from neuropathic calcification and other skeletal abnormalities in the vertebrae of people with SCI[112].

pQCT scans in people with paraplegia revealed significantly lower tibial total BMD[144][146], trabecular BMD, cortical BMD, and cortical thickness, as shown in

Figure 2.2, alongside similar periosteal and increased endosteal circumferences[146] compared to controls. These findings in cortical parameters further confirm the proposed mechanism of cortical thinning by endocortical resorption[114].

BMD and BMC at the distal and proximal tibia epiphyses[141][154] and in the patella have been reported to be significantly less than those in controls. Sabo et al. reported that the cortical area at the distal femur and proximal tibia was lower than that in controls but no difference in cortical BMD was reported in this study[154]. The loss in volumetric BMC was more pronounced at the distal and proximal tibia than in the diaphysis[141][146].

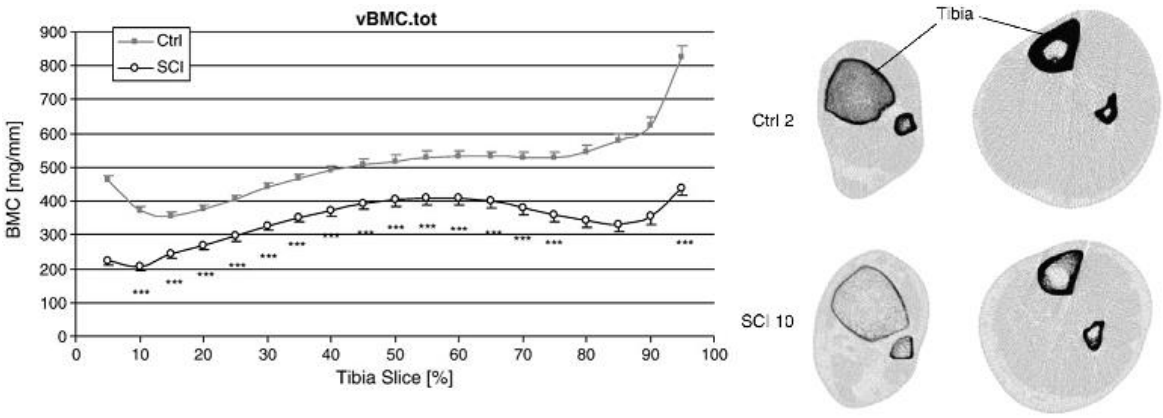

Figure 1.13: (Left): compares volumetric BMC at different sites along the tibia (starting from distal tibia at 5% of the tibial length and moving toward the proximal epiphysis in steps of 5% (up to 95% of the tibia length) between controls and participants with SCI. It also shows the more pronounced bone loss at the epiphyses compared to the diaphysis between the two groups. (Right): shows pQCT images of the tibia distal epiphysis (left column) and diaphysis (right column) in an uninjured control (upper row) and an individual with SCI (lower row). The decrease in trabecular BMD and the cortical thinning at epiphysis can be seen clearly, alongside the cortical loss/trabecularisation in the diaphysis[144]. (Reproduced with permission)

2.5 Factors influencing rate and magnitude of bone loss following SCI

It has been shown that there are inter-individual and site-specific differences in the rate of bone loss after SCI(Figure 2.3), with some individuals approaching published BMD fracture thresholds within only 1 year post-injury (around 67% loss in the distal tibia trabecular BMD) while others experience minor BMD reductions within

the same period(around 1% in distal tibia trabecular BMD)[140]. Intra-individual differences have also been reported in another study where bone loss was greater at the proximal tibia compared to the distal tibia and more pronounced in the epiphyses than in the diaphysis (see Figure 2)[144], with evidence that inter-site variance in bone loss may be related to bone geometry[138][144].

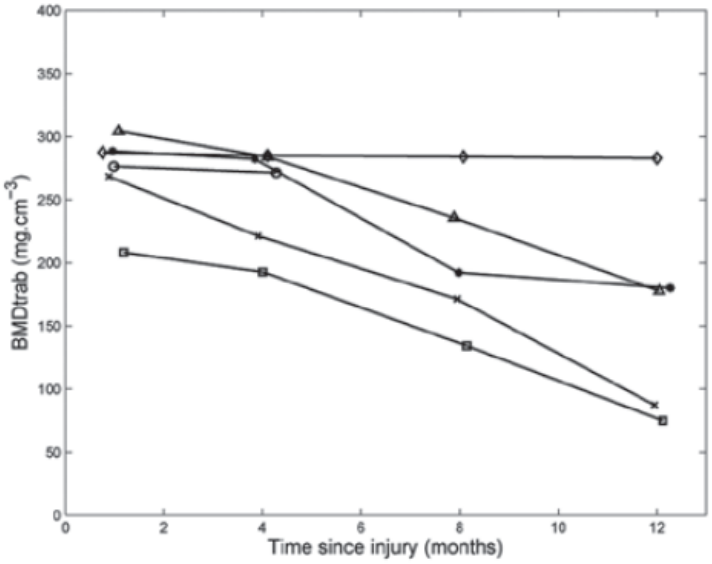

Figure 1.14: Trabecular BMD of the distal tibia measured in six participants shortly after complete SCI, and 4, 8 and 12 months postinjury[140]

An inverse relationship was found between BMD and time since injury [147][152][159]. Unsurprisingly, bone loss has also been found to be influenced by the type, level and completeness of injury (and resulting function). Lower BMD has been reported in the upper extremities [114][127][159] and lumbar spine[127] in people with tetraplegia, compared to those with paraplegia. Patients with complete SCI have been found to have significantly lower BMD than those with incomplete injuries (BMD= -2.29 ± 0.51 in complete versus -0.12 ± 0.22 in incomplete, $P< 0.05$) [159][150]. Bone loss has also been found to be influenced by the level of injury so that individuals with higher spinal lesions tend to have lower BMD (in the affected skeletal sites) compared to those with lower lesions[115][151][157], but this primarily depends on the injury completeness. While it is likely that function linked

to lesion level affects the extent of bone loss, to date these associations have not been reported in the literature.

Neither age[142][150] nor sex have been found to have an effect on bone parameters after SCI[138]. However, a significantly lower cortical BMD has been reported in females compared to males in one study, suggesting a possible effect of gender on cortical bone parameters which was proposed to be influenced by the postmenopausal females participating in this study[138]. The possible effect of spasticity on BMD seems to be unclear with two studies reporting less loss in BMD in spastic compared to flaccid patients[159][160] and other studies reporting no differences in BMD[151][149][161][162] between the two groups. However, in one of the studies that reported a positive effect of spasticity on BMD[159], the 41 tested participants were a mixture of individuals with paraplegia and tetraplegia, and complete and incomplete SCI. It is clear that the 2 groups (spastic and flaccid) were not matched for these influencing factors.

2.6 Fragility fractures after SCI

Osteoporosis is characterised by reduced BMD and deterioration in bone micro-structure that consequently leads to reduced bone strength and increased susceptibility to fracture[109]. The link to fracture risk has been demonstrated as bone geometry parameters[137] and BMD were found to be lower in participants with SCI who had lower limb fractures compared to those with no fracture history[137][76][151][67].

Fragility fractures are common and unresolved consequences of SCI[33] and are mostly caused by minor trauma[37] during transfer between surfaces or turns in bed[163][38][164] or even during rehabilitation training sessions[165][166]. They are more frequent in patients with SCI compared to the general population(14)and occur predominantly in the lower extremities[76][33]; fewer[36] or even no fractures have been reported in the upper limbs[35][33]. The majority of these fractures occur in the femur and tibia[37] especially at epiphyseal sites at the ankle and knee joints[33][36]. This can be linked to the more dramatic and rapid rate of bone loss

documented in these trabecular bone-rich site compared to that in the cortical-rich shaft.

In general, people with SCI are more likely to sustain bone fractures in the chronic phases starting at a mean of 3 to 8.9 years postinjury[76][37][35](Figure 2.4). Annual fracture rates of individuals with a SCI are double those in uninjured (2% and 1% respectively)[35] but this additional risk is highly site-specific. People with SCI have a 23- fold higher risk of experiencing femur fractures whereas upper limb fracture risk is lower compared to controls[35].

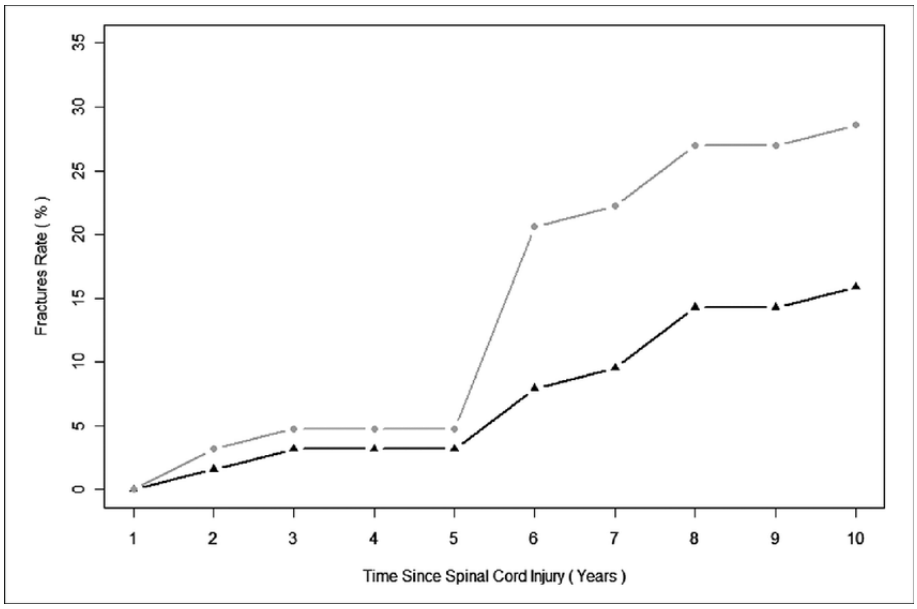

Figure 1.15: Cumulative rate of fracture recorded during the first 10 years postinjury. The black line with triangles represents the rate of patients sustaining new fractures while the grey line with circles represents the rate of newly sustained fractures per patient[37]. (Reproduced with permission)

2.6.1 Fractures risk factors:

Fractures are more frequent in women and also in men with a family or personal history of fractures[35][167]. Fracture risk increases with time since injury[37][167], reaching 4.2% per year in individuals who sustained an SCI more than 20 years ago[76]. Severity of SCI has also been found to be a contributing risk factor, with fractures found to be more common in patients with complete SCI than in those with incomplete injuries[37][36][167]. These factors have been shown previously to

contribute to the magnitude of bone loss following SCI, illustrating the correlation between severity of bone loss and fracture risk in these patients. Other factors associated with high risk of fracture include white ethnicity[167] and higher alcohol consumption[36].

It is worth pointing out that our understanding of many aspects of fragility fractures after SCI such as risk factors and fracture thresholds, is limited by the few studies addressing these issues. More studies should be carried out to obtain clarity which should result in the development of effective interventions.

2.6.2 Complications of fractures:

About 50% of fractures in individuals with SCI are associated with clinical complications[36] [37] such as infections, pressure ulcers[38][36], delayed healing, autonomic dysreflexia, increased muscle spasticity and depression[36][168]. Delayed union can lead to further surgical interventions and therefore prolonged hospitalisation and increased cost[33].

Effective interventions that target bone health and attenuate the rapid decline in bone microstructure and geometry should be incorporated into the patient's treatment plan as soon as they are clinically stable. However, there is currently no evidence that supports the effectiveness of any intervention in preventing fragility fractures.

2.7 Therapeutic Interventions targeting bone loss after SCI

Different physical and pharmacological interventions have been tested in patients to investigate their effectiveness in reversing or attenuating bone loss in the acute and chronic phases, respectively.

2.7.1 Interventions based on electrical stimulation (ES):

Different ES-induced interventions have been employed to improve muscle and bone health and attenuate their deterioration after SCI by eliciting muscle contractions and thereby restoring bone loading. Functional electrical stimulation (FES) techniques applied to the paralysed limbs of people with SCI use surface electrodes to either

activate one muscle group to produce joint extension or flexion [169][170][171][60][172], or induce co-ordinated contractions of two or more muscle groups to produce functional movements such as cycling and rowing[173][174][175][176][177]. While pronounced improvements in body composition [178][179] muscle geometry[180][174][175][181] and functional properties[169][60][181][182][183] have been widely documented, the evidence for the efficacy of FES interventions in attenuating bone loss is equivocal.

Significant site-specific improvements in BMD (ranging between 7-30% increase in BMD) have been reported in some of these studies[170][171][60][175][176][177] as shown in Figure 2.5. In contrast, others found no effect on BMD after undergoing 5-12 months of electrical stimulation-induced training[169][172][173]. Table 1 provides a summary of all studies that used ES- interventions to attenuate the loss in BMD after SCI.

This discrepancy in the documented results of FES on bone health might be due to different factors related to the patient population, intervention protocols and the imaging modalities used to assess changes in bone[184]. For example, a patient's postinjury duration and level of injury seem to influence intervention effectiveness considerably. Studies showed that starting FES interventions within the first few weeks (1-7 weeks) after SCI was effective in attenuating BMD decline in trabecular-rich regions of the femur and tibia[177][182] but with no effect on cortical BMD[173]. Individuals with paraplegia seem to achieve greater FES-cycling power when compared with people with tetraplegia (22.5 W and 4.8 W, respectively)[185]. This is likely due to the preserved control of their upper body which enables them to better coordinate movement with the pedalling action of the lower limbs and thereby delay fatigue in the leg muscles.

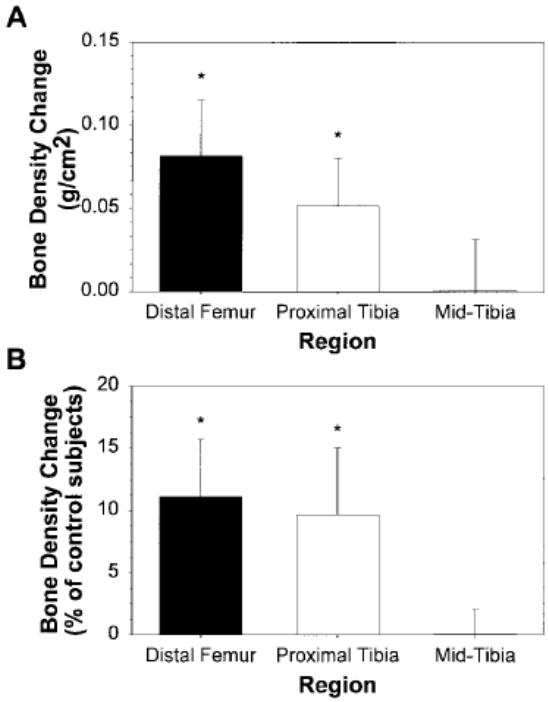

Figure 1.16: Changes in BMD (Mean ± SE) at distal femur, proximal tibia and mid tibia (as absolute values in A and as percentages of uninjured controls values in B) after undergoing 6 months of ES-knee extension intervention[60]. (Reproduced with permission)

The magnitude of the elicited muscle forces and the session duration and frequency also influence the intervention results. This is probably because they determine the magnitude of the mechanical loads acting on bone which need to be large enough to exceed the remodelling threshold and induce bone formation[6]. BMD has been reported to be significantly greater in patients who trained at higher cycling power (≥ 18 W)[185] or received larger compressive loads (150% body weight)[170], compared to that measured at the same sites in patients who trained at lower cycling power (≤ 12 W) and compressive loads (40% BW), respectively. Training paralysed muscles for at least 1 hour per day[175][60], for 5 days per week[60][170][186][171] seems to be more effective in attenuating BMD loss compared to a training protocol of 30 min, or 3 days per week[173][179][187][183]. Furthermore, significant improvements have been shown from training interventions that have lasted at least 6 months[60][185][170][175][186][188][182][174][171] compared to those lasting less than 6 months and reported no change in bone mass[179][189][187][183]. This suggests that bone's response to such interventions is highly dose-dependent.

The magnitude of muscle forces elicited by ES has not been reported in many of these studies[172][175][187][186][188][176][177]. However, some studies measured indirect indicators of muscle force such as joint torques(40 Nm[60] and up to 3 Nm[61]) and applied loads as percentages of body weight[169][170][171][182] (110%, 150%, 90-150% and 100-150% BW, respectively). These achieved loads are comparable to the compressive forces applied on the knees of uninjured individuals during daily activities such as stair descending (123.58% BW) and walking (101.03% BW)[59]. It has also been suggested that a minimum knee extensor torque of 50 Nm is required to achieve standing and walking using FES[60] and at least 35.3–49.2 N/kg muscle force of the knee and hip extensors is required to achieve a sit-to -stand movement[190].

Other studies reported the achieved cycling cadences [183][61] (ranging between 20-60 rpm) and power[179][185][189] (10-18.75 W) as an outcome measure. However, these are not accurate indicators of muscle force and thereby bone loading, because of the inverse relationship between force/load and contraction velocity in skeletal muscles. This concept is demonstrated by considering that high cycling power can be achieved by exerting low force at higher speed as well as by high force and lower speed[170][171]. It is the latter form of high-power cycling (high force, combined with a low velocity) that has been shown to improve bone parameters most effectively in people with SCI[61].

These different ways of measuring the outcome of the aforementioned interventions do not lend themselves well to quantitative comparisons, in terms of the muscle forces produced. Nevertheless, a qualitative comparison between different training approaches (cycling, rowing and resistance training) can be made. Whereas cycling requires stimulating the quadriceps, hamstrings, gluteal muscles and (in some studies) calf muscles, most of the resistance training studies targeted one muscle group such as the quadriceps[170][60] or the soleus[171]. Stimulating one muscle group has been found to mitigate the loss in BMD asymmetrically, on one side/half (posterior) of the lower limb long bones[191][192]. Accordingly, it might be speculated that stimulating the antagonist muscle pairs could have a more homogenous effect on BMD throughout the different areas of the bone.

The imaging modalities used to evaluate the effectiveness of FES interventions are another important methodological consideration. DXA does not distinguish between trabecular and cortical bone and thereby is not able to detect early potential changes in response to training, which are known to occur more rapidly in trabecular than in the cortical compartments[175]. This is illustrated in Figure 2.6, which shows the effect of training on femur trabecular bone using CT[81], with no apparent differences in the cortical shell. Moreover, DXA does not typically provide site-specific scans for regions such as distal femur and proximal tibia which are of clinical interest and are likely to be stressed by cycling exercise.

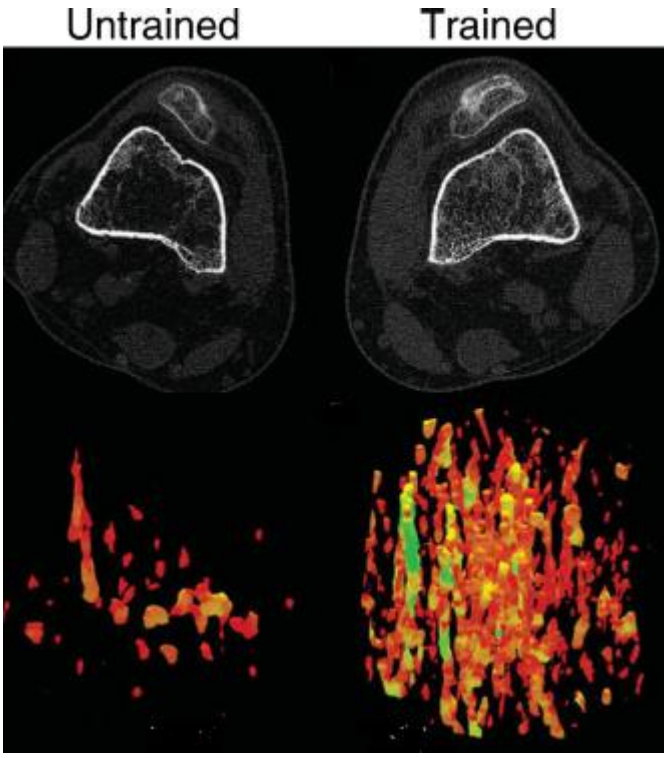

Figure 1.17: Upper panel: CT images of an untrained (left) and trained (right) limbs at 12% of the femur length. Lower panel: 3D reconstruction of the trabecular lattice at the same region showing the greater loss in the untrained compared to the trained limb[170]

Only one study estimated the effect of ES-training on bone strength[176], reporting increases in multiple trabecular and cortical parameters after ES-rowing intervention. However, bone strength (which was estimated using computational modelling from biomechanical indices such as stiffness (-3 SD) and predicted failure load(-3.5SD))

was lower than non-SCI controls; this was proposed to be related to the larger percentage cortical porosity (+4.6SD) and mean pore diameter (+3.7SD)[176]. More studies are required to investigate the effect of ES-interventions on bone strength alongside structural and geometric parameters (as no effect was found in the latter in one study[174]) to assess their effectiveness in preventing fragility fractures.

Different forms of ES- interventions seem to partially reverse BMD when applied intensively over the long-term in people with chronic SCI. However, these improvements have not been shown to lead to fracture risk reduction (which would be considered the desired and clinically relevant outcome). The biomechanical loads/forces elicited by these interventions should be measured and further tested to find out how could they be delivered effectively and safely. Ideally, the ES-interventions would mimic the voluntary muscle loading exerted by able-bodied individuals during daily activities. Additional research is also needed to determine whether it is possible to identify a 'loading dose threshold' above which fracture risk could be significantly minimised.

2.7.2 Other physical interventions:

2.7.2.1 Weight bearing:

Other forms of physical training interventions include weight-bearing activities such as standing and walking, which aim to load the lower limb bones through axial compression, bending and torsional stresses that would normally act on the lower extremities during standing and ambulation.

Most of the reviewed studies (11 out of 16 studies) based on conventional standing training (using standing frames, wheelchairs and leg braces)[193][151][194][128][195][196], exoskeletons[197][198][199][200]or treadmill walking[201][202] did not show improvements in BMD, either during the acute phase[194][196][201] or during the chronic phase[193][151][128][195][197][198][202]. Only four studies showed positive results in bone parameters by preventing bone loss in the acute to sub-acute phase (standing and treadmill walking)[203] and increasing BMD in the chronic phase (treadmill training)[204] (passive standing)[205][206]. The participants recruited were full-time wheelchair users with complete SCI in some of these

studies[193][196][197][198][199][205][206], or had incomplete injuries (or a mixture of both) in other studies [151][194] [128][195] [201][202] [203][204]. Only three studies reported that the recruited participants were already physically active before their recruitment[201][204][206]. Two of these studies reported a positive impact of the intervention on BMD[204][206], which suggests that being active might have improved their muscle health and attenuated their atrophy which in turn optimised their force production and subsequently their impact on bone stimulation.

These improvements range from 7 - 9% larger BMD at different sites in the lower limbs in standing groups compared to controls. However, one of these studies was a single case study recruiting a subject with motor-incomplete SCI (achieving a 20% increase in tibial trabecular BMD)[204], which in itself is known to lead to less bone loss than complete SCI[159][150]. Goemaere et al reported an improvement in femoral shaft BMD but not in the proximal femur[206]. These results were explained by a possible difference between cortical and trabecular bone in the minimal effective strain for initiating bone remodelling (being reached more rapidly in cortical bone)[206], although recent evidence suggests that there are regional variations (regardless of bone type) in strain thresholds within the same bone[207]. In this study, however, the effect of standing was not investigated at the sites that are known to be most prone to fragility fractures in people with SCI (distal femur, proximal tibia and distal tibia). The last study reported attenuation of bone loss based on bone formation and resorption biomarkers but BMD results did not always match the biomarker results[208].

To summarise, to achieve significant improvements in BMD in the lower limbs from standing and walking, long-term training sessions should start within the first few weeks after the injury onset[203][205]. Combining ES with weight bearing activities may have a greater positive effect on bone than performing these activities on their own[204]. While the duration of the ES-training has been shown to have an impact on BMD[209], standing for different durations (<1 hour, 1hour and > 1hour)[128][195] and frequencies (daily standing versus standing for 3 times/week)[206] seems to have no significant effect on BMD. This might indicate that the compression stresses produced during standing alone are insufficient to stimulate lower limb bones even when applied for longer durations.

Similarly, bone stresses delivered through treadmill walking seem to be insufficient to induce adequate bone stimulation. This is, likely because of partial bodyweight support and low treadmill speeds used compared to normal walking speed[201], which would result in lower bone strains[210]. It should also be noted that the atrophied muscles of patients with SCI would produce smaller bone strains when contracting which would make them less effective in inducing osteogenic effects in the bones of the paralysed limbs[199]. Again, as seen with FES interventions, the dosage of the mechanical loading acting on bone that is required to attenuate or reverse bone loss effectively is yet to be ascertained[211].

Additional studies are also needed to determine whether partial body-weight supported treadmill training and other gait rehabilitation orthoses are more effective in terms of bone stimulation than conventional training[201] and whether they could be used safely for long term intervention, as home-based rehabilitation devices[198].

2.7.2.2 Ultrasound

As high-frequency mechanical waves, ultrasound was thought to be a potential technique to stimulate bones with mechanical signals. This concept is based on results of a number of in- vitro trials where low-intensity pulsed ultrasound has been reported to induce osteogenic responses[212][213]. However, in the published literature there is only one study that investigated its effect on bone health in people with SCI. The study found no significant effect of applying pulsed ultrasound for 6 weeks on calcaneal bone loss[214], although the short trial duration likely limited the relevance of the study. Further investigation should be carried out to study the effects of varying ultrasound parameters, as well as, the intervention duration and frequency on its effectiveness in treating bone loss after SCI.

2.7.2.3 Whole Body Vibration

Whole body Vibration systems have been used in people with SCI to deliver vertical or side-alternating oscillations throughout the long bones using a vibrating plate upon which the feet are situated, either while the participant is sitting[215] or passively standing[216]. In the former, the limb is fixed using external compressive loads (35% BW) in order to optimise the transmission and effectiveness of the

vibrations[215]. It has been reported that neither 6 months[216] nor 12 months[215] of applying whole body vibration combined with weight-bearing activities has induced any improvements in BMD or microstructure of the lower extremities. Only one study reported an improvement from whole body vibration (when combined with standing) in BMD (using DXA) in the spine (8.3%) and trunk (5.5%), but not in the lower extremities[217].

Whole body vibration has been incorporated into the rehabilitation programmes of some patients with SCI very recently, but the number of studies that have investigated its effect on bone health is very limited. A summary of the studies that investigated the effect of weight bearing exercises, ultrasound and whole-body vibration on BMD after SCI, can be found in Table 2.

To summarise, most of the physical interventions had limited effects on bone health. This may be related to an inability of current methods to develop or safely apply large internal muscle forces or external forces to bone. For those with chronic SCI, treatments aimed at reversing osteoporosis should be considered, but are less likely to be effective in restoring BMD values to within the normal range[209]. This has been proposed to be due to the weaker bone losing its ability to adapt to applied strains[169] which might be due to the cellular accommodation phenomenon[218] or osteocyte apoptosis[219]. Moreover, patellar tendon stiffness was found to be reduced by up to 77% in people with chronic SCI compared to uninjured controls[220].This would make force transmission from muscles to bones through tendons more difficult and less efficient[220].

2.7.3 Pharmacological interventions (Bisphosphonates):

A wide range of pharmacological interventions are currently available to treat osteoporosis in the general population, such as strontium ranelate[221], denosumab[222], selective oestrogen receptor modulator drugs[223] and bisphosphonates. The latter are the most commonly prescribed treatments for osteoporosis in postmenopausal women[224][225][226] and ambulatory men[227][228], and have been found to be effective in attenuating bone resorption, restoring BMD and preventing fractures[229].

Bisphosphonates are anti-resorptive agents that reduce the bone resorption rate by targeting osteoclasts, inhibiting their activity and subsequently reducing their number in the long term[230] . They are administered either orally on a daily or weekly basis[231] or as an annual single[232][131] or multiple (every month or 3 months)[233][234] intravenous injection. Patients taking bisphosphonates on a weekly basis have been found to be more compliant and to persist with the treatment compared to those who take a daily dose[231].

However, bisphosphonates studies conducted in patients with acute SCI showed mixed results, with 5 out of 9 studies reporting positive effects. Alendronate (weekly for 12 months) has been found to preserve total body and leg BMD[235]. Intravenous bisphosphonates such as zoledronate and pamidronate have been shown to have positive effects on BMD in the lower limbs[234], hip and spine[131][236]. One study reported little effect of disodium dichloromethylene diphosphonate on BMD at the distal tibia[237].

These agents resulted in a lower limb BMD that was 7-17% higher compared to untreated controls. However, it should be pointed out here that in three of these studies, about one third of the participants were classified as having an incomplete SCI (with good preservation of motor function) and were not full time wheelchair users[234][235][236]. With only two studies reporting positive results in participants with complete SCI[131][237]: one reported only a marginal effect on BMC at the distal tibia (7%)[237] while the other reported about 12% greater BMD at the hip[131]. The reduction in bone loss in the acute phase after pamidronate and zoledronate administration (administered once a year) has been reported in some studies to be temporary, lasting 6 months (after the annually administered dose)[232] although the treatment was not discontinued in one study[233]. This might indicate that the dose or the frequency of administration could be further investigated to prolong their effect. Another study reported no effect of the treatment at the knee which might suggest that such treatment is effective in attenuating cortical but not trabecular bone loss[230].

Based on the results reported in these studies, there is little current evidence that supports the effectiveness of bisphosphonates in attenuating/preventing bone loss in

the acute phase of complete SCI at the lower extremities. It was apparent that most studies did not investigate the effect of these treatments in the sites that are most prone to fracture, in part because these sites were only identified relatively recently. However, a number of these studies have been carried out since the fracture-prone sites after SCI were identified and published[76][114][238] and so, this is unlikely to explain all the lack of relevant evidence for the effectiveness of pharmacological intervention to treat bone loss after SCI. Furthermore, these findings are possibly due (in part) to the measurement technique used and the lack of studies that investigated these sites using a 3D imaging technique (e.g. pQCT). Positive changes might have been achieved in the trabecular bone (which responds to interventions faster than cortical bone), but it was not possible to observe it with DXA imaging.

Fewer studies investigated the use of bisphosphonates in the chronic phase of SCI[230][239][240] and one study included a mixed patient group with acute and chronic SCI[241]. The effects of alendronate on leg BMD seem to be influenced by treatment duration. A daily dosage of alendronate administered orally had no effect on lower limb BMD[228] over 6 months, but it attenuated bone loss by 9% [241] when administered for 2 years.

As one of the only two studies that reported significant effects of bisphosphonates in the chronic phase, was a single case study of a patient with incomplete SCI[239], it can be concluded that there is no sufficient data to assess their effectiveness for osteoporosis in chronic SCI. It is clear that the positive effect of bisphosphonates on BMD in the chronic phase of SCI is limited when used as the only intervention. Furthermore, and crucially, it does not exceed keeping BMD within its current level without restoring what has been lost, which could be less effective in the chronic phase (after reaching steady state) compared to the acute phase

A number of issues can be highlighted after reviewing the use of bisphosphonates in people with SCI. Firstly, most of these studies tested these treatments in patients with different ambulatory capabilities and injury severity[230] [234][235][236][242][243] [244]. Some of these studies showed more pronounced improvements in ambulatory compared to non-ambulatory participants [234][244]. Despite the fact that there appear to be comparable numbers of studies with mixed and complete SCI that

reported positive results of pharmacological interventions (4 studies with mixed group (complete and incomplete SCI) and 3 studies of complete SCI only), testing these agents on only non-ambulatory patients with complete SCI would give a clearer indication of their effectiveness in restoring BMD and preventing fractures[131][245]. As a minimum, where mixed patient groups are used, completeness of injury should be included in the data analysis and reporting of findings.

Secondly, very few studies investigated the effect of these treatments on BMD at the sites that are most susceptible to fracture in people with SCI[131][237][241], such as the distal femur and proximal and distal tibia[33]. Furthermore, the use of pQCT (instead of DXA) could provide more quantitative insights into whether cortical bone and trabecular bone respond to such agents to similar or different extents.

Although these studies reported no[230][235] or only acute mild side effects[243][236], osteonecrosis of the jaws[246] and spontaneous fractures[247] are considered as rare side effects of using oral bisphosphonates in osteoporotic patients. Other clinical treatments have therefore been suggested to be tested on people with SCI such as the anti-resorptive denosumab and anabolic therapies such as sclerostin antibodies[131]. Gifre et al reported an increase in lumbar and femoral BMD (8% and 3% BMD respectively) after administering Denosumab in people with acute SCI for 12 months[248]. This was the only study testing Denosumab in people with SCI. Table 3 provides a summary of all studies that used pharmacological treatments to attenuate the loss in BMD after SCI.

2.7.4 Combination treatment interventions:

Despite the reported mitigated bone resorption in the acute phase[131][234][235] and the stabilised BMD in the chronic phase[240][241] in many of these studies , no increase in BMD has been reported, indicating a need for an intervention with an anabolic effect that can be combined (or used sequentially) with anti-resorptive agents to boost their effect[240][249]. The additive effect of anti-resorptive medications combined with the anabolic stimulus of physical activity may explain results from a number of bisphosphonate studies reporting more pronounced improvements in BMD in ambulatory individuals than in full time wheelchair

users[234][244]. Furthermore, combining exercises with anti-resorptive therapies has been reported to have a greater effect on BMD compared to using antiresorptives alone in different models of osteoporosis[250][251] but the number of studies examining such approaches is small[252].

Remembering the significant role of mechanical stresses in preserving/losing BMD might suggest that bisphosphonates should be accompanied by physical training in order to achieve optimum benefits in people with SCI[253]. Hypothetically, by combining these interventions, the anti-resorptive agent would target the inhibition of osteoclastic activity, while the skeletal loading could have a role in simulating osteoblasts, thus mitigating the imbalance between bone formation and resorption caused by the SCI. In a recent study, significant increases in geometric cortical bone parameters (cortical bone volume, cortical thickness index, and buckling ratio) were reported at the distal femur and proximal tibia in the group that had FES-rowing training combined with zoledronate administration compared to the group that performed FES-rowing alone[254]. This is clinically-relevant as reductions in these geometric cortical bone parameters after SCI are thought to be associated with osteoporotic fractures in this patient group[254].

The anabolic parathyroid hormone teriparatide has been also investigated in combination with gait training[249], with vibration[255] and following 12 months of bisphosphonates treatment[242]. The first study reported no effect on BMD (i.e. with gait training)[249]. The second study reported that the combination of vibration with teriparatide did not augment its effect on BMD[255], while in the latter(involving combination with bisphosphonates), an increase in BMC ranging between 3%-15% was reported in different parts of the femur[242]. Studies that tested these combined treatments in people with SCI are summarised in Table 4.

Levels of evidence

After reviewing all available interventions aiming to mitigate bone loss in people with SCI, the quality of these studies was assessed. Only studies that investigated BMD as their primary outcome were included in this assessment, due to the well-documented link between BMD and fracture incidence in people with SCI [67][76].

Furthermore, it is the most commonly measured bone parameter among all studies in the SCI population.

The levels of evidence were assessed following the approach described by Bryson et al.,2009[245] which was based on the Delphi list 9 items. Each of these items (listed in Tables 5,6,7 (provided in Online Resource 1)) was given a subscore of either 1 (not reported), 2 (fair) or 3 (good), except for the randomisation item which was given a score of 0 (if the study was not randomised). Items 5,6,7 were all merged together under one category due to the small numbers of studies that reported blinding of the patient (which was difficult to achieve especially for physical interventions), assessor or the caregiver. All subscores were summed to determine the overall quality rating (poor, fair, good).

All the reviewed studies were either of a poor or fair quality except one study that tested the effect of standing and was of a good quality[196]. For both the ES and the pharmacological interventions studies, 30% were of poor quality while about 70% were of fair quality. Other physical interventions (such as weight bearing) studies showed comparable levels of evidence with 25% poor, 67% fair and 8% good quality. The quality of most of physical interventions studies were found to be limited by the absence of randomisation and blinding which were difficult to achieve due to ethical and practical reasons, respectively. Also, many studies were not controlled, comparing baseline with post-intervention measures, making item 2 (allocation concealment) and item 9 (intention- to -treat analysis) not applicable.

Conclusions

To summarise, this review article has discussed the aetiology, development and consequences of bone loss in people with SCI. Furthermore, the most commonly used imaging modalities to assess bone loss after SCI and available therapeutic approaches have been evaluated.

It is clear that bone loss that develops in the paralysed limbs after SCI is highly site-specific, progressing with different patterns and timelines in cortical and trabecular bone compartments. In addition, rates of bone loss differ substantially between

individuals but to date there is little understanding of the mechanisms responsible for this variation. Most of the physical and pharmacological interventions developed and evaluated to date appear to have a limited effect on bone health, but the poor quality of published studies in this area limits our ability to draw clear conclusions. More high-quality observational and interventional studies, with appropriate outcome measures targeting fracture-prone site, are needed.

Acknowledgments

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Table 1.1: Summary of studies that used ES- interventions to attenuate the loss in BMD after SCI

Study	Training Modality	Electrical stimulation parameters	Produced Stress/power	Training duration/frequency	Injury duration and level	Imaging modality	Changes in bone parameters	Level of evidence
Pacy et al 1988[189]	leg raising against load + bicycle ergometer	6s-6s stimulation-rest, 300µs, 40 Hz. 65-90 V, for leg raising against load ranging from 1.4- 11.4 kg, And 80-125 V for bicycle ergometry.	from 0 to 18.75 W (0-3/8 kilopond)	15 mins, 5 times/week for 10 weeks (leg raising) -15 mins, for 32 weeks (bicycle ergometry), 50 rpm,	1, 3 and 4 years, T4-T6 (3 SCI patients) N.B: 1 patient had hemangioblastoma at T6 for 6 years	DXA	No change in BMC or BMD	-
Rodgers et al., 1991[187]	FNS-induced knee extension (KE)	progressive resistance load on ankle 0-15 Kg	-	6 KE/min/leg, 3 times/ week for 12-18 weeks,	6.4 ± 6.1 years, C4-T10	Central QCT	No change in BMD	Poor
Sloan et al.,1994[183]	FES-cycling (Also participating in physiotherapy)	-	50-60 rpm for patients with incomplete and 30-40 rpm	30 min, 3 times/week, for 3 months	0.2-11.6 years, C5-T12	DXA	No change in BMD (BMD tested in only 2	Poor

			for complete SCI				out of 12 patients)	
Bloomfield 1996[185]	FES-cycle ergometry	monophasic, 350 msec duration at 30 Hz and up to 130 mA;	Cycling power up to 18 W	30 mins, 3 sessions/week, (80 sessions) for 9 months	6 years, C5 to T7	DXA	BMD Increased by 0.047 ± 0.010 g/cm^2 at the	Fair
Mohr et al 1997[174]	FES-cycling	-	Workload 1/8 Kp- 7/8 Kp, 18 ± 2 KJ/session	30 min, 3 days/week, for 12 months followed by 6 months of 1 session/ week	12.5 ± 2.7 years, C6–Th4	DXA	10% increase in PT BMD. This gain faded after 6 months of reduced training	Fair
Belanger 2000[60]	Quadriceps contraction (resisted & unresisted)	300-µsec rectangu-lar pulses delivered at 25Hz with a 5-sec on/5-sec off duty cycle	40 Nm	1-hour a day, 5 days a week, for 24 weeks.	9.6 ± 6.6 years, C5-T6	DXA	Total body scans showed: 30% of lost BMD recovered in DF and PT	Poor

Eser et al, 2003[173]	FES-cycling and passive standing (2 days/week)	peak current =140 mA. Pulse width set 0·3- 0·4 ms, frequency set at 30, 50, and 60 Hz	Power output between 0 and 1 kiloponds	30-min, three times a week for 6 months	4.5 weeks	Central QCT	No effect in tibial cortical BMD	Fair
Chen et al., 2005[186]	FES-cycling	20 Hz; pulse duration, 300 µsec; up to 120mA	-	30 minutes/day, 5 days/week, for 6 months.	At least 2 years and 7 months, C5- T8	DXA	Total body scans showed: BMD at DF and PT increased 11.13%, and 12.92% respectively, but decreased at FN	Fair
Shields et al.,2006[171]	ES-isometric plantar flexion	10 pulse train (15 Hz, 667ms) every 2 seconds.	Compressive loads: 600 N (90% BW) to 1,107 N (150% BW)	4 bouts/day, each consisting of 120 trains, 5 days/week, For 3 years	4.5 months, C5 and T12	DXA	Decline in trained tibial BMD (10%) less than the untrained (25%)	Fair

Shields and Dudley-Javoroski 2006[182]	ES-isometric plantar flexion	0–200 mA, 400 V, 10 pulse train (15 Hz, 667ms) every 2 seconds.	~1–1.5 times BW	4 bouts/day, each consisting of 125 trains, 5 days/week, For ≥2 years	6 weeks, ASIA A	Standard pQCT	31% higher DT trabecular BMD compared to untrained limb	Fair
Clark et al. 2007[172]	ES of quadriceps and dorsiflexors	30 Hz, (tetanic) stimulation: rest ratio 4:8 s, supine position, knee flexed at 20º		15 min sessions, twice daily, over a 5-day/week, for 5 months	3 weeks, C4–T10, (All with tetraplegia)	DXA	Different total body BMD at 3 months only	Fair
Shields and Dudley-Javoroski 2007[169]	ES -isometric plantar flexion	0 to 200 mA at 400 V, 10-pulse train (15 Hz; 667ms) every 2s (125 trains in each stimulation bout)	compressive loads equivalent to 110% of body weight	30min/ day, 5 days a week, for 6 - 11 months	>2 years ASIA A	DXA	No change in PT BMD	Fair
Frotzler et al 2008[175]	FES-cycling	50 Hz, pulse width = up to 500 µs, current amplitude= 80-150 mA	-	58±5 min, 3.7±0.6 sessions/ week for 12 months	11.0±7.1 years	Standard pQCT	Increases in DF epiphysis BMD are: 14.4±21.1% in trabuclar	Fair

							BMD, 7.0±10.8% in total BMD and 1.2±1.5% in CSA	
Griffin et al. 2009[179]	FES-cycling	50 HZ, up to 140mA, 49 rpm	0.71–10.51 W	30 min, 2–3 times/ week for 10 weeks	11 ± 3.1 years, C4-T7	DXA	No difference in BMC	Poor
Lai et al., 2010[177]	FES-cycling	20 Hz; 300 μsec, (electrodesat mid quads and hamstirngs)	-	30 min, mean of 2.4 sessions/week, for 3 months	26–52 days, C5-T9	DXA	Decreased rate in DF BMD less in trained group (2.23% in trained; 6.65% in controls)	Fair
Dudley-Javoroski et al 2012[170]	Compressive loads applied during stance by quadriceps ES	60, 100-pulse trains (20 Hz, 200 μs, up to 200 mA), each train followed by 5 s rest	150% body weight (BW)	30 mins, 5 days a week for 3 years	0.19- 24.23 years, C5-T12	Standard pQCT	BMD in limbs that received 40% BW and untrained was 61.1% of that of 150% BW limbs	Poor

Gibbons et al.,2014[188]	FES-rowing (1 participant)	50 μsec pulse width, 50 Hz, up to 115 mA unramped stimulation.	-	30-45 mins, For > 8 years	13.5 years, T4	Standard pQCT	PT trabecular BMD was higher in trained participant compared to SCI group but less than able-bodied.	Single case study
Gibbons et al2016[176]	FES-rowing	lower limbs exposed to ~ 2700 loading cycles/week	-	3 times/week, 30-min rows at 30 strokes/min,	13.5 years, T4	HR-pQCT	Majority of tibial trabecular and cortical measurements were within ~ 1 s.d.	Single case study
Johnston et al.,2016[61]	FES-cycling (compare low and high cadences)	250μs, 33Hz, and up to 140mA	Low: 20 rpm, 2.9±2.8Nm, High: 50 rpm, 0.8±0.2Nm	56 min, 3 times/week for 6 months	1-27.5 years, C4-T6	DXA and MRI (microstructure)	Greater decreases in alkaline phosphate and N-telopeptide in low cadence	Fair

Table 1.2: Summary of studies that used other physical interventions (without electrical stimulation) to attenuate the loss in BMD after SCI

Study	**Training modality**	**Produced Stress/power**	**Training duration/frequency**	**Injury duration and level**	**Imaging modality**	**Changes in bone parameters**	**Level of evidence**
Biering-Sørensen et al.,1988[151]	Standing or walking using long leg braces	-	For at least 1 hour daily	2-25 years, C7-L3	DXA	No effect on BMC	-
Kunkel et al.1993 [193]	Standing in frame	-	45 min/twice daily for 5 months (144 h over 135 days).	10-39 years, C6-T12, (4 SCI, 2 multiple sclerosis patients)	DXA	No change in BMD	Fair
Goemaere et al.,1994 [206]	Passive Standing using: 1. long leg braces 2. standing frames, 3. standing wheelchairs)	-	Daily standing for 1 hour in 1 group and 3 times/week in the second group	12-118 months. Complete paraplegia	DXA	BMD better at femoral shaft but not proximal femur compared to non-standing.	Fair
Thoumie et al.,1995[198]	Gait rehabilitation with hybrid orthosis	-	2 hours, 3 times/week, for 16 months	15-60 months, T2-T10	DXA	Significant decrease in BMD at femoral neck and no change at lumbar spine	Poor
Needham-Shropshire et	Standing and walking using a device that	-	3 times/week,	At least 6 months, T4-T11	DXA	No significant change in BMD	Fair

al.1997 [199] [200]	combined ES and a modified walker		12-20 weeks, (mean of 143.6 ± 86.4 mins persession)			at FN, neck, and Ward's triangle	
de Bruin et al 1999 [203]	Standing and treadmill walking.	Treadmill speed=1.3 km/h	30min standing, 30 min walking, 5 days /week for 6 months	1-4 weeks, C4-L1	pQCT	Almost no loss in tibia trabecular BMD in trained group compared to -6.9% to -9.4% loss in trabecular bone	Fair
Dauty et al., 2000 [128]	Passive standing	-	Daily for: less than 1 h 1 h More than 1h	68.3 ± 74.7 months	DXA	No effect on BMC	-
Frey-Rindova et al.,2000 [194]	Standing using frame (for complete SCI) And treadmill Walking (for incomplete SCI)	-	At least 30min, 3 times/week for 2 and half years. (Treadmill speed 1.3 km/h)	1-4 weeks	pQCT	No effect on BMD	-
Warden et al.,2001 [214]	Pulsed US	-	20 min, 5 days/week for 6 weeks	1-6 months, C5-T10	DXA	No effect on calcaneal bone parameters	Fair

			US settings: 10 µsec 1.0 MHz sine waves, 3.3 kHz.				
Ben et al.2005 [196]	Standing on 1 leg (on tilt table)	17 Nm dorsiflextion torque	30 min, 3 times/week, for 12 weeks	4±2 months	DXA	Little or no effect on femur BMD	Good
Giangregorio et al.,2005[201]	body weight supported treadmill	-	Less than 1hour, 2 times/week. (48 sessions in 8 months) (speed= 0.7-2 km/h)	2-6 months, C3-C8	DXA and CT	No effect on BMD (proximal and distal femur, PT, spine) or CSA (mid-femur,PT)	Poor
Carvalho et al.,2006[208]	Treadmill gait training	30-50% BW supported	20 min, 2 times/week for 6 months	25-180 months, C4-C8	DXA	Most of the participants showed increased bone formation and decreased bone resorption (BMD results did not always match biomarkers results)	Poor

Giangregorio et al.,2006 [202]	Body weight supported treadmill	-	3 times/week, For 12 months (144 sessions)	1-24 years (all Incomplete)	DXA and CT	No effect on BMD (at proximal and distal femur, PT, spine) or CSA (mid-femur, PT)	Poor
Alekna et al.,2008 [205]	Passive standing in frame	-	For at least 1 hour/day, no less than 5 days/week	8-12 weeks, C2-L1	DXA	Higher BMD in lower limbs after 2 years in standing group (1.018 compared to 0.91g/cm^2)	Fair
Goktepe et al.,2008 [195]	Any form of Standing: More than 1hour 2. Less than 1hour No standing	-	Daily standing	At least 1 year, ASIA A, B	DXA	No significant difference between groups in BMD at PT and lumbar spine	Fair
Coupaud et al., 2009[204]	Partial body-weight supported treadmill training (BWSTT)+ FES on one side	30% BW support -Speed increased from 0.1 m/s to 0.3 m/s	Muscle conditioning over 2 months Target increased for 15 min to 30 min, 3 time/week for 5 months.	14.5 years, T6 (Incomplete) (one subject)	pQCT	Increase of 5% (right) and 20% (left) in DT trabecular BMD. Changes are	Single case study

	(bisphosphonate + Vitamin D prescribed independently)		FES: 40Hz, 40mA, and 117-351μs			negligible in PT and DF	
Davis et al.,2010 [217]	3 phases of training: 1.standing only, 2. partial standing/WBV (foot only on plate) 3.standing with vibration	-	1.Phase1: 40 min, 3 times/week, for 10 weeks 2. Phase2:20/20 mins, 3 times/week 3. Phase3: 7 mins/session, 3 times/week	4 years, T10 (Incomplete), (single case)	DXA	Improvement in BMD in the trunk and spine after phase 3 only. No effect on legs	Single case study
Wuermser et al.,2015[216]	Low-magnitude whole body Vibration+ passive standing	About 76-86 % BW	20 mins, 5 days/week, for 6 months (0.3 g, 34 Hz,50 μm.)	2-27 years, T3-T12	DXA and HR-pQCT	No effect on BMD at PF or microstructure at DT	Fair
Dudley-Javoroski et al.,2016 [215]	Body Vibration	35% BW applied during the vibration training	3 times/week, for 12 months Vibration parameters: 0.6g, 30 Hz, 20 min, three times weekly)	0.1 to 29.2 years, C7-T4	pQCT	No effect on trabecular microstructure or BMD at DT and DF	Fair
Karelis et al.,2017 [197]	Walking with a robotic exoskeleton	-	Up to 60 min, 3 times/week for 6 weeks	7.6 ± 4.6 years, C7-T10	DXA	No significant change in BMD	Fair

	Mean standing time/session: 48.4 min, walking time: 27.0 min

Table 1.3: Summary of studies that used pharmacological treatments to attenuate the loss in BMD after SCI

Study	Treatment	Injury duration and level	Dose, Duration & frequency	Imaging device	Changes in BMD	Supplements	Level of evidence
Minaire et al.,1981 [237]	disodium dichloromethylene diphosphonate	Acute SCI, T1-T12, (All with complete paraplegia),	400 or 1600 mg/day for 3.5 months	Photon absorptiometry	Little effect in BMC at distal tibia (for 400mg)	-	Fair
Pearson et al., 1997 [244]	Cyclical Etidronate	Within 6 weeks, C5-T12,	Orally 800mg/day for 2 Weeks, this was repeated after 13 weeks	DXA	BMD maintained only in ambulatory treated patients.	-	Poor
Nance et al.,1999 [234]	Intravenous Pamidronate	6 weeks, C4-T12	30-mg infusion/month for 6 months	DXA	Greater BMD at hip, femoral and tibial diaphyses, femoral and	Calcium: 1000mg daily	Poor

					tibial epiphyses. (less bone loss in ambulatory)		
Sniger and Garshick,2002 [239]	Alendronate	27 years, C4 (incomplete), (single case)	Daily: 1. Alendronate: 10mg 2.Vitamin D: 400mg 3.Calcium carbonate 500mg, daily for 2 years	DXA	Increased BMD at spine and lower legs	vitamin D: 400mg/d calcium carbonate: 500mg/d	Single case study
Zehnder et al., 2004 [241]	Alendronate	0.1–29.5 years, T1-L3, (all with complete SCI)	10mg + 500 mg Calcium daily for 24 months	DXA	BMD at distal tibia, tibial diaphysis and total hip remained stable compared to control group	Elemental calcium: 500g/d	Fair
Bauman et al.,2005 [233]	Intravenous Pamidronate	22 to 65 days, ASIA A, Acute SCI	60 mg given at 1, 2, 3, 6, 9, 12 months	DXA	No changes in long term (12, 18,24 months)	Calcium: at least 700mg/d in diet	Fair

					although reported early (1,3,6 months) reduction in bone loss in total leg BMD		
de Brito et al 2005 [230]	Alendronate	13.1–255.7 months, ASIA A, B, C	10 mg (+1000mg Calcium), Daily for 6 months	DXA	General increase in BMD	Calcium: 1000 mg/d	Fair
Mechanick et al.,2006 [243]	Intravenous Pamidronate	Acute SCI, AIS A, B, C	90 mg over 4 hours (single dose)	-	Reduced bone resorption biomarkers, BMD not tested	-Calcium: 1,000 mg daily -calcitriol: 0.25 µg daily	-
Gilchrist et al 2007 [235]	Alendronate	Within 10 days, C4-L2	70mg once weekly, For 12 months	DXA	Total and hip BMD was 5.3% and 17% greater in intervention group respectively.	-	Fair

					Effects sustained for more 6 months after treatment discontinued		
Shapiro et al.,2007 [232]	Intravenous Zoledronate	10-12 weeks, C2 to T12,	4 or 5 mg (Administered once)	DXA	BMD and CSA increased at proximal femur only at 6 months, and for 12 months at the femoral shaft	Calcium: 800mg vitamin D: 800 IU (both from diet)	Fair
Bubbear et al.,2011 [236]	Intravenous Zoledronate	Within 3 months, C4-L3	4 mg (administered once)	DXA	Higher BMD at total hip (12.4%) trochanter (13.4%), and lumbar spine (2.7%) up to 12 months	-	Fair

Bauman et al.,2015 [131]	Intravenous Zoledronate	Within 3 months ASI A, B (All with compete SCI)	5 mg (administered once)	DXA	Reduction of BMD loss at the hip but not at the knee	Calcium carbonate: 1250mg/d vitamin D: only for participants with levels <20 ng/ml	Poor
Haider et al.,2019 [242]	Teriparatide (in previous study) followed by oral alendronate	15± 9 years, ASI A, B, C (C1-L5),	Teriparatide: 12-24 months alendronate: 70 mg once weekly for 12 months	DXA	Significant increase in aBMD at the spine 2.5% And in BMC at femoral epiphysis, metaphysis, and diaphysis, 15%, 7.7%, 3.0%, respectively. -no clear results at the tibia	Vitamin D (cholecalciferol 1000 IU) daily -calcium carbonate: 1000 mg daily	Fair

Gifre et al 2016	Denosumab	15±4 months, C4-T8 (ASIA 12A, 1B, 1C)	60 mg every 6 months for up to 12 months	DXA	Increases in lumbar (8%) and femoral BMD (3%)	Calcium and Vitamin D	Fair

Table 1.4: Summary of studies that used physical interventions combined with pharmacological treatments to attenuate the loss in BMD after SCI

Study	Intervention	Protocol	Physical Training parameters	Injury duration and level	Imaging device	Changes in BMD	Level of evidence
Gordon et al., 2013 [249]	Parathyroid hormone and gait training	20 µg/day and robotic-assisted stepping for 6 months, followed by 6 months of teriparatide alone.	40 minutes/ session, 3 times/week, at speed 2.0-2.5 km/h, <50% BW support	>1 year, C1-T10	DXA (hip and spine) and MRI (microarchitecture of distal tibia)	No change in spine and total hip BMD. Positive anabolic effect significant at 3 but not6 months	Fair
Edwards et al.,2018 [255]	3groups: 1-Teriparatide + sham vibration 2-placebo+ vibration 3- Teriparatide + vibration	Teriparatide: 20µg/d Vibration:10min/d -Additional 12 months of Teriparatide treatment	Vibration: 30 Hz, acceleration amplitude= 0.5 g	19±13.8 years, ASIA A, B, C, D.	DXA and CT	Increase in groups that used teriparatide: 4.8% - 5.5% increase in spine BMD -Vibration did not augment teriparatide effect -Small increase in knee cortical bone in all groups. - the additional 12 months of Teriparatide resulted in 7.1% -14.4% increase from baseline),	Good
Morse et al., 2019 [254]	Intravenous Zoledronate and FES-Row	12-month FES-rowing	30 min, 3 days/week at an intensity of	0.4-37.9 years,	DXA for BMD, QCT	Greater cortical bone volume, cortical thickness index and buckling ratio at proximal tibia and distal femur metaphysis	Fair

	program and single dose of zoledronate	75% to 85% of peak heart rate	75% had a motor-complete injury,	

2.10 Literature update

Updates in the published literature since the review article was published in October 2020[58], are summarised below. This update includes all the most recent studies on the testing and treatment of bone loss following SCI.

A recent survey collected from 82 professionals (from 25 countries) in SCI medicine found that BMD following SCI was more likely to be tested during the chronic phase (51%) or following a fragility fracture (43%), rather than in the early phases (acute or sub-acute) with only 20% testing BMD during the first 3 months postinjury[256]. The majority of these professionals (70%) reported testing BMD at the hip using DXA[256], and not in the area prone to fracture in this patient group, which was in line with the patterns reported previously in the published literature review. These findings highlight the inconsistencies in the detection of bone loss in people with SCI, and further emphasise the need for standardised clinical practice that is tailored for this site-specific and rapid form of bone loss.

One recent cross-sectional study investigated bone microstructure in the distal tibia, fibula and radius using high-resolution peripheral quantitative computed tomography in individuals with SCI (0.7 - 18.6 years postinjury) and able-bodied controls[257]. Loss in total volumetric BMD was reported in the tibia and fibula sites, but not in the radius, with the loss in the fibula reported to be about 50% that of the adjacent tibia in both paraplegia ad tetraplegia groups[257]. Bone microstructure deteriorated in both cortical (thinner cortices, higher porosity) and trabecular (thinner and disconnected trabeculae) compartments in the tibia, but only in the trabecular compartment in the fibula despite the large proportion of cortical bone in this fibular site[257]. These results highlight the heterogeneity of bone loss and the different response to the SCI-induced unloading between the tibia and fibula, which was reported in other cross-sectional studies[258]. However, longitudinal changes in the fibula following SCI were investigated for the first time in our recently published paper that will be presented in the following chapter.

Regarding FES interventions, two recently-published studies assessing rowing training have reported positive effects on BMD. Electrically stimulated rowing training-that commenced within the first 2 years postinjury and lasted for 26 weeks-led to a slower rate of decline in pelvic and total BMD compared to arms-only

rowing training[259]. A reduction in femoral and tibial trabecular bone loss was also reported after 90 sessions of rowing in people with motor-complete SCI (grades A and B on the ASIA Impairment Scale) over a period of 9-12 months[260]. In both studies, training was performed for 30 mins, 3 times/week. On the other hand, in a recent case study, no change in BMD was reported following 12 month of cycling (6-month isometric stimulation followed by 6 months of FES cycling)[261]. However, evidence of the efficacy of different forms of physical activity in improving BMD after SCI remains scarce and inconsistent[262].

Only two new pharmacological intervention studies were found, which tested intravenous zoledronic acid[263] and alendronate[264]. These were administered within the first 3 and 8 weeks postinjury, respectively. Zoledronic acid helped maintain the BMD in the distal femur but not in the proximal tibia, and did so at 4-months, but not at 12 months postinjury[263]. A weekly dose of alendronate administered for 1 year resulted in reduced bone loss at the hip[264]. These results support a recent published systematic review which concluded that early use of bisphosphonates following SCI attenuates BMD loss in the hip and lumbar spine, but not in the distal femur[265].

Reviewing the literature revealed the modest and conflicting effects of most of the available interventions on bone health following SCI. Effectiveness of ES-training interventions seems to be limited, partly, by the low muscle forces induced from the paralysed atrophied muscles. Therefore, one of the aims of this thesis is to develop and test a training approach that maximise the elicited muscle force and probably increase bone stimulation. This approach will be developed and tested first on healthy uninjured individuals (Chapter 5), before testing its effects on muscles and bones of people with SCI (Chapter 6).

After reviewing the published literature in the areas of the aetiology, pattern and testing of SCI-induced bone loss, some aspects appear to require further investigations in order to improve our understanding of the characteristics of this complex form of bone loss. Therefore, another aim of this thesis is to develop a more detailed characterisation of localised bone loss in the paralysed limbs following SCI. This will be pursued by focussing on filling some of the gabs in the literature within two areas.

Firstly, bone loss following SCI is known to be site-specific, affecting mostly bones at the knee and ankle joints in the paralysed limbs. These inter-site differences in bone loss have been widely documented[149][114], but bone changes within different regions at the same bone site have not been studied before. In addition, the rate of bone loss following SCI shows a large degree of inter-individual variation, with BMD losses ranging between 1% and 67% evident 12 months after injury[140]. Investigating the homogeneity of bone changes would help develop better characterisation of the bone loss, which in turn, would help identify areas of greatest (or maybe fastest) bone loss and develop better targeted treatments. These areas could also help detect rapid bone loss earlier and potentially, inform physicians to develop clearer guidelines for the testing of bone health in people with SCI. Regional and temporal variation in bone loss in the tibia will be investigated and discussed in Chapter 4 of this thesis.

Secondly, bone loss in the paralysed lower limbs was tested and widely explained in the literature in the tibia and femur bones, but not in the fibula. Few studies investigated the cross-sectional changes in the fibula but no study reported bone changes longitudinally. Investigating the longitudinal changes in the fibula and compare it to the neighbouring tibia might help explain the lower incidence of fracture in the fibula and potentially further improve our understanding of the mechanisms driving this extensive bone loss. Fibular response to disuse in people with SCI will be assessed in the next chapter (Chapter 3).

Chapter 3:

Fibula response to disuse: A longitudinal analysis in people with spinal cord injury

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Mini Abstract

Fibular response to disuse has been described in cross-sectional but not longitudinal studies. This study assessed fibular bone changes in people with spinal cord injury. Fibular bone loss was less than in the tibia, and were not correlated together. This might explain low fibular fracture incidents in these patients.

Abstract

Purpose: Cross-sectional studies suggest that the fibula responds differently to loading and disuse compared to the tibia. While tibial bone changes following spinal cord injury (SCI) have been established in longitudinal studies, fibular changes remain unexplored.

Methods: Fibular and tibial bone parameters were assessed in 13 individuals with SCI (aged 16-76 years). Peripheral quantitative computed tomography scans were acquired at 4%, 38% and 66% distal-proximal tibia length at 5 weeks and 12 months postinjury. Changes in 4% site total bone mineral content (BMC), total cross-sectional area (CSA) and bone mineral density (BMD), and 38% and 66% sites total BMC, total CSA, cortical BMD and cortical CSA were assessed using paired T-tests. Relationships between bone loss in the two bones at equivalent sites were assessed using paired T-tests and correlation.

Results: At the 4% site, fibular total BMC and BMD losses were less than tibial losses (-6.9±5.1% and -6.6± 6.0% vs -14.8 ±12.4% and -14.4±12.4%, p=0.02 and p=0.03, respectively). Similarly, at the 66% site, fibular BMC losses were less than those in the tibia (-2.0±2.6% vs -4.3±3.6%, p=0.03), but there was no difference at 38% (-1.8±3.5% vs -3.8±2.1%, p=0.1). No correlation was observed for BMC changes between the two bones (all p>0.25).

Conclusion: These results support cross-sectional evidence of smaller disuse-related bone loss in the fibula compared to the tibia. These results may in part explain lower incidence of fibula fractures in individuals with chronic SCI. The lack of association between losses in the two bones, might point to different underlying mechanisms.

Keywords: Fibula, Disuse Osteoporosis, Mechanoadaptation, Spinal Cord Injury, pQCT

Declarations

Funding

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Conflicts of Interest

Shima Abdelrahman, Mariel Purcell, Timo Rantalainen, Sylvie Coupaud, Alex Ireland declare that they have no conflict of interest.

Availability of data and material (data transparency)

The bone scans and datasets produced and analyzed during this study are available from the authors.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

3.1 Introduction

The human fibula has a much smaller cross-sectional area and lower bone mineral content compared to the neighbouring tibia[266]. It supports only 5-19% of the shank axial loading but this load proportion increases with load magnitude[267].

The tibia increases in size and density after harvesting of the fibula for humerus reconstruction [268], emphasising the important mechanical role that fibula plays. Moreover, the greater fibular strength (for lateral bending) in soccer players compared to untrained controls , and the dramatic increase in the size of the fibula after transplanting it to replace an excised tibia[269], further indicate that the fibula has the capacity to adapt to an altered loading environment. However, this adaptation seems to be different in training [270] and disuse[258] compared to the tibia.

Cross-sectional studies suggest that fibula bone loss following spinal cord injury (SCI) is modest and confined to epiphyseal regions[258], whereas the tibia undergoes extensive loss along its length[115][144]. However, to date, the fibula's response to disuse has not been explored in longitudinal studies. The absence of disuse related loss could help explain the low incidence of fibula fractures in individuals with SCI (approximately 1/5 of the number of fractures reported in femur and tibia)[238]. In addition, greater understanding of the fibula shaft's apparent protection from disuse-related losses could lead to strategies to prevent loss in other bone regions.

Therefore, in this study, fibular and tibial bone parameters were assessed and compared in individuals with SCI within 12 months following their injury. Our aim was to describe longitudinal disuse-related changes in the fibula during the first 12 months of SCI, and to compare these changes to those in the tibia. We hypothesised that, in line with previous cross-sectional observations, bone loss in the fibula would be much smaller than that in the tibia and only evident in the epiphysis.

3.2 Methods

Twenty-nine inpatients (aged 16-76 years) with motor-complete SCI (grades A or B on the American Spinal Injuries Association Impairment Scale (AIS)) at the Queen Elizabeth National Spinal Injuries Unit (UK), were recruited for this study. Longitudinal changes in the tibia in these individuals have been reported previously[138]. The main exclusion criteria were age <16 years; recent bone

fracture and continued ventilator dependency at week 5 post-injury. Ethical approval for the study was obtained from the NHS Research Ethics Committee. Further details on patient recruitment and scanning protocols for that study have been described previously[138].

pQCT scans (XCT3000, Stratec Medizintechnik GmbH,Germany) were obtained by a single operator from these 29 participants within the first 5 weeks (baseline) and at 4,8 and 12 months postinjury and were analysed for longitudinal changes in bone parameters at the tibia throughout the first year of injury[138]. Of these, a subgroup of 13 individuals with complete sets of baseline and 12-month scans, were included in this study, as these were the only two timepoints considered in this investigation of the fibula.

A quality control scan was conducted using the manufacturer phantom before each scanning session. Slice thickness was 2 mm and the voxel size was set to 0.5 mm in accordance with previous pQCT studies in SCI[114][139]. Scans of the tibia and fibula were taken at 4%, 38% and 66% of tibial length (from the distal reference point). These scans were analysed using an ImageJ plugin (National Institutes of Health, Maryland, USA)[271][272]. Epiphyseal parameters calculated at the 4% site were the total bone mineral content (BMC), total cross-sectional area (CSA) and bone mineral density (BMD). Total rather than trabecular BMD was examined at this site due to the thick cortex and small trabecular area evident at distal fibula sites[258]. The parameters calculated at 38% and 66% (diaphyseal) sites were total BMC, total CSA, cortical BMD and cortical CSA. Given the thin cortex in individuals with SCI at epiphyseal sites, thresholds of 120 $mg.mm^{-3}$ and 150 $mg.mm^{-3}$ were used to separate bone and soft tissue at the epiphyseal and diaphyseal sites, respectively. These thresholds are not standard for pQCT use but were determined after testing multiple threshold values on all scans. These thresholds used were the ones that allowed the ImageJ code to automatically detect the cortical cortex on all participants scans. Short-term error of tibia and fibula pQCT scans assessed using similar thresholds in paired scans from twenty-five individuals by our group was very low. In both bones, coefficient of variation of total BMC was less than 0.6% whereas for no other parameter was this value greater than 1.5 %.

The normality of the data has been assessed using the Anderson-Darling normality test. Where data were normally distributed, parametric tests were performed to assess changes in all bone parameters Where data were not normally distributed, non-parametric Wilcoxon Signed rank test was used. Analysis was performed on Minitab Statistical software (Minitab, version 19). In addition both absolute and relative changes in the same bone variables in tibia and fibula were compared using paired T-tests, with relative changes calculated as the percentage change from baseline. In addition, relationships between normalised (%) bone loss in the two bones at the equivalent site were assessed using Spearman correlation, paired T-tests and Wilcoxon Signed test (for tibial BMD at 4%).

3.4 Results

Descriptive statistics of bone parameters at baseline and 12 months post-injury at 4%, 38% and 66% of tibial length in fibula and tibia are summarised in Table 3.1. All the data were normally distributed, with the exception of the tibial total BMD at the 4% site

At the 4% site, both total BMC and BMD declined in both bones over the time-course of observation. However, tibial BMC and BMD losses (-14.8 ±12.4% and -14.4±12.4%, both $P < 0.05$) were greater than those observed in the fibula (-6.9±5.1% and -6.6± 6.0%, $p=0.02$ and $p=0.03$, respectively). Both bones maintained their total area at this site ($p=0.6$ for the tibia; $p=0.8$ for the fibula) (Figure 3.1(a)).

Table 2.1: Descriptive statistics (Mean, standard deviation (SD), median, interquartile range (IQR)) of bone parameters at baseline and 12 months post-injury at 4%, 38% and 66% of tibial length in fibula and tibia

Bone / Scan site and Parameter	Fibula								Tibia							
	Baseline				12 months				Baseline				12 months			
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR
Distal Tibia 4%																
BMC (mg/mm)	104.5	26.9	100.8	45.7	97.3	25.6	95.9	41.8	418.1	50.1	420.9	50.3	356.9	72.1	378.5	106.6
BMD (mg/cm^3)	562.2	92.6	580.2	110.8	525.4	94.1	524.7	124.5	-	-	334.5	30.4	-	-	301.0	60.9
Total CSA (mm^2)	186.2	36.9	184.5	57.4	186.7	41.6	177.8	56.1	1268.4	137.6	1290.8	167.1	1263.6	149.1	1257.5	170.4
Distal Tibia 38%																
BMC (mg/mm)	120.6	19.9	119.3	37.1	118.4	20.2	117.8	41.5	431.3	55.2	439.7	63.2	415.2	57.7	415.4	58.6
Cortical BMD (mg/cm^3)	892.8	36.7	899.2	50.3	892.6	38.6	879.4	68.0	971.8	28.7	983.7	41.2	947.3	27.3	957.6	53.0

Total CSA (mm^2)	154.0	28.5	155.5	53.5	151.6	28.1	151.5	51.8	509.1	57.7	500.5	72.3	504.2	57.6	499.8	73.3
Cortical CSA (mm^2)	134.3	22.5	130.3	42.5	131.8	21.9	129.5	40.4	440.0	53.0	442.5	47	434.1	53.7	431	43.5
Distal Tibia 66%																
BMC (mg/mm)	101.2	17.6	101.7	34.1	99.2	17.7	97.6	34.0	480.0	57.5	474.2	75.8	459.3	59.4	449.1	84.8
Cortical BMD (mg/cm3)	834.6	52.5	835.0	80.4	842.8	59.5	853.7	67.3	834.9	49.1	839.6	66.0	811.8	57.9	801.4	62.6
Total CSA (mm^2)	129.4	22.6	134.5	36.8	126.8	23.3	130.3	38.1	763.8	100.1	765.0	158.8	760.7	96.9	775.9	168.6
Cortical CSA (mm^2)	120.4	19.5	123.5	31.6	117.1	20.3	119.1	36.4	564.3	66.2	560.3	100.8	553.4	60.7	538.6	89.6

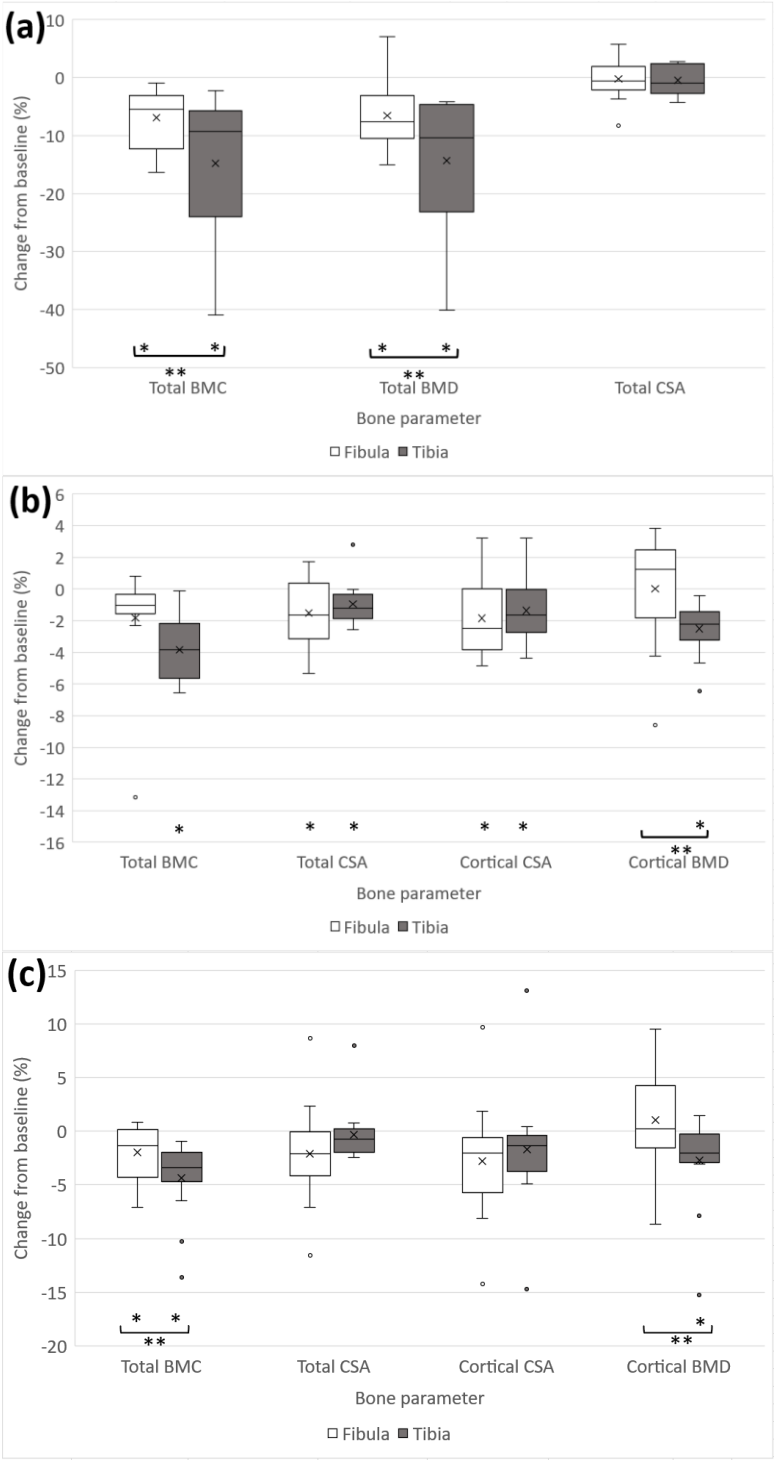

Figure 2.1: Box plots of change at 12 months post-injury relative to baseline values in: (a) Total bone mineral content (BMC), total bone mineral density (BMD), and total area (CSA) at the distal 4%, (b) total BMC, total CSA, cortical CSA and cortical BMD at 38% site, (c) total BMC, total CSA, cortical CSA and cortical BMD at 66% site (distal to proximal) of tibia and fibula bones. Plot shows mean(x), median, IQR, lowest and highest changes. (*) Indicates significant change in bone parameter at 12 months, (**) Indicates significant difference between tibial and fibular percentage changes

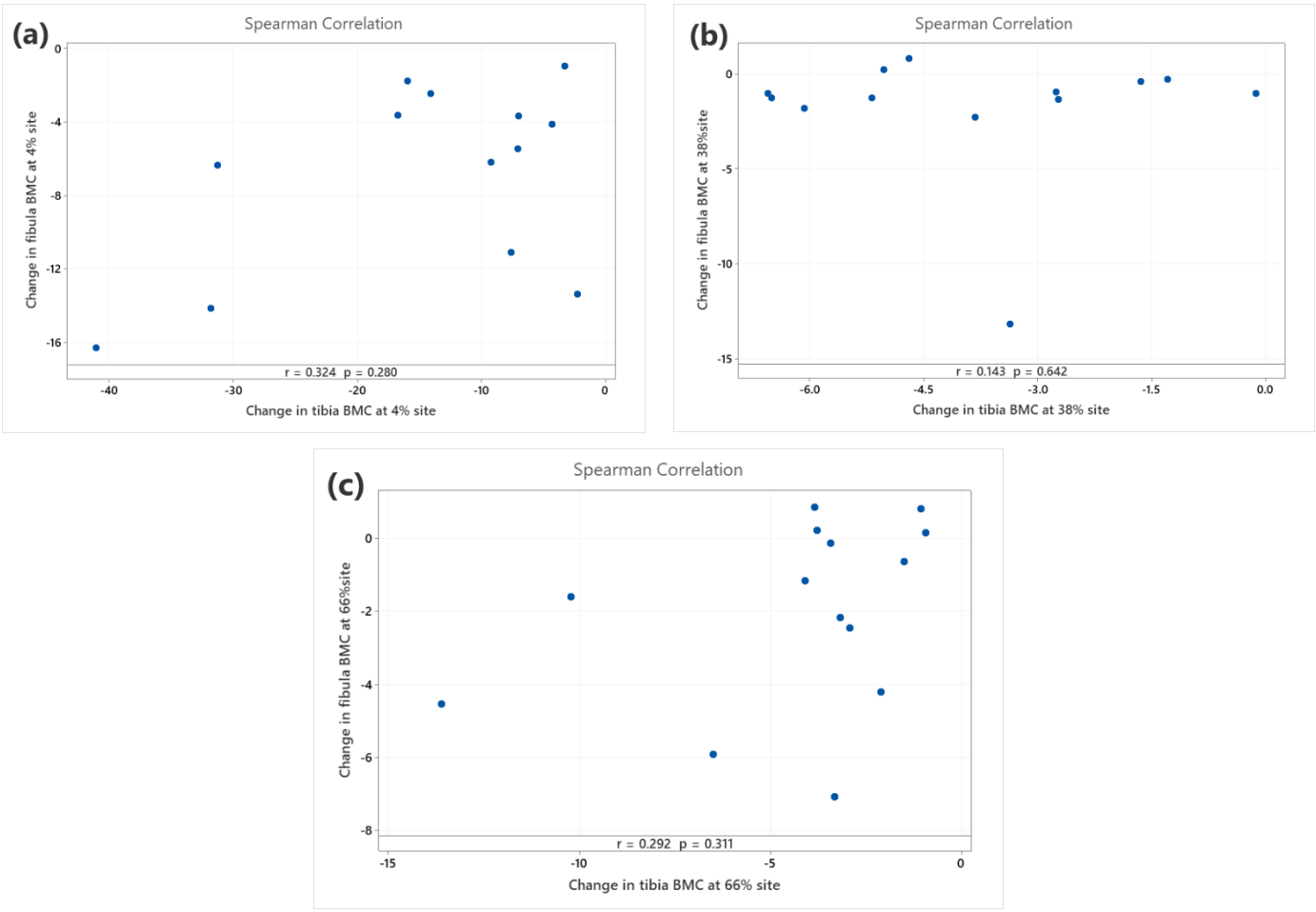

Figure 2.2: Matrix plots of correlation results of changes in total bone mineral content (BMC) between tibia and fibula at (a)4%, (b) 38% and (c) 66% of tibial length between baseline and 12 months post-injury

In the diaphyses, BMC decreased at both tibial sites (38%,66%), with tibial losses at 66% being twice those in the fibula (-4.3±3.6% vs -2.0±2.6%, p=0.03). However, whilst fibular losses were similar in magnitude at both sites, the statistical evidence of a loss at 38% was weak (p=0.06) and there was no evidence of a difference in loss between the tibia and fibula (-3.8±2.1% vs -1.8±3.5%, p=0.1), partly due to the larger standard deviation (as can be seen in Figure 3.1(b, c)). Differences in BMC loss resulted from greater cortical BMD losses in tibia than fibula at both sites (-2.5±1.6% vs 0.0 ±3.6% and -2.7±4.2% vs 1.03±4.7%, p=0.01 and p=0.02,

respectively). In contrast, whilst CSA and cortical CSA decreased in both tibia and fibula at the 38% site (-0.9±1.4% vs -1.5± 2.1% and -1.4±1.9% vs -1.8±2.4%, p=0.5 and p=0.63, respectively) and were maintained at the 66% site in both bones (-0.3±2.6% vs -2.1±4.7% and -1.7±5.7% vs -2.8±5.4%, p=0.07 and p=0.4, respectively), these changes were similar in both bones. No evidence of a correlation was found between changes in BMC between the two bones (all p>0.25, Figure 3.2).

In secondary analyses, we investigated whether the relative proportions of cortical and trabecular bone could contribute to observed differences in bone loss between the tibia and fibula. In the distal tibia, cortical and trabecular bone made up 69±3% and 31±3% of total BMC respectively (full data not shown). Whilst percentage bone loss from the cortical component (18±6%) was slightly larger than from the trabecular component (14±7%), this difference was not significant (P = 0.32). In contrast, bone at the distal fibula was mostly cortical (99.7±0.7%), and losses from this component (7.2±5.6%) were smaller than those observed at the distal tibia (P < 0.001). As expected, at both tibia and fibula diaphyseal sites the bone was almost entirely cortical, with >98% cortical content at all sites.

3.5 Discussion

The aim of this study was to describe longitudinal changes in the fibula bone in response to disuse during the first 12 months of SCI, and to compare these changes to those in the tibia. Across epiphyseal and diaphyseal sites, fibular bone loss was less than 50% of that at the corresponding tibia site which supports the results from previous cross-sectional studies[258][273][257]. Losses in the tibia and fibula within each participant were not correlated with each other. The loss of BMC that was evident at the fibular shaft (38% and 66%, respectively) contrasts with a previous cross sectional report that observed no difference in BMC in the fibular shaft in chronic SCI[258]. In both the fibula and tibia, bone losses were more prominent at the distal end compared to the shaft, which supports findings from cross-sectional studies[274][114].

Previous evidence suggests that: i) relative changes in fibula loading are greater than those in the tibia, ii) the fibula supports a substantial portion of shank loading during

physical activity and iii) the fibula is able to change its size and mass dramatically in response to increased loading [267][268][269]. Therefore, it is perhaps surprising that disuse-related bone losses are less than half those in the neighbouring bone. In addition, the lack of correlation reported here between the tibia and fibula suggest further that they are affected by different mechanisms. Evidence for ii) and iii) could be considered robust, particularly for ii) when we consider the occurrence of fibula stress fractures in athletes. However, proposition i) is based on cadaveric data and, to date, the in vivo loading environment of the fibula is unknown. In addition, previous data describe static loading conditions, and it is well established that the rate of force application is a key determinant of bone mechanoadaptive response. Therefore, assessment of fibula deformation in vivo would improve our understanding of fibula's mechanoadaptive response.

The mechanisms leading to less pronounced bone loss in the fibula compared to the tibia are not fully understood, but structural differences between the two bones have been considered. The endocortical surface, with its higher rate of bone turnover is larger in the tibia, and previous studies showed that between-site differences in endocortical circumference are strongly correlated with site-specific loss in the tibia [274][144]. However, when normalised to bone size the surface:area ratio is greater in the fibula than in the equivalent sites in the tibia suggesting that this does not contribute to observed inter-bone differences. For the two diaphyseal sites, the percentage loss was identical. However, it was statistically evident at the 66% and not at the 38% due to the greater dispersion in BMC changes at 38% which appeared to be related to one outlier. This outlier was checked and was found to be generated by movement artefacts, which could also be seen in few other scans. Therefore, it was decided to keep it in the dataset, as removing this scan will require removing all others scans with movement artefacts.

Divergent responses of the distal tibia and fibula to disuse could alternatively be explained in part by the greater trabecular component in the distal tibia, that is known to show a more rapid response to disuse (in absolute terms) compared to the cortical component[115][114]. In secondary analyses we considered the relative proportions of trabecular and cortical bone in addition to relative losses in the two bone regions. At the distal tibia, 31% of bone mass was trabecular whereas the proportion was

negligible in the fibula. However, percentage bone loss was higher in the distal tibia cortical component than in the distal fibula and these losses were also more than twice as large as those observed in the cortical component of the distal fibula. When considering that at both tibia and fibula shaft sites the bone was almost entirely cortical, it seems clear that the relative proportions of trabecular and cortical bone cannot explain the differences in bone loss between the tibia and fibula at any site. Whilst caution must be used when assessing cortical bone at the distal tibia using pQCT due to the thin cortical shell and associated partial volume effect, it is reassuring that our findings of similar loss in cortical and trabecular components is similar to a previous report using high-resolution pQCT[257].

That modest fibula response to disuse may explain the low incidence of fibula fractures in patients with SCI, who tend to experience fragility fractures mostly in the distal femur and tibial epiphyses[33]. Moreover, a deeper understanding of the mechanisms that lead to these smaller fibular deficits in disuse could help us develop therapies to mitigate or treat osteoporosis. Understanding these different responses to disuse can also provide more insights into neuro-skeletal interactions that are yet to be fully understood.

To the best of our knowledge, this study is the first to explore the fibula's response to disuse following SCI longitudinally. Using pQCT to assess changes in tibia and fibula was beneficial in measuring volumetric BMD and cortical parameters. However, it was limited by the pQCT's inability to accurately assess cortical BMD in bones with cortical thickness smaller than 1.5mm (which is prevalent in this patient group), due to partial volume effect. Within-individual comparisons enabled a characterisation of disuse-related loss in the fibula which has not been possible in previous cross-sectional studies. However, the absence of an uninjured control group in this longitudinal study, prevented a direct comparison to determine whether the bone losses observed in the fibula differed from typical age-related changes. Whilst tibial changes are clearly far greater than those observed in controls[275], no comparable data exists for the fibula. In the only longitudinal study of the fibula bone in older adult athletes (in whom disuse does not contribute), the fibula changes in the shaft are not entirely dissimilar[56]. Therefore, further controlled studies or alternative disuse models should be examined.

3.6 Conclusions

Fibula bone losses following SCI are less pronounced than in the neighbouring tibia. This is despite the substantial contribution which the fibula makes to shank loading, and evidence that the fibula has the capacity to adapt in response to increased loading. The losses in the two bones are seemingly not related, suggesting that they may be influenced by different mechanisms. Alternatively, the differences in their mechanical loading *in vivo* which have not been revealed by previous *ex vivo* studies may contribute. In contrast to previous cross-sectional reports, some loss of bone mass was observed in the fibula. These results may in part explain lower incidence of fibula fractures in individuals with chronic SCI. Further study of the biomechanics of the two bones is required.

Chapter 4:

Regional and temporal variation in bone loss during the first year following spinal cord injury

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Abstract

Osteoporosis is a consequence of spinal cord injury (SCI) that leads to fragility fractures. Visual assessment of bone scans suggests regional variation in bone loss, but this has not been objectively characterised. In addition, substantial inter-individual variation in bone loss following SCI has been reported but it is unclear how to identify fast bone losers.

Therefore, to examine regional bone loss tibial bone parameters were assessed in 13 individuals with SCI (aged 16-76 years). Peripheral quantitative computed tomography scans at 4% and 66% tibia length were acquired within 5 weeks, 4 months and 12 months postinjury. Changes in total bone mineral content (BMC), and bone mineral density (BMD) were assessed in ten concentric sectors at the 4% site. Regional changes in BMC and cortical BMD were analysed in thirty-six polar sectors at the 66% site using linear mixed effects models. Relationships between regional and total loss at 4-month and 12-month timepoints were assessed using Pearson correlation.

At the 4% site, total BMC ($P = 0.001$) decreased with time. Relative losses were equal across the sectors (all $P > 0.1$). At the 66% site, BMC and cortical BMD absolute losses were similar (all $P > 0.3$ and $P > 0.05$, respectively) across polar sectors, but relative loss was greatest in the posterior region (all $P < 0.01$). At both sites, total BMC loss at 4 months was strongly positively associated with the total loss at 12 months ($r=0.84$ and $r=0.82$ respectively, both $P < 0.001$). This correlation was stronger than those observed with 4-month BMD loss in several radial and polar sectors ($r=0.56$-0.77, $P < 0.05$).

These results confirm that SCI-induced bone loss varies regionally in tibial diaphysis. Moreover, bone loss at 4-months is a strong predictor of total loss 12-months postinjury. More studies on larger populations are required to confirm these findings.

4.1 Introduction

People with complete spinal cord injury (SCI) experience extensive and rapid bone loss in their paralysed limbs[58]. The distal femur and proximal tibia lose up to 52% and 70% of BMD after only one year following injury[128]. This dramatic decline in bone density and quality leads to fragility fractures which occur mostly at the knee and ankle joints[37][33], putting these individuals at risk of developing secondary medical complications[36]. People with SCI are at 23-fold higher risk of experiencing femur fractures compared to the uninjured population[35]. Studies have shown that bone loss continues throughout the first years following SCI[138][141], until it reaches a steady state around 3-8 years postinjury[114]. Whilst there are mixed results of the effectiveness of physical interventions in mitigating bone loss[58], anti-resorptive drugs provide positive effects on BMD in people with acute SCI[264]. Administering bisphosphonates early after SCI decreases bone loss in the hip[264][265] and lumbar spine at 12 months[265]. Thus, detecting and targeting bone loss during the early months following SCI could potentially help prevent further loss.

Bone loss after SCI is characterised by substantial inter-individual and regional variation. The rate and magnitude of bone loss has been shown to vary widely among individuals with SCI, with trabecular BMD loss at the distal tibial varying between 1-65% one year postinjury [140]. Bone loss has been shown to be more pronounced at the epiphyseal compared to the diaphyseal sites[138][144] and bone scan images appear to show regions with greater bone loss compared to others within the same bone cross-section[144]. However, this intra-site variation in bone loss has not been objectively characterised before. If regional variation is confirmed, regions with higher rate of bone loss could be assessed and used to predict fast bone losers. An ImageJ plugin[271][272] has been developed which allows regional analysis of bone parameters in concentric or radial sectors.

Therefore, we aimed to characterise regional bone loss in the first year following SCI. In addition, to assess the relationships between early stage (four months postinjury) regional and total bone changes and those observed at twelve months postinjury. We hypothesised that bone loss following SCI varies regionally. In

addition, these regions with rapid early-stage bone loss would be predictive of total bone loss at one-year postinjury.

4.2 Methods

Twenty-nine inpatients (aged 16-76 years) with motor-complete SCI (grades A or B on the American Spinal Injuries Association Impairment Scale (AIS)) at the Queen Elizabeth National Spinal Injuries Unit (UK), were recruited for this study. Longitudinal changes in the tibia in these individuals have been published an a previous report[138]. The main exclusion criteria were age <16 years; recent bone fracture and continued ventilator dependency at week 5 post-injury. Ethical approval for the study was obtained from the NHS Research Ethics Committee. Further details on patient recruitment and scanning protocols for that study have been described previously[138].

Bone scans were obtained by a single operator using pQCT (XCT3000, Stratec Medizintechnik GmbH, Germany) from these 29 participants within the first 5 weeks (baseline) and at 4,8 and 12 months postinjury and were analysed for longitudinal changes in bone parameters at the tibia throughout the first year of injury[138]. Of these, a subgroup of 13 individuals with complete sets of baseline, 4-month and 12-month scans, were included in this study. Two, four and seven participants were excluded from the analysis for not having their bone scans at baseline, four months and twelve months, respectively.

Scans were taken at 4% and 66% of tibial length (distal-proximal). These scans were analysed using the pQCT plugin on ImageJ (National Institutes of Health, Maryland, USA)[271][272]. Epiphyseal parameters calculated at the 4% site were the total bone mineral content (BMC), total cross-sectional area (CSA) and bone mineral density (BMD). BMD was also calculated at 10 anatomical concentric sectors, starting from the center of the bone (Sector 1) and moving toward the cortex of the bone (Sector 10). The parameters calculated at 66% diaphyseal site were total BMC, periosteal circumference, endocortical circumference and cortical BMD. Mean periosteal and endocortical circumferences (Circumference $_{mean}$) were calculated for each subject using the formula:

$$\text{Circumference}_{mean} = 2 * \pi * R_{mean}$$

R_{mean} is the mean periosteal/endocortical radius calculated by averaging the 36 radii values (which were calculated by the plugin for the 36 polar sectors). BMD and cortical BMC were also calculated for the 36 polar sectors.

Given the thin cortex in individuals with SCI at epiphyseal sites, thresholds of 120 $mg.mm^{-3}$ and 150 $mg.mm^{-3}$ were used to separate bone and soft tissue at the 4% and 66% sites, respectively as in previous studies[276][144]. Coefficient of variation of total BMC and BMD in both sites in twenty-five individuals ranged between 7-15%.

Normality was assessed using Shapiro-Wilk test. Linear mixed effects models with time and sector (10 concentric sectors for 4% site bone variables, and 36 radial sectors for 66% site variables) as fixed factors and participant as a random factor were constructed. Site-by-time interactions were also examined, indicating differences in bone change between baseline and follow-up between the sectors. Where interactions terms were not evident ($P > 0.1$), the interaction term was removed. To account for baseline differences in bone parameters between sectors, analyses were also repeated with data normalised for baseline values. The relationships between individual sector losses and total bone losses at 4 months with total loss at 12 months was also examined at both sites using Pearson correlation. Data are presented as mean (SD), except when not normally distributed when median (IQR) is presented.

4.3 Results

Descriptive statistics of bone parameters at baseline, 4 months and 12 months post-injury at 4% and 66% of tibial length are summarised in Table 4.1. All values were normally distributed except the 4% site total BMD at 4 months and 12 months. One scan at the 4% site was removed from the analysis due to movement artefacts. An estimate of the bone cross-sectional shape/circumference was drawn by plotting all the mean periosteal radii for the 36 sectors at the 4% site. This was repeated for periosteal and endocortical radii to draw the cortical bone of the diaphyseal 66% site (Figure 4.1(upper), Figure 4.2(a)).

At the 4% site, total BMC ($P = 0.001$) but not total CSA ($P = 0.28$) decreased with time. Total BMD decreased with time across all sites, and values in the most peripheral sector were greater than all other sectors (all $P < 0.001$). There was also a site-by-time interaction for total BMD such that absolute losses in the outermost sector were greater than in other sectors (all $P < 0.05$) except sector 9 ($P = 0.074$). However, when adjusted for baseline values relative losses were similar across all sectors (Figure 4.1(lower), all $P > 0.1$).

Table 3.1: Descriptive statistics (Mean, standard deviation (SD), median, interquartile range (IQR)) of bone parameters at baseline, 4 months and 12 months post-injury at 4% and 66% of tibial length

Time point / Scan site and Parameter	Baseline				4-month				12-month			
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR
Distal Tibia 4%												
BMC (mg/mm)	418.1	50.1	420.9	50.3	398.0	57.6	402.7	75.1	356.9	72.1	378.5	106.6
BMD (mg/cm^3)	328.9	19.1	334.3	31.6	-	-	331.7	38.0	-	-	301.0	60.9
Total CSA (mm^2)	1268.4	137.6	1290.8	167.1	1256.1	154.7	1274.8	222	1263.6	149.1	1257.5	170.4
Distal Tibia 66%												
BMC (mg/mm)	480.0	57.5	474.2	75.8	477.0	58.5	473.3	79.0	459.3	59.4	449.1	84.8
Cortical BMD (mg/cm3)	834.9	49.1	839.6	66.0	833.1	54.2	837.3	61.8	811.8	57.9	801.4	62.6
Total CSA (mm^2)	763.8	100.1	765.0	158.8	755.6	96.4	754.3	153.6	760.7	96.9	775.9	168.6
Cortical CSA (mm^2)	564.3	66.2	560.3	100.8	562.2	67.2	550.8	102.6	553.4	60.7	538.6	89.6

periosteal circumference	96.2	6.6	95.7	10.0	96.0	6.4	95.9	9.9	96.3	6.6	98.2	11.2
endocortical circumference	47.7	6.3	48.7	9.1	48.2	6.0	48.9	9.9	48.7	7.3	48.1	9.5

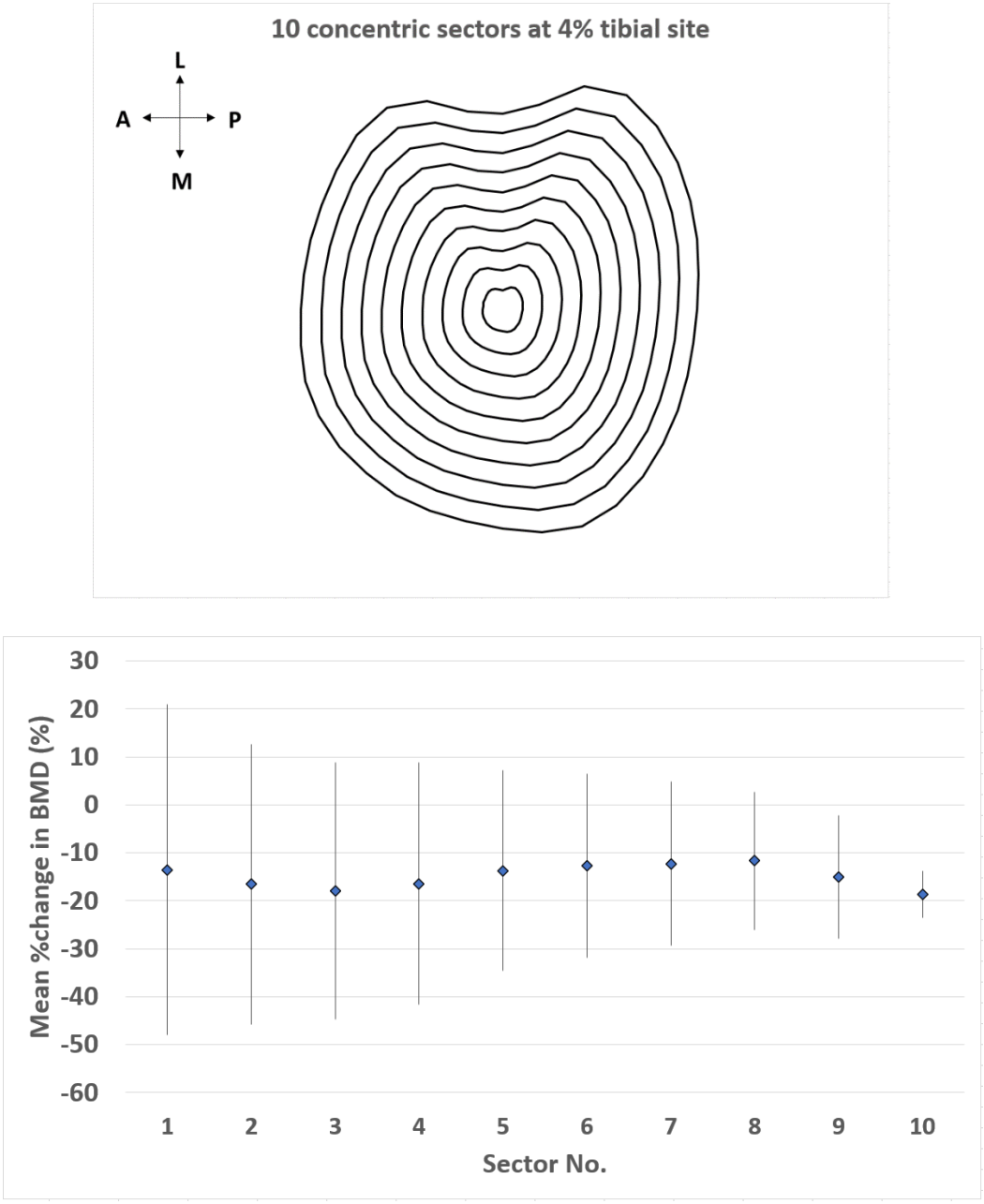
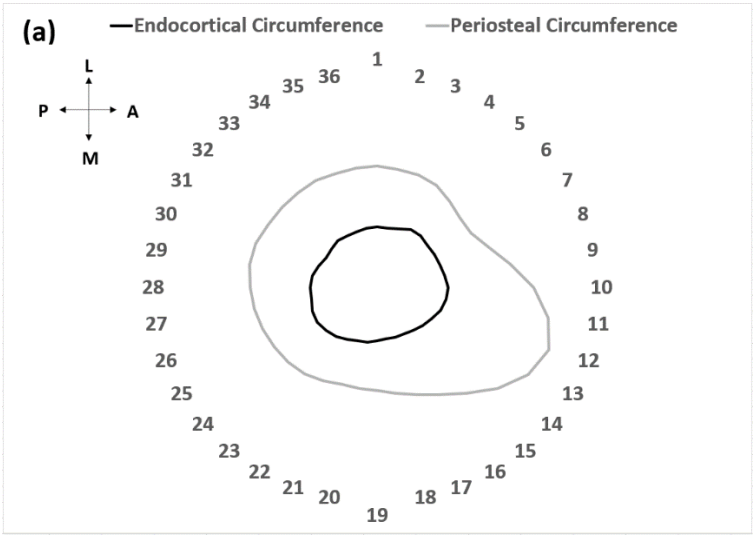


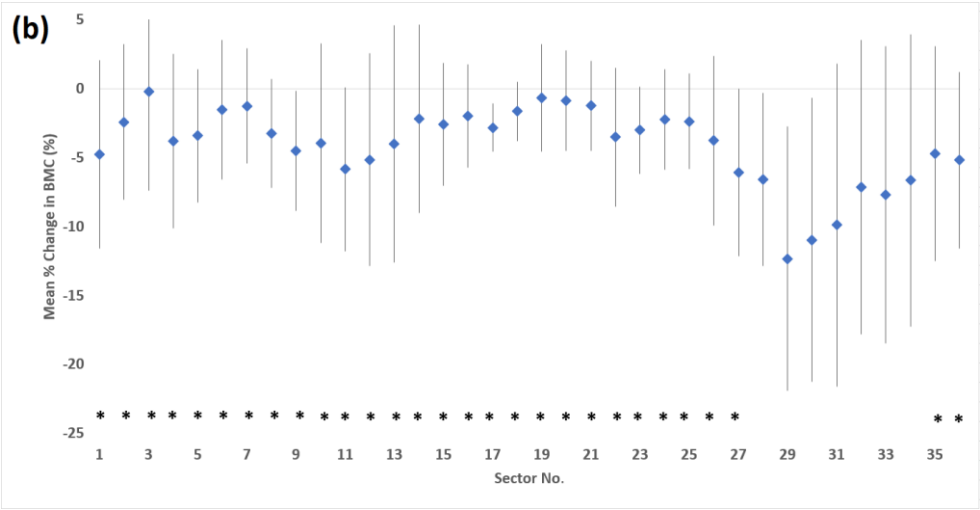
Figure 3.1: (Upper) An estimate of the bone cross section shape at the 4% site in baseline scans, showing the 10 concentric (sector 1 is innermost sector, sector 10 is outermost sector) (lower) Mean percentage change in BMD and 95%CI at the 10 anatomical concentric sectors at 4% distal tibia at 12 months postinjury (Error bars indicate ±SD).

At the 66% site, total BMC decreased with time across all sites, and values in sector 30 (posterior region) were lower than those in sectors 1-9 (lateral region) and 16-24 (medial region) (all $P < 0.001$) and 25-28 (posterior-Medial) (all $P < 0.05$), and lower than sectors 11-14 (Anterior region) (all $P < 0.001$). There was no evidence of a site-by-time interaction suggesting that absolute BMC losses across all sectors were similar (all $P > 0.3$). However, analysis of baseline adjusted values showed that relative losses were greater in sector 30 than sectors 1-27 (Lateral, anterior and medial) and 35-36 (Anterior) (Figure 4.2, all $P < 0.01$ except sector 27 where $P = 0.04$).

To examine density and geometrical changes underlying these total BMC results at the 66% site, we repeated the analyses for cortical BMD, periosteal circumference and endocortical circumference. For cortical BMD, similar results to BMC were observed as values decreased with time across all sites ($P = 0.013$) and were higher in sector 30 (posterior) than sectors 8-17 (Anterior and medial regions), and lower than values in sectors 1-6 (Lateral and lateral-anterior), 21-26 (medial-posterior) and 32-36 (posterior-lateral) (all $P < 0.05$) but no site-by-time interaction was observed (all $P <0.05$). However, baseline-adjusted relative losses were greater in sector 30 (posterior)than sectors 1-9 (lateral and anterior), 17, 25-26, 34 and 36 (Figure 4.2, all $P < 0.05$).

The uneven bone loss across the bone cross section can be seen in some of the bone scans shown in Figure 4.3, with bone loss being more pronounced not only at the posterior region but also in the anterior region. The loss can be seen across the bone cross section from periosteal to endocortical surfaces.


(a)
Endocortical Circumference
Periosteal Circumference
L
P
A
M
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
26
27
28
29
30
31
32
33
34
35
36


(b)
Mean % Change in BMC (%)
5
0
-5
-10
-15
-20
-25
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
Sector No.

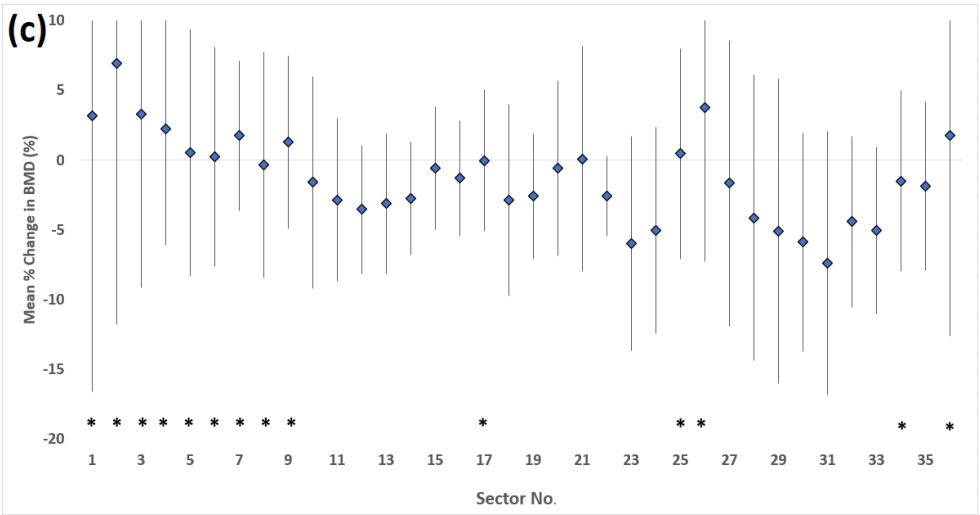

(c)
Mean % Change in BMD (%)
10
5
0
-5
-10
-15
-20
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
Sector No.

Figure 3.2: (a) An estimate of the bone cross section shape at 66% site, showing the 36 polar sectors,(b) Mean percentage change in BMC (upper) and (c) BMD (lower) at 12 months postinjury at the 36 polar sectors at the 66% diaphyseal site. (*) indicates the sectors that have less loss compared to sector 30 (Error bars indicate ±SD)

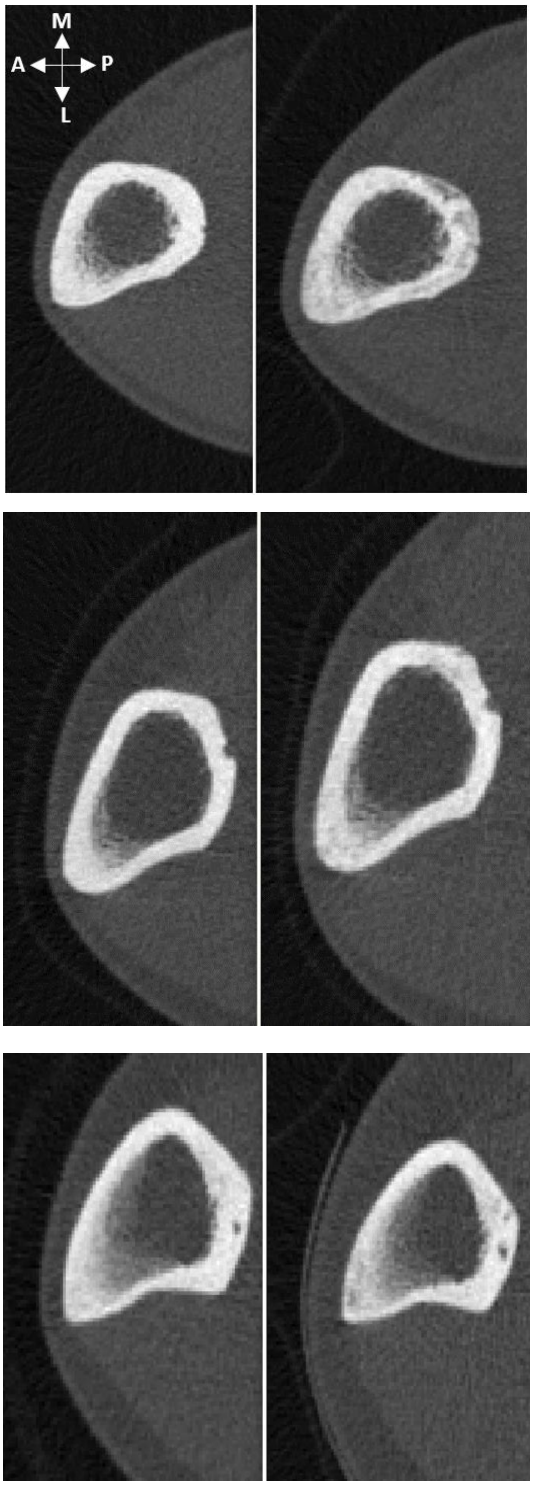


Figure 3.3: pQCT scans of tibial shaft (at 66%) from three individuals with SCI obtained at baseline (left column) and at twelve months postinjury. Scans show more pronounced bone loss at the posterior and anterior sites of tibia cross-section

There was no effect of time on periosteal circumference ($P = 0.914$) but absolute values in sector 30 (posterior)were greater than sectors 1-8 (lateral and lateral-anterior), 17-26 (medial and medial-posterior) and 32-36 (posterior and posterior-lateral) and lower than 10-15 (anterior and anterior-medial) (all $P < 0.05$) (Figure 4). There was no evidence of a sector-by-time interaction (all $P > 0.05$). $P > 0.2$. Endocortical circumference increased with time ($P = 0.003$), and values in sectors 5 and 8-13 (anterior), were higher and 16-22 (medial) and 25-29 (posterior) lower (all P 0.05) than in sector 30 (Figure 4).

BMD loss in concentric sectors 3-10 at 4 months was positively associated with total BMC loss at 12 months (r=0.62-0.77, $P < 0.05$) but not at sectors 1 and 2 ($P > 0.2$). These relationships were slightly weaker than those observed between total BMC loss at 4 months and at 12 months (r=0.84, $P < 0.001$) (Figure 4.4).

BMD loss at 4 months in sectors 1, 3, 12-14, 30 and 36 was positively correlated with total BMC loss at 12 months (r=0.56-0.67, $P = 0.05$) but not at other sites. The strength of the correlation was weakly positively associated with the bone loss *i.e.* those regions in which greater losses were observed had closer relationships with total losses at 12 months (r=0.35, $P = 0.037$). As with the 4% site, the relationships between 4-month sector losses and 12-month total losses were weaker than those observed between total BMC losses at 4 and 12 months (r=0.82, $P < 0.001$) (Figure 4.4).

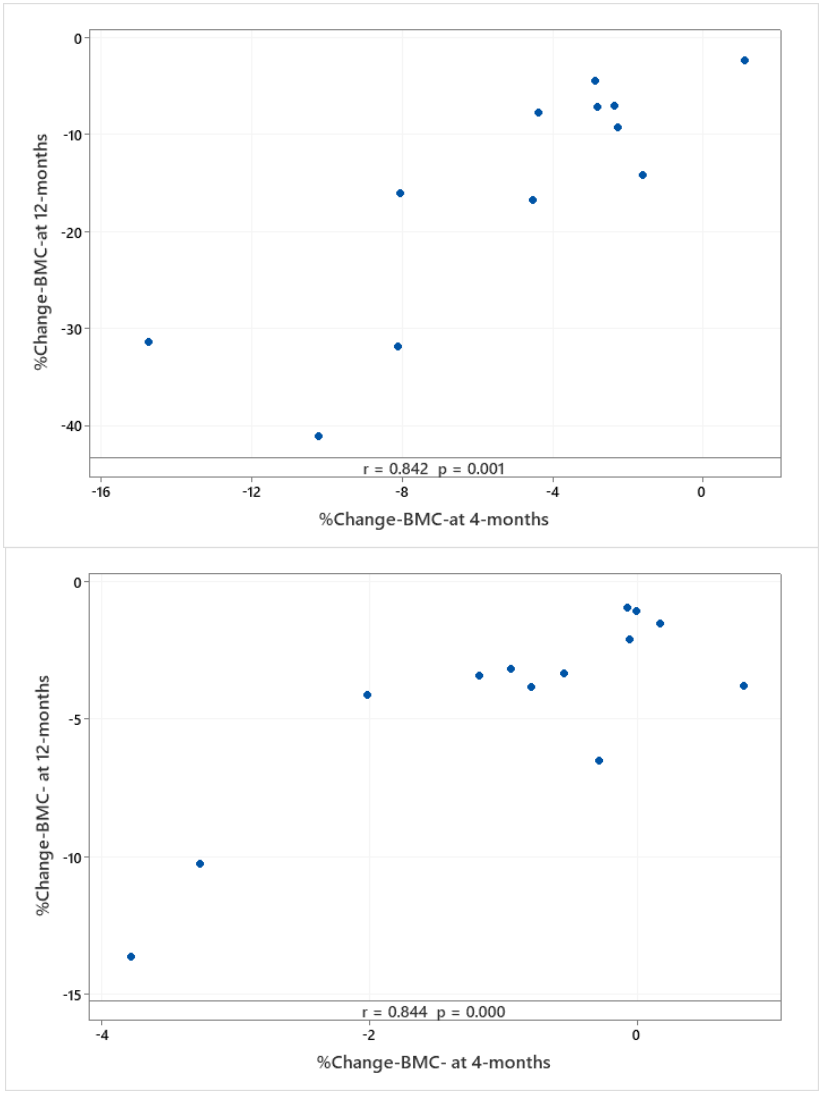


Figure 3.4: Correlations between total BMC losses at 4-months and 12-months total BMC losses for 4% (upper) and 66% (lower) sites

4.4 Discussion:

The aim of this study was to investigate whether bone losses following SCI vary regionally within the tibia. In addition, to assess whether total and regional bone loss at the four-months postinjury are associated with total loss at twelve months postinjury.

Whilst absolute losses in BMD were greater in the outermost region of the distal tibia cross-section, relative (*i.e.* percentage) losses were equal across the bone. In contrast, both total BMC and cortical BMD decreased equally in absolute terms in all sectors

at 66% site, but relative loss was greatest in the posterior region compared to other sectors across the bone. In addition, total BMC loss at four months postinjury was highly correlated with loss at twelve months postinjury, whereas associations with regional loss were less strong.

Greater absolute losses in BMD at the outermost sector in the distal tibia can be explained by the predominantly cortical bone component of that region which has higher density compared to the predominant trabecular component in other sites. Even when adjusted for this discrepancy, losses in the outer sector were similar to those in other sectors, which contradicts the assumption that trabecular loss is faster and greater than cortical losses[76]. This finding corroborates the results of another recent HR-pQCT-based study which reported similar deficits in bone mass in trabecular and cortical bone at distal tibia following long-term spinal cord injury[257]. A comparable relative bone loss across different regions at this site may be due to the similar mechanical stresses (that are mostly compressive) experienced at this site.

As previously reported, the loss in total BMC at the diaphyseal 66% was largely due to cortical BMD loss rather than decreased CSA[138]. This is in contrast to longer-term studies which suggest that bone mass loss is mostly due to the reduction in cortical wall thickness[114]. Bone loss appears to be more pronounced in the posterior and anterior regions compared to other parts of the bone[144] similar to the regional variation reported in age-related bone loss in uninjured population[277]. This outcome is not well understood but could probably be explained by different form or magnitude of loading that is experienced by this region of the bone. An asymmetric response to training was reported at the tibial[278] and femoral[191] posterior regions, and regional differences in habitual loading might underly asymmetrical responses to disuse once they are removed. This decline in the posterior and anterior regions could also be explained by the relatively high endocortical circumference: area ratio at this region, which was suggested to be associated with greater bone loss following SCI in a cross-sectional study[144]. The low BMD may indicate a higher porosity at this region compared to other regions, which would typically provide more surface for bone resorption.

In agreement with other reports, endocortical circumference increased while the periosteal circumference showed no change[146][279]. One study reported decreased periosteal circumference in people with SCI compared to able-bodied controls [144]. However, the SCI group in that study had sustained their injury between 9-32 years ago, which might suggest that this decline in periosteal circumference occurs later in the chronic phase. Alternatively, that group differences reflect a blunting of the usual age-related increase in periosteal circumference observed in uninjured adults[280]. Given that area increases by the square of the radius, relatively large changes in area equate to small changes in circumference. Hence, some minor changes in periosteal circumference and regional variations in both circumferences may not have been detected by our pQCT assessments.

Bone losses four months postinjury were strongly correlated with losses at twelve months postinjury. Previous studies have identified huge variation in bone loss following SCI, with trabecular BMD losses of 1-65% observed twelve months after injury[140]. That fracture incidence in SCI, as with uninjured individuals, increases dramatically with degree of BMD loss[281] means that those with rapid bone loss are at high risk of fracture even relatively shortly after injury. However, a recent study found that 51% of SCI medical professionals would only test for bone loss in the chronic phase, or only after a fragility fracture occurs (43%)[256], which highlights the inconsistency in the detection of bone loss following SCI. Our finding of the importance of the four months bone scans further emphasises the need for clinical guidelines for early bone scans and probably preventive treatments to reduce fracture risk.

A previous study used statistical shape modelling to predict bone loss in SCI[282], in order that individuals at risk could be identified and early treatment commenced. Whilst these models identified individuals at risk of bone loss, these relationships were less strong than those observed in the current study. In addition, that the total BMC measures found to be strong predictors of twelve-month bone loss are available from the pQCT manufacturer's software without additional analysis increases their clinical viability. Further assessment of the predictive value of bone assessments in

the early postinjury stages should be conducted, and if appropriate, the effects of early-stage interventions in those at risk of rapid bone loss should be assessed. These studies should also consider other regions that are prone to fracture in this group such as the proximal tibia and distal femur.

To our knowledge, this is the first study to assess regional bone loss within tibia cross-sections. A longitudinal assessment in injured individuals represents stronger evidence than that available from cross-sectional comparisons of injured and uninjured individuals. Whilst repeated measures assessments are a powerful statistical technique even with small participant numbers, these assessments were performed in a relatively small cohort. Therefore, we may have been underpowered to detect modest variations in regional loss, which reinforces our call for these assessments to be replicated in a larger cohort.

We observed regional variation in tibia bone loss following spinal cord injury. Absolute losses in the distal tibia were greatest in outer region of bone, although relative losses were similar. Conversely, whereas absolute decreases were similar across radial sectors in the tibia shaft, relative losses were greater in the posterior region. We also found that bone losses at four months postinjury were strongly correlated with bone loss twelve months after injury. More studies on larger populations are required to help assess the utility of early-stage bone assessments in identifying rapid bone losers and implementing early treatments to prevent greater bone loss.

Chapter 5: Able-bodied study

5.1 Introduction

Surface electrical stimulation of skeletal muscles is a commonly used technique for rehabilitation and muscle strengthening applications[283]. It has been widely used to stimulate individual muscle groups to produce functional movements in paralysed limbs to attenuate muscle atrophy and disuse osteoporosis in individuals with spinal cord injury (SCI)[52][58]. Despite its well-documented advantages to restore muscle size and strength in this population, its effect on bone health remains limited[58]. This is partly due to the limited peak joint torques and associated muscle forces elicited by electrical stimulation in individuals with SCI due to muscle atrophy and deconditioning. Typical stimulated knee extension torques are 5-50 Nm in individuals with SCI[284], compared to voluntary values of 147-280 Nm in uninjured young adult males[285][286].

Commonly, only one group of an agonist-antagonist pair muscles e.g. knee extensors or flexors are stimulated at one time during electrical stimulation. Simultaneous recruitment of opposing muscle groups should increase the forces acting on bone, thereby providing a greater stimulus for bone formation[6]. High-intensity stimulation can result in large net joint torques which in rare cases have led to bone fracture in individuals with SCI[165]. Importantly for future applications in reducing fracture risk in high-risk populations such as people with SCI, in our dual-stimulation approach the opposing actions of the two muscle groups should hypothetically neutralise or reduce the net joint torque thereby improving safety and reducing discomfort. In addition, the proposed method can be applied when individuals are seated with little specialist equipment required. This can reduce cost and inconvenience, increase adherence and thereby exercise efficacy.

Patellar tendon elongation increases with knee extensor muscle force[287], providing a non-invasive indicator of function during stimulation when other techniques such as electromyography are not applicable. Assessment of knee torque and patellar tendon elongation during single and paired muscle stimulation would provide information on the individual and net contributions of the two muscle groups.

Therefore, we applied a novel approach to simultaneously stimulate antagonistic muscle pairs; knee extensors (quadriceps) and flexors (hamstrings) and assessed joint torque, tendon elongation and comfort. We hypothesised that dual stimulation would lead to reduced net joint torque, greater combined torques with a similar comfort level compared to single muscle group stimulation.

5.2 Methods

5.2.1 Participant recruitment:

This study was conducted at Manchester Metropolitan University between February-July 2019. It was granted a favourable ethical opinion by the Science and Engineering Research Ethics and Governance Committee (Reference Number: 5739) in February 2019. Participants were recruited through adverts on Twitter, university webpages, school email list and word of mouth at Manchester Metropolitan University.

Potential participants were eligible if they were healthy adults (male and female) aged between 18-45 years. Exclusion criteria were: (i) spinal cord injury or other clinical conditions affecting mobility e.g. cerebral palsy; (ii) skin condition affecting skin on legs and thighs (e.g. eczema, dermatitis); (iii) having metallic implants or other contraindications for MRI scanning; (iv) current musculotendinous or joint injury.

5.2.2 Consent

A participant information sheet was provided to each eligible candidate upon enquiry. Those interested in taking part were then invited to a 2-hour testing session in the musculoskeletal lab at Manchester Metropolitan University. When the participant arrived at the lab, the protocol was explained to them before taking their written informed consent.

5.2.3 Knee torques:

A Cybex dynamometer (Cybex, New York, USA) **(Figure 5.1)** was used to measure knee extension and flexion torques. The centre of rotation of the knee was aligned with the pivot point of the dynamometer lever. The shin was strapped tightly into the

dynamometer lever. The participant's trunk was strapped to the dynamometer chair to minimise the movement of upper body and keep the hip joint at 90^{o} flexion, whilst the knee joint angle was set at 45^{o}. This angle was chosen as it resulted in higher hamstring torques due to reduced muscle slackness, thereby decreasing the difference in maximal torques between the opposing muscle groups.

Figure 4.1: Cybex dynamometer used to measure knee torques

5.2.4 Ultrasound:

Sagittal scans of the patellar tendon were taken using a linear array, 7MHz ultrasound probe (Aloka SSD-5000SV, Tokyo, Japan). After applying the conducting gel on the knee surface, the ultrasound probe was placed and strapped on the knee in a position that provided a clear view of the patella apex and the tibia tuberosity **(Figure 5.2).** The tendon length was measured as the distance between the patella and the tibia tuberosity as shown in **Figure 5.3.**

For assessment of maximum voluntary contraction (MVC) torque the participant was asked to flex their leg (right), gradually increasing the strength of contraction until reaching MVC. They were asked to reach this peak within five seconds and maintain

this level for two seconds before they were asked to relax. This step was repeated three times with 1-2 minutes rest between contractions. The typical error for tendon length and maximal elongation measurements taken in our laboratory was previously reported[288] to be 0.6 mm and 0.1 mm, respectively.

Figure 4.2: Measuring patellar tendon elongation using ultrasound probe placed on participant knee during electrical stimulation of thigh muscles

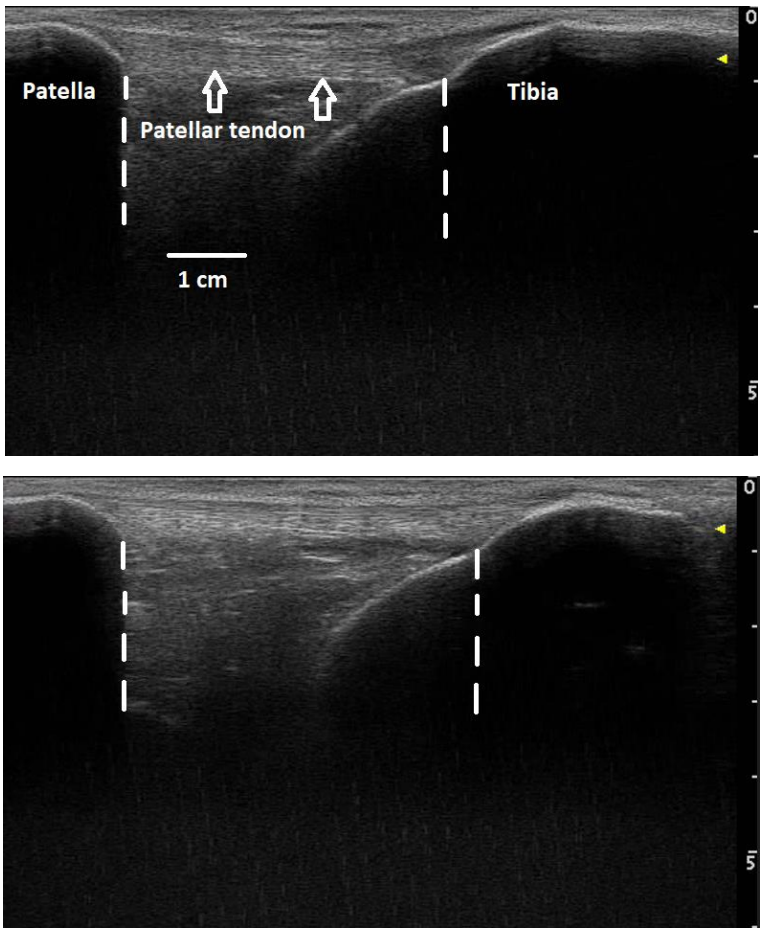


Figure 4.3: Ultrasound scans showing the patellar tendon at rest (top) and stretched (bottom) during quadriceps muscle electrical stimulation (current intensity=45 mA)

5.2.5 Electrical stimulation:

Before applying the electrical stimulation, the distance between the anterior superior iliac spine and the upper surface of patella was measured while the participant was sitting on the dynamometer chair. On the quadriceps, the cathode electrode was placed at 20% and the anode at 70% of that distance according to the configuration suggested by a previous investigation of electrode placement[53]. On the hamstrings, the electrodes were placed on equivalent positions under the thigh. One pair of 5cm x 10cm electrodes was placed on each muscle group and connected to the stimulator as shown in **Figure 5.4**. An electrical stimulator (EV-906A, The Tens company, Stockport, UK) was used to produce an electrical current (biphasic square pulses, 20-45 mA, 70 Hz, 300 μs) with a two-second on ten-sec off duty cycle.

First, the hamstrings were stimulated with current intensity set at 5 mA first before it was increased in 5 mA increments up to the maximum intensity tolerated by the participant. The participant was asked after each stimulation level whether they were happy to proceed. After completing the hamstrings stimulation, quadriceps were then stimulated while the ultrasound probe was placed on the knee to measure the patellar tendon elongation during both the voluntary and the electrically-stimulated knee extension. After measuring quadriceps MVC and the torques and the associated ultrasound scans, both hamstrings and quadriceps were stimulated simultaneously. They were stimulated using the same current intensity (5mA), which was then increased for both muscles until the participant decided to stop the test.

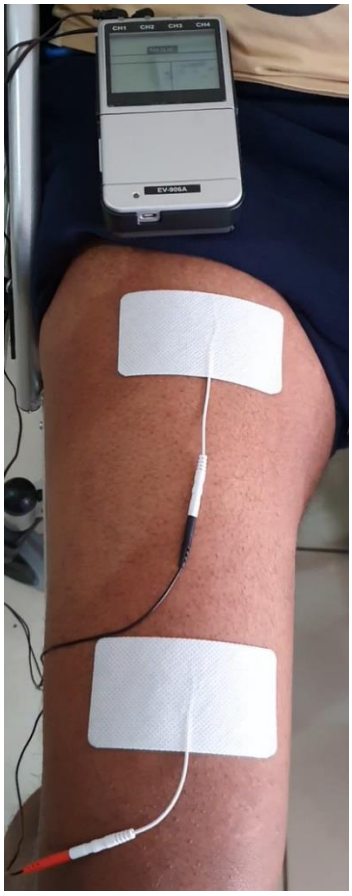


Figure 4.4: Electrical stimulator connected to 5x10 cm surface electrodes

5.2.6 Discomfort

During both single and dual electrical stimulation, discomfort was assessed using the visual analogue pain scale shown in **Figure 5.5**. Participants were given a sheet in which they could write down a number between 0 (no pain) to 10(worst pain possible), based on their pain level.

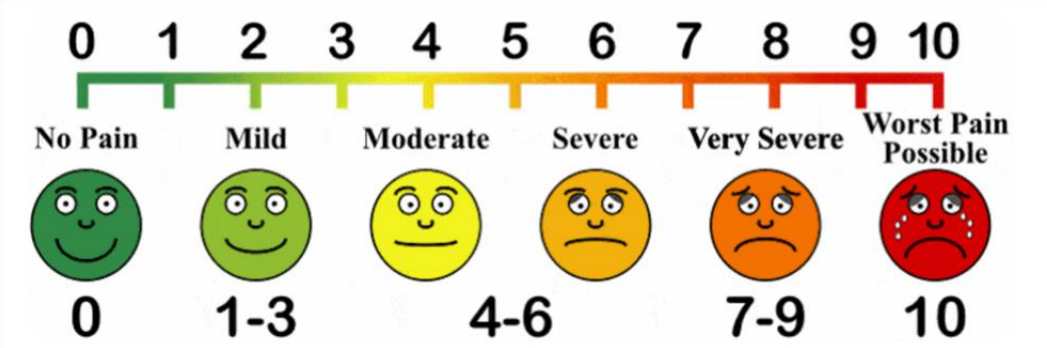


Figure 4.5: The visual analogue pain scale used to assess discomfort during electrical stimulation

5.2.7 Data analysis:

Knee extension and flexion torques were recorded from the dynamometer via LabChart software (LabChart 8 Reader, ADInstrumenets, Dunedin, New Zealand) (Figure 5.6).

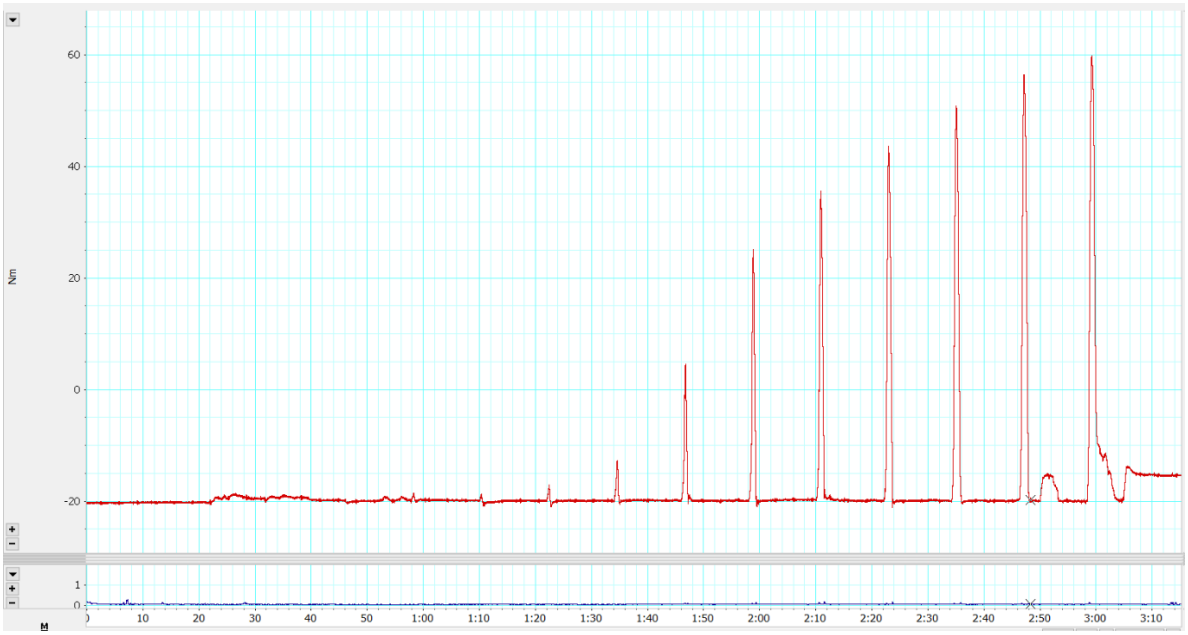


Figure 4.6: knee torque recorded from Cybex dynamometer on LabChart software during dual stimulation. X and Y axis present time (in minutes) and torque (in Nm)

Patellar tendon elongation was measured from ultrasound scans during quadriceps MVC, quadriceps stimulation and dual stimulation using ImageJ software (ImageJ 1.52a, National Institutes of health, USA, available at: https://imagej.nih.gov/ij/).

Multiple screenshots were taken from the ultrasound video of the muscle contraction (during each current intensity) at different time points, starting just before the contraction occurred (when the muscle was relaxed) and then after each five frames, until the contraction was complete. The tendon elongation was calculated from the formula below:

Tendon Elongation = tendon length during muscle contraction (t_n) - tendon length during relaxation (t_o)

Where: t_n $(n=1,2,3,4,\ldots)$ is the time point during contraction taken after 5 frames, so that if the point t_o corresponds to the frame during muscle relaxation, then the time point t_1 will corresponds to the fifth frame after t_o, and t_2 to the tenth frame and so on. Then a relationship was established and depicted between the frame number and the tendon elongation. Another relationship was depicted between the calculated elongation and the knee torque.

5.2.8 Statistical Analysis

Paired t-tests were used to assess quadriceps and hamstrings MVCs and to assess the difference in tendon elongation during single and dual stimulations. Differences in knee torques produced during the three stimulation types (single stimulation of quadriceps, single stimulation of hamstrings and dual stimulation) were assessed using one-way ANOVA test. ANOVA mixed effects model was used to test the effect of stimulation type and current intensity on discomfort/pain level, with 'Participant' set as a random factor, 'Stimulation type' as a fixed factor and the 'current Intensity' as a covariate or continuous variable.

5.3 Results

5.3.1 Participants

7 uninjured participants (2 males,5 females, mean age 32.1±5.7y) were recruited in this study. They were all active and fit individuals with 167.9 ± 6.3 cm hight and 62.3 ± 8.8 kg body mass. A summary of the participants basic characteristic is shown in Table 5.1. Normality was tested using Anderson-Darling test and all participants characteristics, knee torques, and tendon elongation (at 45 mA) were normally distributed. Maximum voluntary contraction (MVC) produced by the quadriceps was greater than that produced by the hamstrings (127.8± 64.8 and 79.6± 48.6 Nm, respectively, $p<0.001$).

Table 4.1: Basic characteristics of participants

Characteristic / Participant	Sex	Age (years)	Weight (kg)	Height (cm)
AB1	male	35	77.4	178
AB2	male	27	71.6	174
AB3	female	34	60.7	167
AB4	female	34	54.4	160
AB5	female	41	55.2	169

AB6	female	25	57.3	164
AB7	female	28	59.5	163.5

5.3.2 Electrically stimulated knee torque

Table 5.2 shows knee torques (elicited at 45mA) as absolute values and as normalised values with respect to the participant's MVC. Knee extension and flexion torques were normalised with respect to quadriceps and hamstrings MVCs, respectively, while dual stimulation torque was normalised with respect to quadriceps MVC, as it was a negative torque (knee extension torque). The maximum ES torque was -115 Nm knee extension torque (64% MVC), produced by AB1 at 90 mA. The maximum current intensity reached in this study (by AB2) was 100mA. However, the comparisons between single and dual stimulation were tested at 45 mA, as this was the maximum intensity reached by all participants allowing a full data set of knee torques (during knee extension, flexion and dual stimulation) for comparison.

At 45 mA, single stimulation of quadriceps and hamstrings produced -25.6± 23.6 Nm and 7.6± 2.4 Nm knee extension and flexion torques, which were equivalent to about 20% and 11% quadriceps and hamstrings MVC, respectively. During single muscle stimulation, torques elicited by knee extensor were 100-310% greater than those elicited from knee flexors at different current intensities (Figure 5.7), but the statistical evidence was weak ($p=0.09$ and $p=0.08$ for absolute and normalised torques, respectively, at 45 mA, and $p=0.07$ at 55 mA (6 participants)). Generally, there was no difference between torques elicited during knee extension, flexion and dual stimulation at 45 mA. ($p=0.17$, $p=0.4$ for normalised torques).

Table 4.2: Elicited knee torques recorded during Quadriceps and hamstrings MVC, single and dual electrical stimulation (at 45 mA) N-Quads, N-Hams and-Dual are the normalised torques with respect to MVC for quadriceps, hamstrings and dual-stimulation, respectively.

Knee torque (Nm)	MVC-Q	MVC-H	Quadriceps stimulation		Hamstrings stimulation		Dual Stimulation	

Participant				N-Quads (%MVC)		N-Hams (%MVC)		N-Dual (%MVC)
AB1	-181	113	-20	11.0	12	10.6	-11	6.1
AB2	-250	171	-73	29.2	8	4.7	-61	24.4
AB3	-95	44	-6	6.3	7	16.0	-7	7.3
AB4	-69	39	-20	28.8	4	10.2	-27	38.9
AB5	-80	42	-38	47.7	8	18.9	-56	70.3
AB6	-103	63	-5	4.8	6	9.5	-9	8.7
AB7	-116	84	-17	14.7	8	9.5	-6	5.1
Mean	-127.8	79.6	-25.6	20.4	7.6	11.3	-25.3	23.0
SD	64.8	48.6	23.6	15.6	2.4	4.7	23.8	24.3

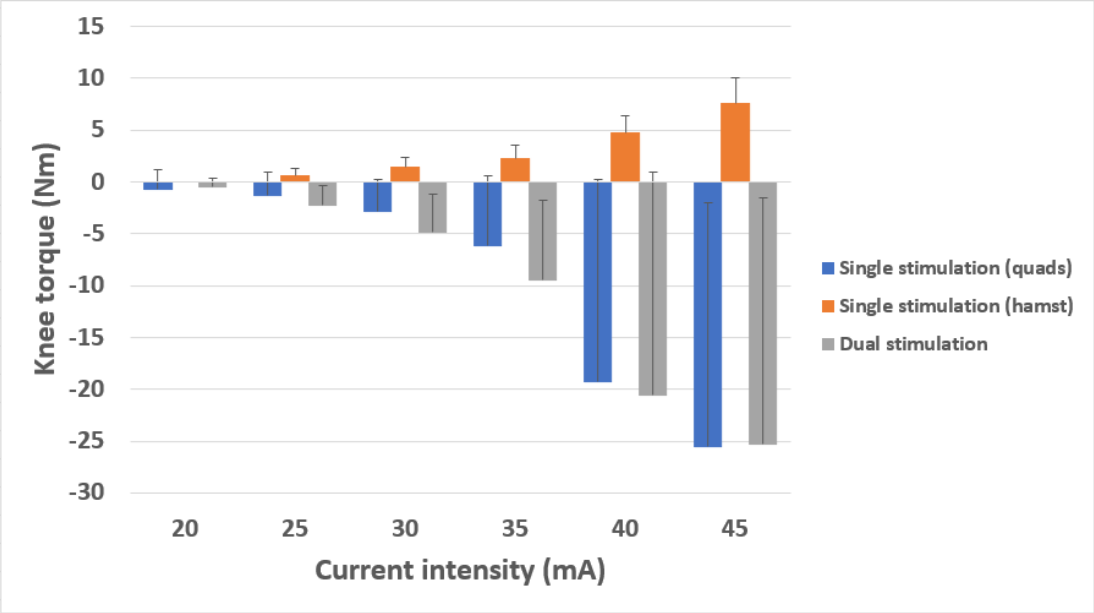


Figure 4.7: Mean and SD of the elicited knee torque during single stimulation of quadriceps and hamstrings, and dual stimulation of both muscles simultaneously (only positive values of SD are presented here)

5.3.3 Tendon elongation:

Patellar tendon elongation was measured at 45 mA during single and dual stimulation (Figure 5.8). Although tendon elongation was 67% greater during dual stimulation, the statistical evidence for the difference was weak ($p=0.1$).

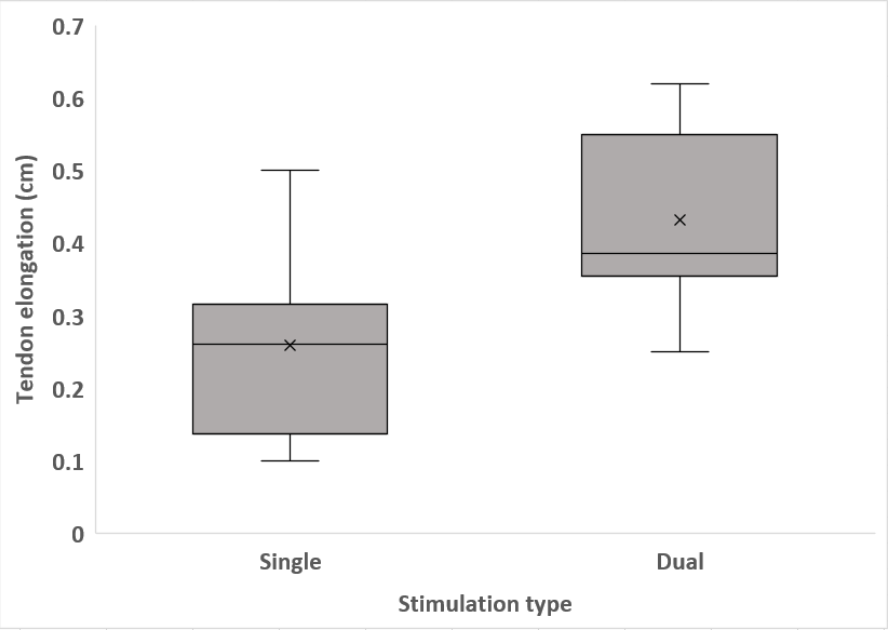


Figure 4.8: Box plot of the patellar tendon elongation at 45 mA current intensity during quadriceps single stimulation and dual stimulation

5.3.4 Discomfort

ANOVA mixed effects model revealed an effect of both current intensity and stimulation type on discomfort/pain level ($p<0.001$ for both). Discomfort results depicted in **Figure 5.9** shows that discomfort increases with increased current intensity during different stimulation types. Mixed effects model results also revealed greater discomfort level during dual stimulation compared to quadriceps single stimulation ($p<0.001$) with no difference in discomfort levels during single

stimulation of quadriceps and hamstrings (p=0.4). The maximum tolerated current intensity ranged between 50-90 mA for quadriceps single stimulation and between 45-65 mA for dual stimulation. The maximum discomfort level reported by one participant was 9 (very severe) during dual stimulation. Many commented that dual stimulation felt like their muscle was being 'squeezed', which made it less comfortable compared to single stimulation.

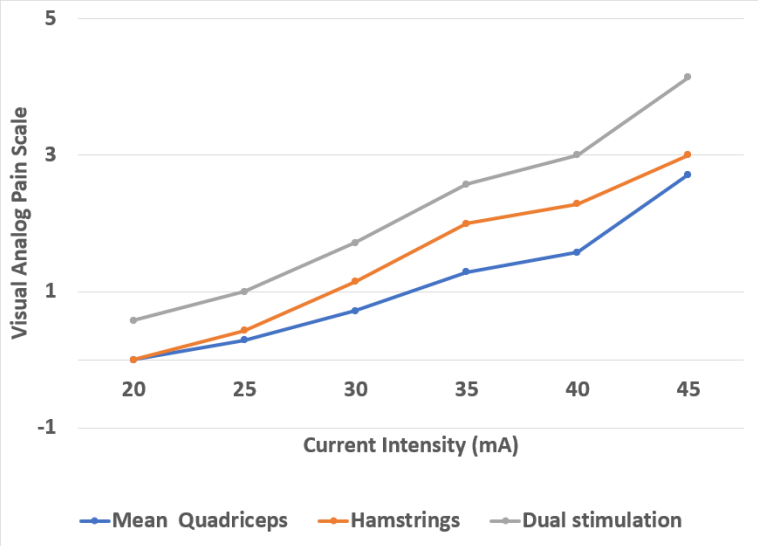


Figure 4.9: Mean discomfort levels at different current intensities during Single stimulation of quadriceps and hamstrings and dual stimulation

5.4 Discussion and conclusion

In this study, the effect of simultaneous stimulation of antagonistic muscle pairs on thigh muscles output was assessed in seven adult participants without SCI. There was no difference in the knee torque produced during dual stimulation compared to those produced during single stimulation of quadriceps and hamstrings, with a tendency for higher quadriceps torques compared to the hamstrings torque. Similarly, there was no difference in patellar tendon elongations measured at 45^{o} during quadriceps single stimulation and dual stimulation.

Quadriceps MVC was greater than hamstrings, despite performing the tests at 45^{o} knee flexion which was expected to reduce hamstrings muscle slackness and improve its output. This may have been due to the patellar tendon moment arm length being at its maximum at this knee angle[42], which could have subsequently increased knee extension torque. This supports other studies reporting greater quadriceps torques compared to that of hamstrings[289][290].

Similar to the MVC, the electrically-stimulated quadriceps torque was more than 3-fold that of the hamstrings but the evidence for this difference was weak. Single stimulation of quadriceps and hamstrings produced about 25% and 9.5% of their voluntary MVCs, respectively, using 45 mA current intensity. Another study reported similar results using 40 mA square waveform, which produced about 22% quadriceps MVC[291]. These muscle strength results are less than those reported using sine waveform[291]. A number of studies reported higher muscle forces produced using sine waveforms compared to square, inferential and Russian waveforms[291][292]. However, the handheld stimulator used in this study, like most of the commercially available stimulators for home/personal use, produced only square waves.

Stimulation waveforms are one of the main factors that influence electrically-elicited muscle output[54][292]. Other factors include pulse duration, with pulses of longer durations producing greater knee extensor torque compared to those with shorter durations (a 450μs pulse produced greater knee torque compared to 150 μs and 250μs pulses)[293][294]. When comparing the output of one of these studies that used the same square waveform used in our study but with 450 μs pulse duration, 75% MVC was produced in that study using 74 mA[293], which could not be achievable (using 300 μs pule duration) in any of the participants in our study even at 100 mA. It is worth noting that this study also used higher frequency compared to our study (100Hz and 70 Hz, respectively), which was also reported to influence elicited torques[295]. Therefore, using longer pulse duration and higher frequency might help produce higher knee extension torque.

Knee extension and flexion torques are also influenced by the hip joint/position with higher torques reported with flexed compared to extended (supine position) hip joint[296][297], which subsequently influenced the decision to keep the hip joint angle flexed at 90^{o} during the testing sessions in our study. Another factor which has been carefully considered when developing our protocol was the electrode placement[53][298][299], with the configuration suggested by Vieira et al[53] being the one that elicited the largest knee extensor torque compared to other studies[299][300].

In contrast to the factors mentioned above, neither stimulation duration (3 vs 10 seconds)[294] nor electrode size or shape[301] have been found to have effects on the electrically-elicited muscle output.

Knee torque produced during dual stimulation of quadriceps and hamstrings did not differ significantly when compared to single stimulation of quadriceps. This was also reflected by the comparable patellar tendon elongation between single and dual stimulation. This is a positive result indicating that dual stimulation does not result in large net joint torques which may cause fracture in individuals with low bone mass. No other studies that tried simultaneous stimulation of quadriceps and hamstrings were found in the literature to compare our results with as most studies test only knee extensors[302][303][55].

It was expected that dual stimulation would reduce the net knee torque as a result of the opposite muscle actions of the quadriceps and hamstrings. However, the hamstrings torque was too low to reduce the difference between the knee extension and flexion torques. Balancing out the net knee torque might require applying higher currents on hamstrings than quadriceps to offset the large difference and further reduce limb movement. Increasing stimulation level on the hamstrings may be limited by discomfort in those with intact sensory function. Alternatively, the quadriceps stimulation level could be reduced without compromising the high muscle force which is crucial to induce bone formation. Therefore, stimulation levels

should be tested and optimised for each individual so that so that muscle forces are maximised and net knee torque is minimised.

Discomfort levels reported by participants were higher during dual stimulation compared to single stimulation, which limited the number of torque data points that could be measured from all seven participants. The effect of stimulating waveform on pain level has been reported by some studies. Square wave, which was used in this study as the only waveform produced by our stimulators, was reported to be the least comfortable waveform compared to sine and Russian waveforms, with sine wave producing the least pain at the same stimulation level[291][292]. The current required to achieve 10%MVC knee extension was 3-times lower using sine waveform compared to the square and Russian waveforms, which would lead to less pain caused by the stimulation[292]. Although the literature shows the effectiveness of using sine wave over square wave in terms of the produced muscle force and discomfort, it was not possible to test it out or use it in the clinical study due to the lack of commercial simulators that produce sine waveforms.

Assessing discomfort/pain produced by RAMP-ES is relevant for those with paralysis to protect them from skin burns or sensitivity. Although RAMP-ES training approach was primarily aimed at those with SCI, it can also be used with those with intact sensation during prolonged bed rest or following hip fractures or arthroplasty to prevent muscle atrophy.

Simultaneous stimulation of opposing muscle groups appears feasible, without resulting in increased knee torques that could increase risk of fracture. It is a novel training approach that requires further tests to improve its efficacy. Current intensity levels used for RAMP-ES training would be a trade-off between maximising the muscle forces and minimising the net knee torque and should be decided for each individual before starting the training. It is also important to assess using sine wave instead of square wave for this simultaneous stimulation intervention to try to optimise the produced muscle output and minimise the associated discomfort.

Chapter 6: Clinical Study of RAMP-ES stimulation

6.1 Introduction

People with SCI experience dramatic bone loss in their paralysed limbs[32], which puts them at high risk of sustaining fragility fractures[33]. Fracture rate in people with SCI has been reported to be twice that in the able-bodied population, with about 23-fold higher risk of experiencing femur fractures compared to the general population[35]. In particular, the distal femur and proximal tibia regions are common fracture sites in individuals with SCI. At least 50% of these fractures are associated with clinical complications such as infections and pressure ulcers[36]. Different electrically-stimulated interventions have been developed to help the paralysed muscles contract and stimulate the long bones that they attach to, such as electrically stimulated cycling and rowing[175][176]. However, the improvements in bone health achieved by these interventions have been limited by the low muscle forces produced by the atrophied and weak muscles, that could probably be below the threshold required to induce bone formation[6]. Both the quadriceps and hamstrings muscles cross the knee joint, so simultaneous stimulation of these muscle groups could provide a potent stimulus for bone adaptation at these sites by applying compressive stresses to the distal femur and proximal tibia regions most at risk of bone loss and fracture in this population. The applied compressive stresses could improve bone strain at these sites and, subsequently, enhance bone remodelling to increase BMD.

The aim of this study was to evaluate the effectiveness of the Recruitment of Antagonistic Muscle Pairs using electrical stimulation (RAMP-ES), which was tested on able-bodied individuals at Manchester Metropolitan University and was found to be feasible and safe to use (Chapter 5). The effect of four months RAMP-ES training on muscle size, muscle strength, fat fraction and bone health of people with chronic SCI was assessed.

6.2 Materials and Methods:

6.2.1 Participant Recruitment

This study (R&I ref: GN20NE049) was given favourable ethical opinion by West of Scotland Research Ethics Committee 4 (REC reference:20/WS/0051). All NHS, R&I and Caldicott approvals were received in April 2020, however, and due to the Covid 19 pandemic, the final green light to resume the study and start advertising and recruiting participants was given in November 2020.

Potential candidates for the study were identified from the Queen Elizabeth National Spinal Injuries Unit (QENSIU) database by the Spinal Consultant on the study. A letter of invitation and a copy of the Participant Information Sheet for the study were sent to individuals fitting the main inclusion/exclusion criteria in the QENSIU database.

Inclusion criteria were: (i) Motor and sensory complete (ASIA Impairment Scale grade A) spinal cord injury (SCI) leading to paraplegia or tetraplegia (from low cervical SCI); (ii) 1 year or more post-injury; (iii) age range 16-65 years; (iv) living in Greater Glasgow and surrounding areas.

Primary Exclusion criteria were: (i) Incomplete SCI (ASIA Impairment Scale grade B,C or D); (ii) cervical level SCI at C6 or above; (iii) less than 1 year post-injury; (iv) flaccid paralysis; (v) metallic implants at the scan and/or stimulation sites (vi) skin conditions (e.g. eczema, dermatitis); (vii) current use of bisphosphonates; (viii) recent training with electrical stimulation on lower-limb muscles (i.e. within the last 3 months); (ix) fracture(s) in either of lower limbs in the past 5 years; (x) pregnancy.

Additional exclusion criteria determined from baseline assessments were:

• Trabecular bone mineral density of <150mg/cm3 at the distal femur and/or <100mg/cm3 at the distal tibia, as determined from pQCT bone scan.

• Muscle spasticity indicated by Modified Ashworth Scale grade 3 or more, as assessed by the research physiotherapist.

6.2.2 Consent process

Individuals who met the inclusion criteria were invited to attend the QENSIU for baseline assessments after they completed and signed the consent form. Participant consent was obtained by the Chief Investigator. The candidate was asked to confirm that they read and understood the PIS, and whether they had any questions before being asked to initial the boxes on the consent form.

6.2.3 Baseline Musculoskeletal Assessments

6.2.3.1 Bone scans

Bone scans were performed by the Chief Investigator using a research pQCT scanner (XCT3000, Stratec Medizintechnik GmbH, Germany) at the QENSIU during a scanning session totalling approximately two hours. Scans were obtained at two sites at the knees (4% length of the femur (distal-proximal) (DF); -4% length of the tibia (proximal-distal) (PT)) and at one site above the ankles (4% tibia length (distal-proximal) (DT)) of both lower limbs. Voxel size was 0.5 mm for tibia and 0.3 mm for femur.

The participant was asked to transfer from their wheelchair to a bed. Tibia length was then measured using a measuring tape, and this measurement was inserted into the scanner computer. Then a sliding sheet was put underneath the participant to help move them into and out of the scanner. The scanning protocol started by moving the first leg inside the scanner ring, while placing the other leg on a side table. The first site to be scanned was the distal tibia. After scanning the DT, the patient was moved further into the scanner to get into the required position to scan the PT and DF (For DF and PT, the knee was positioned just distal to the scanner ring). A towel was wrapped around the leg, between the limb and the holder clamps, to protect the skin. After acquiring the scans for the first leg, the patient leg was moved out of the scanner using a bed sheet and the whole process was repeated for the other limb.

6.2.3.2 Muscle spasticity assessment

The purpose of this assessment was to evaluate the muscle spasticity level. This assessment was performed by the physiotherapist in the research team. The physiotherapist held the participant’s leg and then flexed and extended it a number of

times to monitor and assess their spasticity level. The modified Ashworth scale which measures the increase in muscle tone (using a six-point scale) was used[304].

Only those candidates with Ashworth scores of 2 or less were eligible to take part in the study. Volunteers were required to satisfy both bone scan and muscle spasticity conditions in order to be eligible to proceed to the next phase of the study.

6.2.4 Electrical stimulation

6.2.4.1 Muscle conditioning phase (duration: 4 weeks)
Following baseline assessments, participants underwent a phase of muscle conditioning using electrical stimulation (ES). A portable electrical stimulator (EV-906A, Med-Fit, Stockport, UK) was used to produce an electrical current (biphasic square pulses, current amplitude: 5-100 mA, frequency: 50 Hz, pulsewidth:350μs) that was delivered to the thigh muscles (quadriceps and hamstrings) via 2 pairs of self-adhesive surface electrodes (5x10 cm) (SKU:SA20, Med-Fit UK, Stockport, UK). The stimulation was applied to one limb while the other (contralateral) limb served as a within-subject control.

The distance between the proximal edge of the patella and the anterior superior iliac spine (ASIS) was measured using a measuring tape while the participant was seated. One pair of surface electrodes was placed on the skin over the quadriceps and one pair over the hamstrings to deliver the electrical current to the paralysed muscles as shown in Figure 7.1. To stimulate the quadriceps muscles, the cathode and anode electrodes were placed on the thigh at approximately 20% and 70% of the patella-ASIS distance, respectively, as described by Vieira et al 2016[53]. For the hamstrings, the electrodes were placed in equivalent positions under the thigh. This positioning has been tested previously and found to be generating the highest muscle torques in a study on able-bodied participants at Manchester Metropolitan University (Described in Chapter 3).

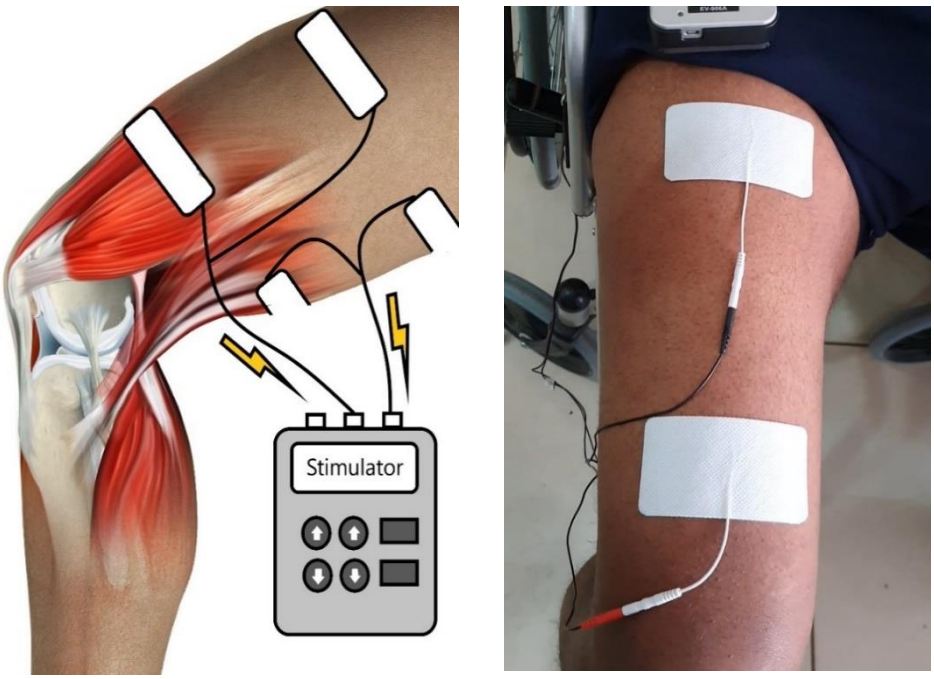


Figure 5.1: The stimulator delivered an electrical current to the paralysed muscles via surface electrodes placed on the quadriceps and hamstrings

At the beginning of the training session, muscles were stimulated using low current values (10-15 mA) which were gradually ramped up in 5 mA increments. During the muscle conditioning phase, the stimulating current was applied individually to the quadriceps and hamstrings. The first session was conducted in the Treatment Room of the Step-Down Unit at the QENSIU. After checking that both muscle groups were responding adequately to the stimulation (i.e. achieving sustained tetanic contractions), the participant was given the option to continue their sessions at home, 3 days per week for 4 weeks. All necessary instructions were provided to the participants in the form of secure Microsoft OneDrive links to videoclips showing how to don/doff the equipment; and a home-training manual, alongside a training diary to complete. At the end of these 4 weeks, participants were invited to carry out the pre-intervention assessments at the QENSIU.

6.2.4.2 RAMP-ES Intervention Phase (duration: 4 months)

The 4-month RAMP-ES intervention phase began after completing the 4-week muscle conditioning. During the RAMP-ES intervention sessions, the thigh muscles

(quadriceps and hamstrings) were stimulated at the same time. Participants carried out RAMP-ES sessions at home, with a pre-programmed stimulator (EM-6300P, Med-Fit, Stockport, UK) using optimal current levels decided during a previous muscle assessment session. To help participants replicate the electrode placement during at-home sessions, photographic references of the electrode positions were used, which were taken during the first hospital sessions. This was determined as the current levels that generated visibly strong contractions with minimal leg movement. Participants were encouraged to undergo the stimulation for 15-30 mins per day, 3-5 times per week and to keep a record of their session in a training diary.

A mid-training muscle assessment session was performed in Week 12 (week 8 of the RAMP-ES intervention period), to assess the effect of these 8 weeks of RAMP-ES intervention on muscle force and compare it to that at the end of the previous muscle conditioning period. This visit was also used to assess whether the RAMP-ES current levels needed to be modified for the final 8 weeks of training. This have been required to ensure strong yet balanced contractions (minimal limb movement) from both knee extensors and flexors.

6.2.5 Muscle assessments

6.2.5.1 Muscle strength assessment

Muscle assessment sessions took place in the Step-Down Unit Treatment Room at the QENSIU. The torques produced by the quadriceps and the hamstrings to extend and flex the knee joint, respectively, during ES were measured using the Biodex dynamometer (System 3, Biodex Medical Systems, US).

During these assessments, the participant transferred first from their wheelchair to a height-adjustable plinth before sliding across to the padded dynamometer chair (see Figure 7.2). Sitting upright, their trunk was then strapped to the chair to secure their upper body. Surface electrodes were then placed on top and bottom of one thigh, in the same configuration as used during the previous assessments and training phase. The leg was then strapped to an attachment in the dynamometer lever and the centre of rotation of the knee was aligned with the pivot point of the lever. The electrical current was applied for each muscle group, individually, and gradually increased

from 20 mA up to 100 mA in 5 mA increments, to produce isometric contractions. This muscle assessment was carried out at the following timepoints:

• At the mid-muscle-conditioning phase **(Week 2)**

• At the end of the muscle conditioning phase, prior to starting the RAMP-ES training phase **(Week 4)**

• Mid-RAMP-ES training phase **(Week 12)**

• End-of RAMP-ES training phase **(Week 20)**

The timeline of all procedures and assessments that were carried during the 20 weeks duration of the study is summarised in a flow chart in Figure 7.8.

Figure 5.2: Left: setting for the dynamometery session to measure ES-induced knee torques. Right: Set-up used for participants to transfer from the height-adjustable plinth to the dynamometer chair

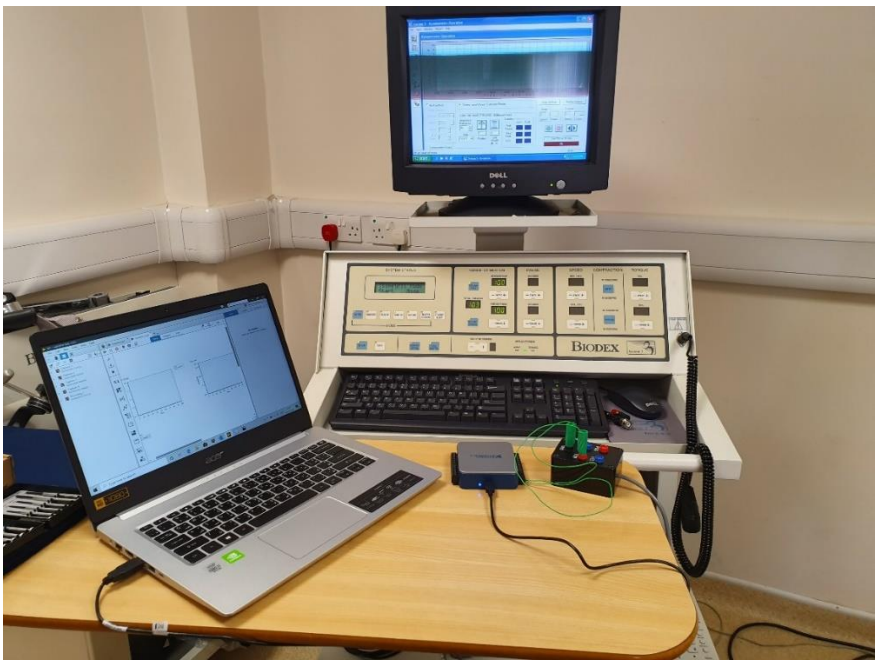


Figure 5.3: A DAC was used to the obtain the torque output from the dynamometer into the LabVIEW programme where it was read and stored as a .csv file

Torque measurements were obtained in real time at a sampling frequency of 1000Hz, from the dynamometer using a data acquisition card (DAC) (EV-906A, National Instruments, Texas, US), which was connected to a LabVIEW programme (LabVIEW NXG4.0.0, National Instruments, Texas, US) as shown in Figure 7.3.

This programme (Figure 7.4) recorded the output voltage(V), converted it to torque (Nm) values, displayed it in real time and allowed it to be saved as CSV files. The equation used to convert dynamometer voltage values to torque values was:
Torque (Nm) = (Voltage (V) + 0.0012)/0.0073
This equation was acquired by applying different torque values on the dynamometer lever ranging from (0-100 Nm) and reading the voltage output using an initial LabVIEW programme that read and saved the dynamometer voltage output.

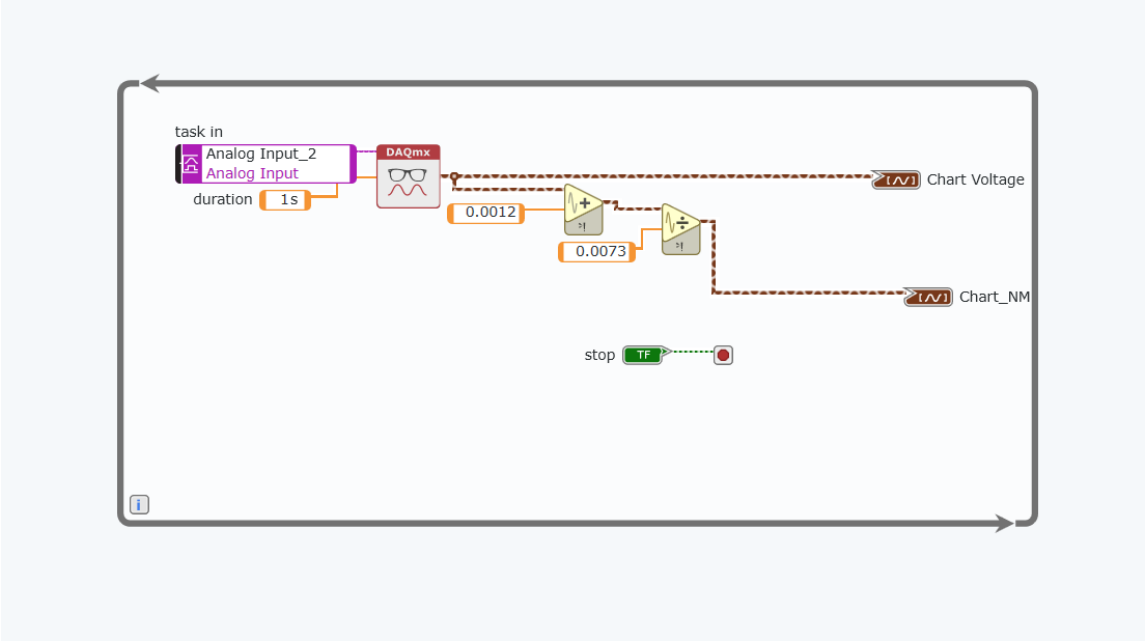


Figure 5.4: LabVIEW programme (VI) used to record and save real time torque results from the dynamometer

6.2.5.2 MRI assessment of muscle

After completing the muscle conditioning phase and before progressing to the RAMP-ES intervention phase (16 weeks), participants underwent an MRI scanning session using a 3T MRI scanner (Prisma, Siemens, Erlangen, Germany) with an 18-channel anterior array coil and 32-channel spine array at the Glasgow Clinical Research Facility within the Queen Elizabeth University Hospital. The scans were obtained by the hospital research MRI technician team using a six-point VIBE Dixon sequence at 50% of the femur length (voxel size: 1.1×1.1×2.0 mm) to assess the effect of RAMP-ES on muscle cross-sectional area (CSA, mm^2) and fat fraction (%).

Coefficient of variation used to analyse muscle outcomes was calculated using the formula:

$$\text{Coefficient of variation} = (\sigma/\mu) * 100$$

σ – the standard deviation

μ – the mean

Coefficient of variation for muscle CSA outcome ranged between 0% to 2%.

6.2.5.3 End of RAMP-ES training musculoskeletal assessments

Repeat bone and muscle assessments were carried out after the RAMP-ES intervention was complete. These post-intervention assessments included pQCT bone scans, muscle and knee MRI scans and a final dynamometry muscle assessment. The follow-up pQCT scans were carried out at the DF and PT only because these were the bone sites that were the most relevant in relation to the stimulated thigh muscles.

6.2.6 Analysis and Statistics:

6.2.6.1 pQCT scans

Bone parameters were calculated from the pQCT scans using the manufacturer's analysis software (XCT550, Stratec Medizintechnik GmbH, Germany) and the pQCT plugin on ImageJ (National Institutes of Health, Maryland, USA). The purpose of using two different software packages was to compare the outcomes obtained by the widely-used manufacturer's software and the most recently developed I mageJ plugin, which use two different protocols to calculate bone density. The bone parameters calculated were trabecular density (TrD), total density (BMD) and total area (ToA). The Chief Investigator used the manufacturer's analysis software that calculates bone density from the inner 45% area of the bone. In contrast, the ImageJ plugin calculated it using the total CSA which was identified automatically based on a threshold set by the user. The threshold used was 120 mg/cm^3 for both DF and DT sites. This threshold was previously used in the literature[258] and accurately identified the bone and separated it from the surrounding soft tissue without underestimating the bone CSA in all participants scans. Using higher thresholds lead to parts of the bone CSA being unidentified by the software, due to the thin bone cortex and the amount of bone lost in these participants.

Whilst the manufacturer's software calculated bone parameters at the three bone sites (DF, PT, DT), the ImageJ plugin was only used to calculate them at the DF and DF. It was not possible to use this plugin to calculate bone parameters at the PT due to the irregular shape of the bone that made it challenging for the software to identify the bone area automatically. Results were calculated and compared at baseline and after completing the 4-months RAMP-ES intervention.

6.2.6.2 Muscle strength

Due to the large size of the recorded torque files, another piece of software was developed by a data scientist to open and plot the recorded torque outputs. The code was developed with a simple user interface that allowed loading, plotting, resampling and exporting torque csv files (Figure 7.5). After loading the csv, the programme presented the status (loading/loaded/data resamples, etc), sampling frequency (Hz), file length in seconds and the date and time when the torque was recorded.

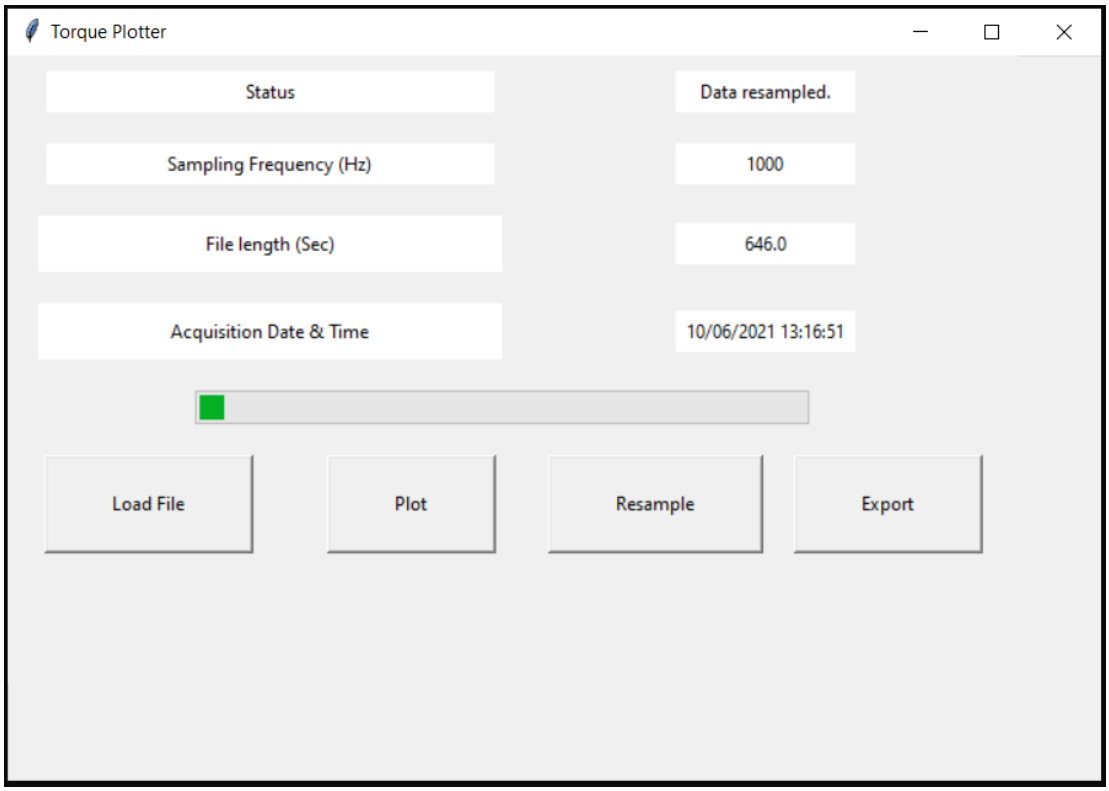


Figure 5.5: User interface of the Torque Plotter programme that read and plotted torque csv files

After plotting the torque recordings (Figure 7.6), the torque amplitude produced by each electrical current pulse was read. Filtering out the noise and fixing the signal drift were challenging due to the noise frequency being comparable to that of the torque signal and the lack of technical support that could be provided by the

department in NHS premises. All torque results for knee flexion and extension are presented in Appendix A.

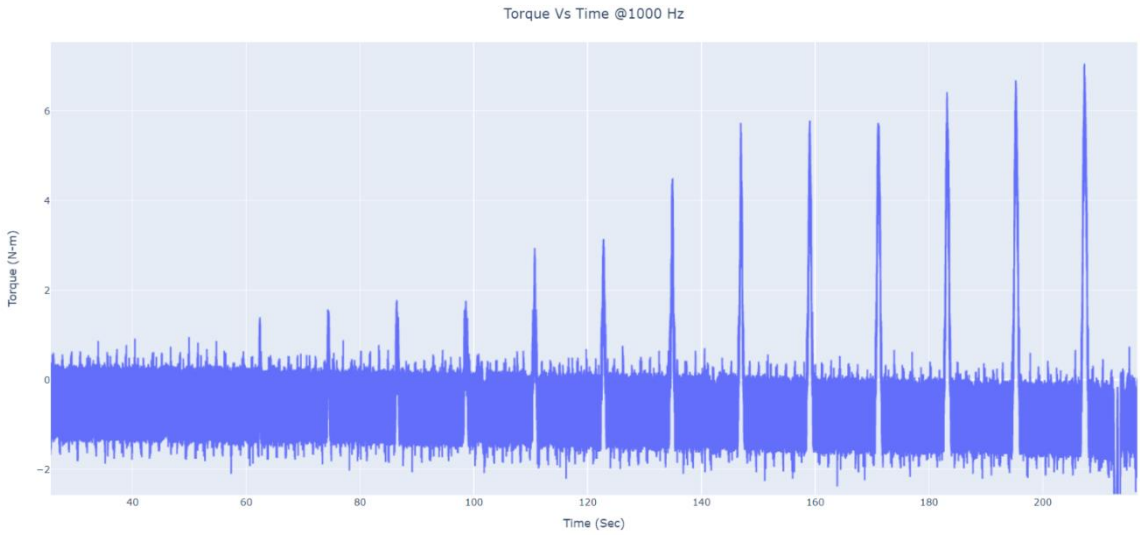


Figure 5.6: Plot of the torque outputs measured by the dynamometer after produced by electrical stimulation of the quadriceps/hamstrings muscles

6.2.6.3 MRI muscle and fat outcomes

In order to compare muscle/fat outcomes before and after the RAMP-ES intervention, mid-thigh scans were analysed. Anatomical features were identified and used to ensure matching of scan sites. Scans were then analysed with MATLAB software (MathWorks Inc., San Mateo, California, USA), using a MATLAB code that was developed and provided by the hospital MRI Physics team. A number of dots (about 40-50 dots) have been drawn manually around the selected muscle group, before the program drew a line connecting these dots to select the Region of Interest (ROI). Muscles on the scans were divided into 3 ROIs: quadriceps, hamstrings, and "other muscles" (which included any other muscle apart from quadriceps and hamstrings) as shown in Figure 7.7. The MATLAB code then calculated the CSA (mm^2) and the fat fraction (%) of the selected muscles. This analysis was repeated three times by the same investigator to assess short-term error. Coefficient of variation was no more than 1.3% and 1.9% for all muscle CSAs in the trained and untrained limbs, respectively.

Normality was tested using Anderson-Darling tests. Changes in muscle CSA, fat fraction, knee torque and BMD were assessed using paired T-tests if found to be normally distributed. ANOVA mixed effects model was used to analyse muscle and bone parameters on Minitab with participants as a random factor and leg and time as fixed factors.

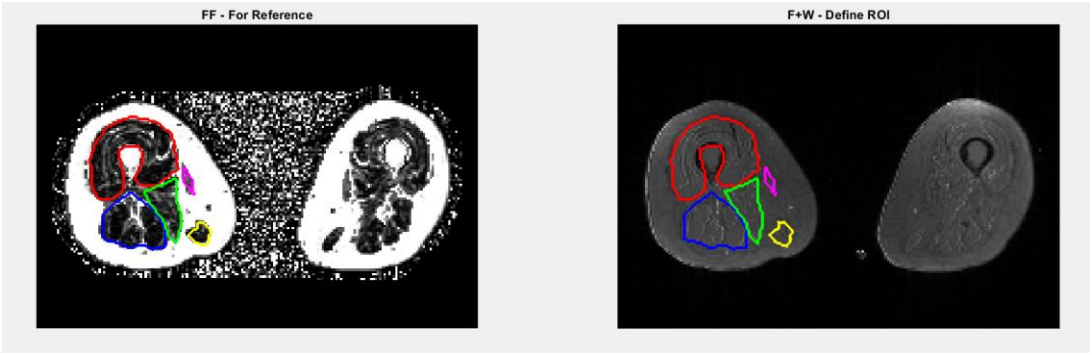


Figure 5.7: Muscles selected on MRI scans and divided into: quadriceps (red), hamstrings (blue) and "other muscles" (green, yellow and pink) on MATLAB for CSA and fat fraction analysis

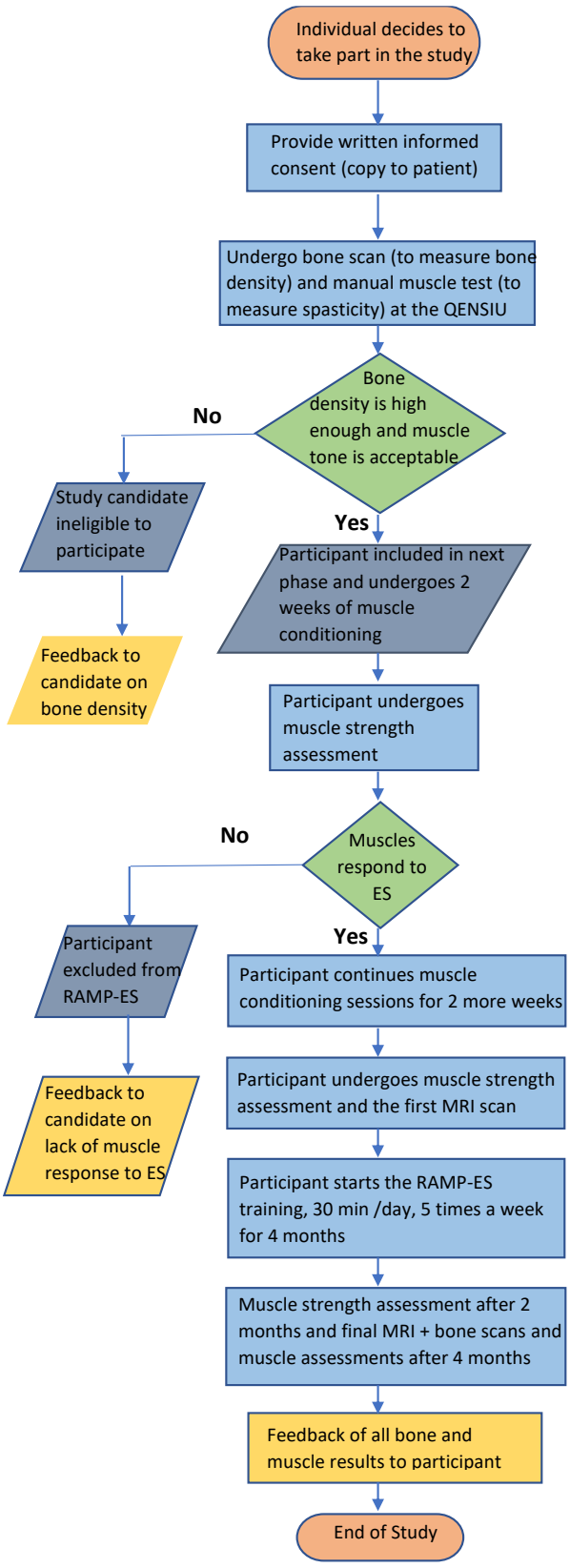


Figure 5.8: Flow chart summarising all the procedures and assessments conducted in the RAMP-ES clinical study

6.3 Results

6.3.1 Study Participants

The invitations and study information documents were sent to 56 potential candidates who were identified from the QENSIU database by the administrative staff using the study inclusion and exclusion criteria. As shown in Figure 8.1, 4 out of the 9 received responses were eligible to undergo the baseline assessments after being contacted by the project Chief Investigator. Following the baseline assessments of bone mineral density (BMD) and spasticity, one potential candidate was deemed ineligible based on the minimum trabecular BMD threshold set for inclusion in the study. pQCT scans of this candidate also revealed signs of heterotopic ossification at DF of both limbs which had not been previously dignosed (Appendix B). Three candidates with BMD and levels of spascity fell within the set safe levels were eligible to start the muscle conditioning phase. Table 8.1 shows their basic characteristics.

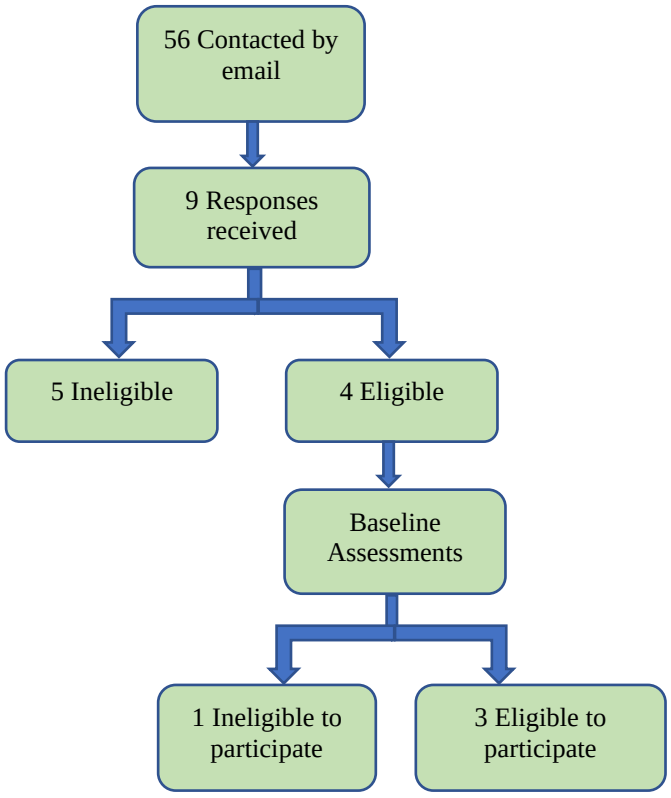


Figure 5.9: Participants recruitment process.

Table 5.1: Basic characteristics of the patients enrolled on the study

Participant	**Age (years)**	**Sex**	**Time since injury (years)**	**Level of Injury**	**AIS Grade**
P1	55	Female	5.1	C6	A
P2	54	Male	4.9	T2	A
P3	59	Male	2.1	T4	A

6.3.2 Training compliance

Table 8.2 shows training logs which were provided by all three participants for both phases of the training. Participants completed 4 weeks of muscle conditioning, carrying out 3 sessions/week before starting the 4-month RAMP-ES intervention. Participants P1, P2 and P3 completed 18, 21 and 16 weeks of RAMP-ES, respectively, carrying out a mean of 3.9 sessions/week. P3 trained 5 times/week for 16 weeks. Training compliance was 100% with all three participants commenting that it was convenient to use at home and was easy to use on their own without requiring help from others.

Table 5.2: Training logs for all participants during the muscle conditioning and RAMP-ES phases

Training / phase / Participant	Muscle Conditioning				RAMP-ES intervention			
	No. of sessions	Training duration (weeks)	Current amplitude* (mA)		No. of sessions	Training duration (weeks)	Current amplitude (mA)	
			Quadriceps	Hamstrings			Quadriceps	Hamstrings
P1	12	4	60	90	54	18	60	99
P2	17	7	85	99	75	21	85	99
P3	14	4	85	65	80	16	95	65

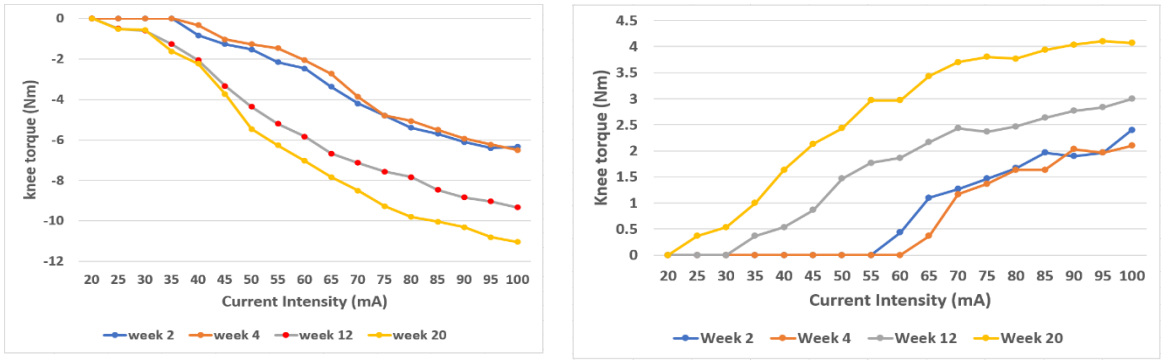
*(Reached by end of training)

6.3.3 Muscle outcomes

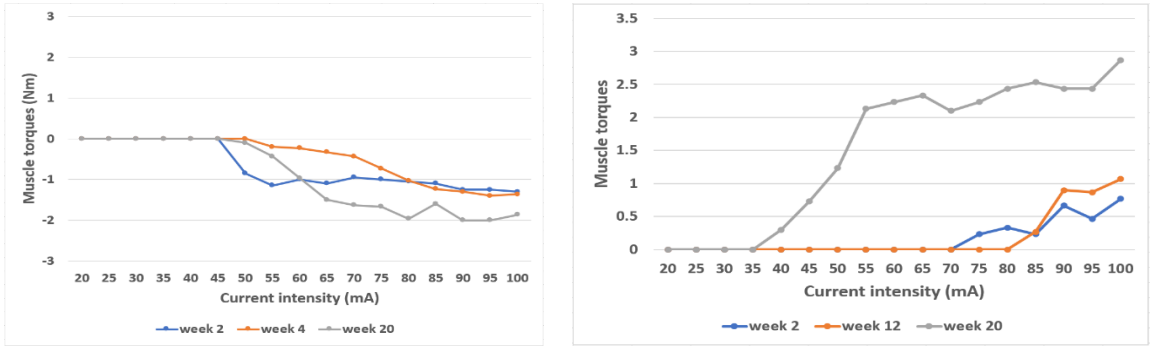
6.3.3.1 Muscle strength

All muscle and bone outcomes were normally distributed. Figure 8.2 shows all recorded knee extension and flexion torques for all the three participants at week 2,4,12 and 20 of the intervention. Table 8.3 shows the maximum knee flexion and extension torques recorded at 100mA at week 2 and after completing the intervention.

(a)



(b)



(C)

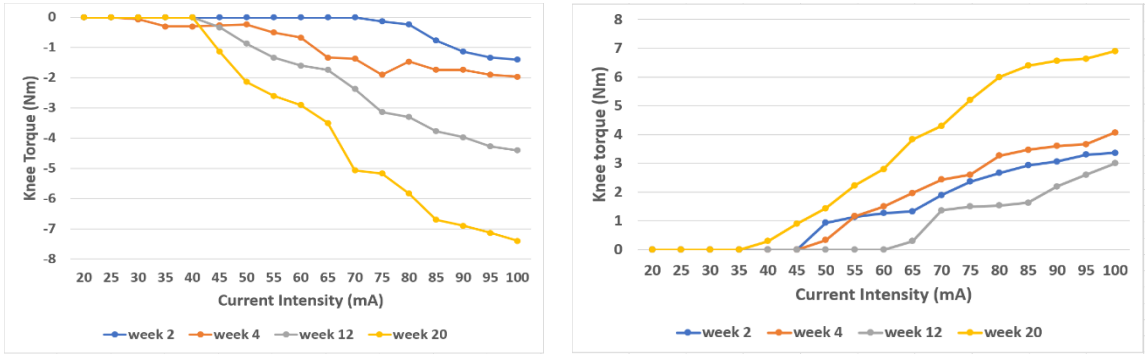


Figure 5.10: Electrically-elicited knee extension and flexion torque for Participants P1 (a), P2 (b) and P3 (c) during week 2,4,12 and 20 of the intervention

Torque readings at week 12 is missing for P2 due to unexplained difficulty in reading their knee torques. Their muscles were unresponsive to the stimulation and produced weak contractions that were too small to induce knee extension or flexion. All connections and stimulator output were checked. P1 and P3 achieved the highest absolute knee extension and flexion torques (-11 Nm and 7 Nm), respectively (Table 8.3). The highest relative increase in knee extension and flexion torques was achieved by P3 and P2 (429% and 263%), respectively.

Knee flexion torque at the end of the intervention period was 71-263% greater than that recorded at week 2 (p=0.05). Whilst knee extension torque also increased by 46-429 %, the statistical evidence for this change was weak (p=0.15, Figure 8.3).

Table 5.3: Knee extension (Quadriceps) and flexion (hamstrings) torques recorded at 100mA at week 2 and week 20

Knee torque (Nm) / **Participant**	**Quadriceps**		**Hamstrings**	
	Week 2	**Week 20**	**Week 2**	**Week 20**
P1	**-6.3**	**-11.0**	**2.4**	**4.1**
P2	**-1.3**	**-1.9**	**0.8**	**2.9**
P3	**-1.4**	**-7.4**	**3.4**	**6.9**

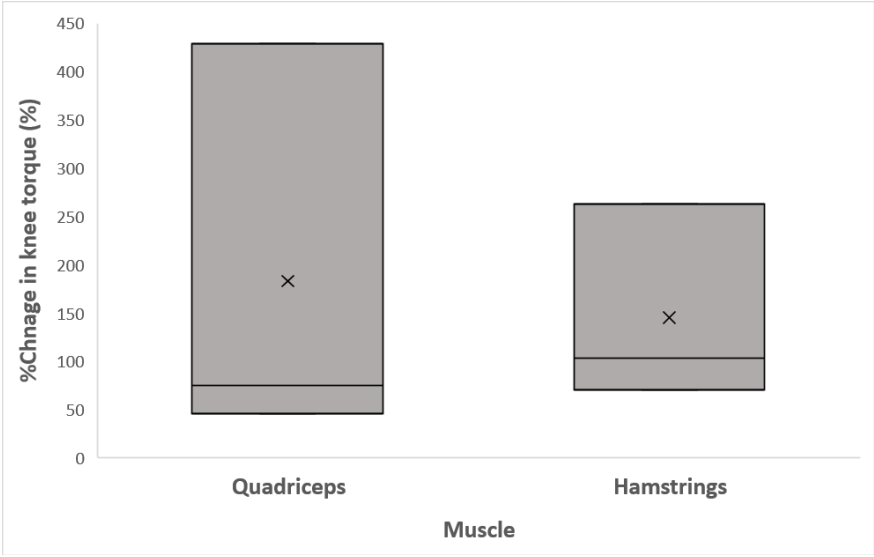


Figure 5.11:. Percentage changes in knee extension (quadriceps) and flexion (hamstrings) torques between week 2 and after completing the RAMP-ES intervention

6.3.3.2 Muscle CSA

The total CSA of all thigh muscles in the trained leg of all participants increased by 7.3-19.8%, although the statistical evidence for this was weak (p=0.07) (Figure 8.4), unlike the untrained limb that showed no change in the muscle CSA (p=0.6) Table 8.4. These results were supported by those from the fit mixed effects model which revealed a tendency to a difference in the muscle CSA between the trained and untrained limbs over the training period, which did not reach statistical significance (p=0.06). The LSC for muscle CSA was 3.4%, which indicates an increase in the muscle CSA (compared to the LSC for muscle CSA outcome) in trained legs of all participants, while showing no change in the untrained muscles.

CSA of quadriceps and hamstrings muscles also increased in all participants except in the hamstrings of P3. P2 had the largest relative increase in muscle CSA, although their number of training sessions was less than P3. Figure 8.5 shows the percentage changes in the CSA of the quadriceps and hamstrings muscles in the trained limb which also showed no change (p=0.1, p=0.2, respectively). But the model results suggest a tendency for a difference in the quadriceps CSA between the trained and untrained limbs over time, p=0.05) unlike the hamstrings changes that were similar

between the two limbs (p= 0.4). All CSA fat fraction results are presented in Appendix C.

Figure 5.12: Percentage change in muscle CSA in the trained and untrained limbs after completing the RAMP-ES intervention

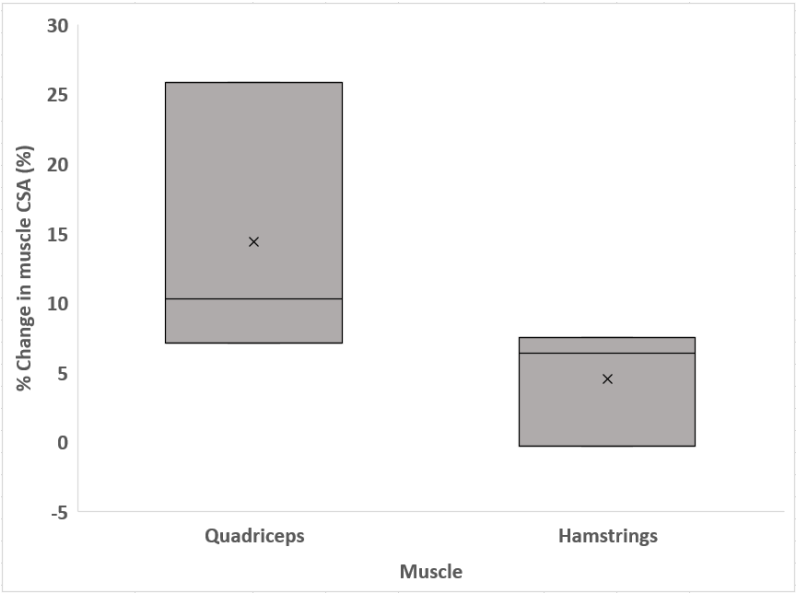


Figure 5.13: Percentage changes in CSA of the quadriceps and hamstrings muscles in the trained limb after completing the RAMP-ES intervention

6.3.3.3 Fat fraction

Table 8.4 shows fat fraction (%) at midthigh in the trained and untrained limbs before and after completing the RAMP-ES intervention and Figure 8.6 shows the percentage change in fat fraction (%) after completing the intervention. Similar to muscle CSA results, paired T-tests revealed no significant change in the fat fraction in both the trained and untrained limbs (p=0.14, p=0.8) which was supported by the model results that showed no difference in fat fraction between the two limbs over time (p=0.2).

The coefficient of variation for fat fraction measurement ranged between 0% and 6%. The LSC for the fat fraction was 5.4%, which indicates a reduction in fat fraction in the trained limb of P1 and P2 but not P3 who had a reduction of 5.2%. No difference was found in fat fraction levels when analysing the change in the quadriceps and hamstrings separately (p=0.2 for both muscles) (Figure 8.7).

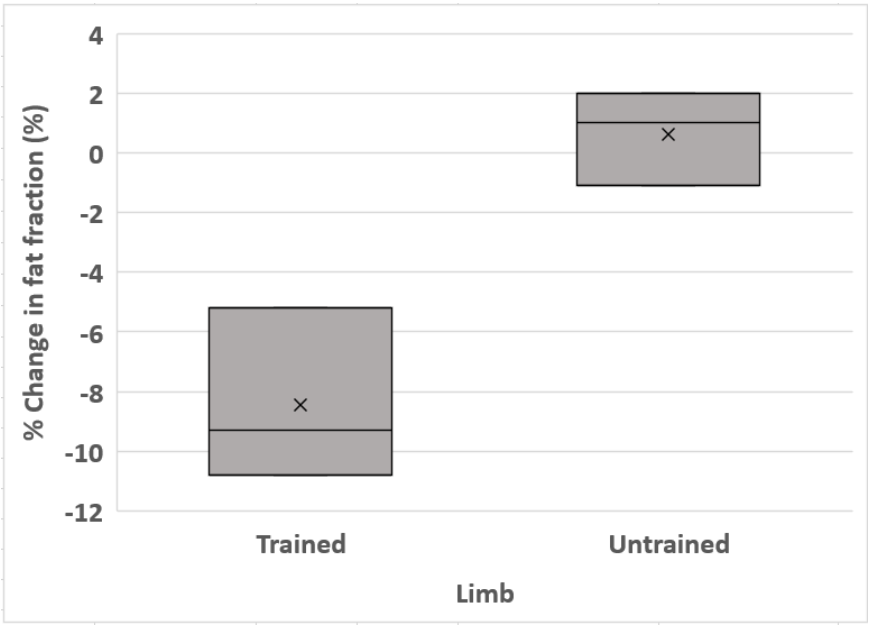


Figure 5.14: Percentage change in fat fraction (%) at midthigh in the trained and untrained limbs after completing the RAMP-ES intervention

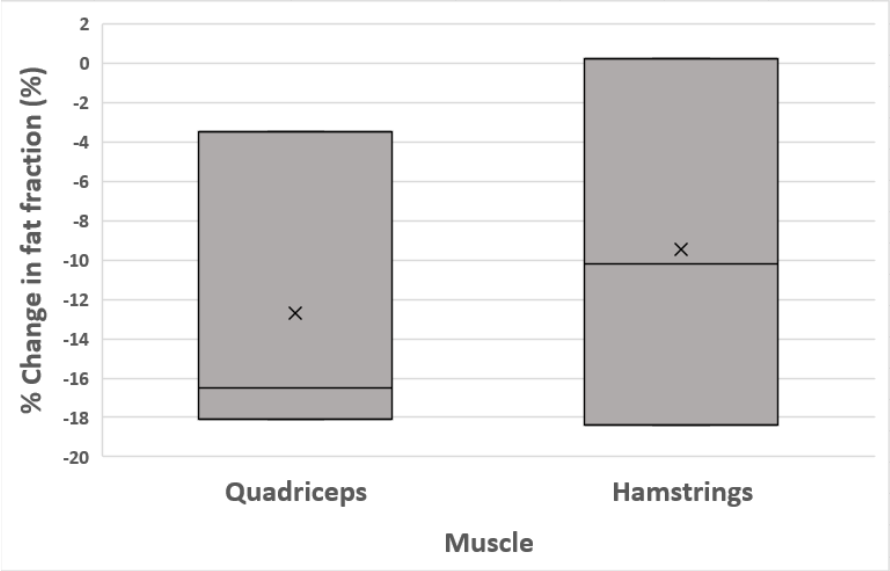


Figure 5.15: Percentage change in fat fraction of the quadriceps and hamstrings in the trained limb after completing the RAMP-ES intervention

6.3.4 BMD

Absolute values of total and trabecular BMD in both limbs. before and after completing the RAMP-ES intervention are presented in Table 8.5. The total BMD at the DF reduced by a range of -0.6% to -6.9% and changed by -4.5% to 0.5% in the trained and untrained legs, respectively with P1 experiencing the greatest loss in total BMD at this site. Paired T-tests showed no difference in total BMD at the DF during the training period in both trained and untrained limbs (p=0.3 for both). These results were further supported by the model results that showed no difference between the two limbs (p=0.8). The location and irregular shape of the PT led to a difficulty in obtaining the repeat scans in the same location as the first scans. Therefore, For P1 and P2, the repeat scans were obtained at slightly different location as shown in Figure 8.8. For P3, although the analysis showed a 1% increase in BMD at the trained limb, the total bone mass did not change at this site (6.07 gm/cm). Repeatability of pQCT measures in people with SCI has been shown previously to be high[305][114].

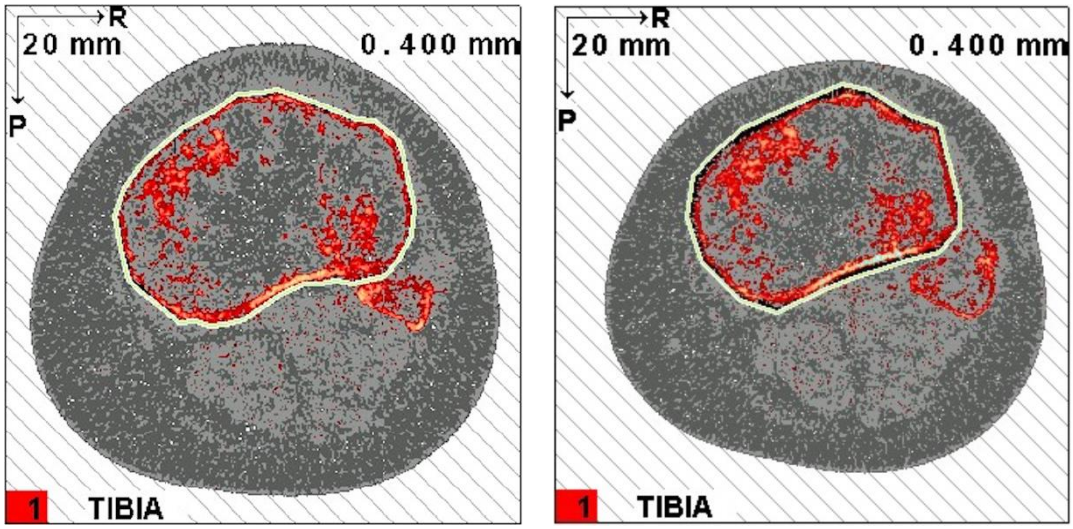


Figure 5.16: pQCT scans of the PT of the participant P1 before (left) and after (right) completing the RAMP-ES intervention showing the slight differences in the scan location between the 2 time points

Table 5.4: Muscle total CSA and fat fraction at mid-thigh in the trained and untrained limbs pre- and post- the RAMP-ES intervention

Muscle parameter / Participant	**Muscle CSA (mm^2)**				**Fat Fraction (%)**			
	Trained		Untrained		Trained		Untrained	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
P1	5546	5952	4353	4236	24	22	28	28
P2	6415	7683	5933	6027	35	31	33	32
P3	9503	10444	9166	9365	13	12	15	15

Table 5.5: Total CSA, BMD and Trabecular BMD of the trained and untrained limbs at baseline and after completing the RAMP-ES intervention

	Trained Limb						**Untrained Limb**					
	Baseline		Post-Intervention		%Change		Baseline		Post-Intervention		%Change	
	Total BMD (mg/cm^3)	Tr.BMD (mg/cm^3)	Total BMD (mg/cm^3)	Tr.BMD (mg/cm^3)	Total BMD (mg/cm^3)	Tr.BMD (mg/cm^3)	Total BMD (mg/cm^3)	Tr. BMD (mg/cm^3)	Total BMD (mg/cm^3)	Tr.BMD (mg/cm^3)	Total BMD (mg/cm^3)	Tr. BMD (mg/cm^3)
P1												
DF	168	179	157	168	**-6.5**	**-6.1**	158	163	151	157	**-4.4**	**-3.7**

PT	83	48	98	58	**18**	**20.8**	87	49	93	56	**6.9**	**14.2**
P2												
DF	202	218	200	216	**-1.0**	**-1**	197	205	194	201	**-1.5**	**-2.0**
PT	168	147	165	145	**-1.7**	**-1.4**	130	94	130	95	**0**	**1.1**
P3												
DF	237	220	236	221	**-0.4**	**0.5**	235	230	236	232	**0.4**	**0.9**
PT	188	121	190	122	**1**	**0.8**	181	124	135	76	**-25.4**	**-38.7**

6.4 Discussion

The effect of RAMP-ES training on muscle and bone health was assessed in three participants with chronic, complete SCI. The 4 months of RAMP-ES intervention resulted in increased muscle size, increased knee flexion torque and reduced fat fraction. However, due to the low participant numbers the statistical evidence for these changes was weak and did not translate into positive effects on bone mineral density at fracture-prone sites.

All three participants completed the 20 weeks of the study and recorded their home sessions in training logs. The flexibility and convenience of this training probably contributed to the high participant compliance. Participants completed all training and testing sessions without any adverse events. P3 trained for 5 times/week while P1 and P2 trained for 3 times/week. However, this increased number of training sessions did not appear to have a clear effect on their muscle and bone outcomes when compared to the other two participants. This might appear to contradict a number of studies in which participants trained for 5 times/week and reported improvements in bone health[170][186], while those with 3 times/week training protocols reported no effect on bone health[173][179]. However, it is worth noting that all of these studies that report positive effects, involved a training period of 6 months or more (and up to 3 years). This might indicate that the training duration might have a more pronounced effect on bone response compared to the training frequency.

The total change in muscle CSA and fat fraction were analysed and reported in all thigh muscles, because the surface electrodes configuration was designed to deliver currents to all muscles in the thigh. This study showed that the RAMP-ES intervention increased total thigh muscles CSA by 7.3% to 19.8%. This corroborates the findings of many studies that reported increases in lean muscle mass[179][259] and muscle CSA[180][178][175][306] following FES training. Some studies reported 20% to 35.5% increase in muscle CSA following 8 weeks[180][178] and 12 months[175] of training. The higher percentage increase reported in the one study, in which participants trained for only 2 days/week, might be due to the shorter

postinjury duration (<1 year)[180] compared to our study, which might have led to the muscles being more responsive to ES.

This increase in muscle CSA was also associated with a non-significant decrease in fat fraction by 5.2% to 10.8% in the trained leg muscles. This agrees with two FES studies that reported about 10% and 16.7% decrease in leg fat following 8 weeks and 12-months of ES-cycling, respectively[178][175].

SCI and the subsequent lack of physical activity lead to dramatic loss of muscle CSA (14–16% within the first 6 months postinjury)[101], and increased intramuscular fat percentage that can be up to 4-fold that of uninjured individuals[104]. These changes in body composition have clinical consequences and can lead to metabolic and cardiovascular diseases[66]. The loss in lean muscle mass after SCI results in reduced metabolic rate, and reduced energy expenditure[66][307], with high intramuscular fat mass being a contributing factor to developing glucose intolerance[308]. People with SCI are at higher risk of developing glucose intolerance or insulin resistance[66] with type II diabetes prevalence being 3-times higher in people with spinal cord injury compared to uninjured population [309][310]. Diabetes in people with SCI has been associated with higher prevalence of vascular conditions such as stroke and coronary artery diseases, compared to those with SCI without diabetes (49% and 24%, respectively). In a recent study, heart disease was reported to be the second highest mortality cause (after respiratory disease) among people with SCI in the USA[311]. Therefore, muscle gain and fat loss reported in this study may have significant clinical importance for people with SCI and should be further investigated in future research.

The knee torques increased by 46-429 % in all participants by the end of the intervention. The steady increase in knee torques throughout the training months indicates that greater muscle forces could be achieved if the training duration increased. Most studies of ES-interventions either do not report muscle forces or report a surrogate measure instead (for example, form of body weight percentage[58]), which makes it difficult to compare their outcomes to those of this study. But few studies reported increases in muscle strength after ES-cycling[183].

In relative terms, P3 experienced the greatest knee extension torque gain (> 400%), compared to baseline), which could be a result of the higher number of sessions

carried out by this participant compared to the other two participants. However, this increase in muscle strength did not reflect the resulting fat fraction loss which was the smallest among the participants. This was probably due to the already small fat fraction and large muscle size of this participant at baseline, compared to the other participants

This study showed no evidence of a positive effect on bone outcomes at the knee (which is a fracture-prone site in this patient population). The lack of positive effect on bone health can be attributed to a number of factors. The short duration of training could be one of the main contributing factors. Improvements in bone outcomes have been reported previously in studies following 6 months or more of training[186][259][175][58]. In contrast, training interventions that lasted less than 6 months had no positive effect on bone outcomes[179][183]. Regular long-term rowing training has been shown to mitigate the loss in trabecular bone density and microstructure[312]. In that same study, the rowed distance was found to have greater effect on trabecular BMD than Foot force at the distal tibia [312], which further highlights the importance of training duration.

Another factor could be the low muscle forces produced. Despite the steady increase in knee torque throughout the training months, the maximum knee torque reached at the end of the training period (1.9 to 11.0 Nm and 2.9 to 6.9 Nm, for knee extension and flexion torques, respectively) remained far less than that produced in able-bodied people. For example, standing and walking requires a minimum torque of 50 Nm[60]. This low level of muscle force could be lower than the remodelling threshold required to stimulate bone formation[6]. Another factor could be the time postinjury at which the training started. Two studies showed positive effects of ES-rowing on participants with SCI who started their training within the first 2 years following injury[312][260]. Both P1, P2 were about 5 years postinjury when they started their training while P3 was 2 years and 1-month postinjury. However, there is not enough evidence in the literature which suggests that starting training interventions within the first 2 years would contribute to the effectiveness on bone health.

Despite the lack of effect on total BMD, there could be a positive effect on bone trabecular microstructure that has not been investigated in this study. Johnston et

al,2016 reported an improvement in trabecular bone microarchitecture after 6 months of cycling, even though this intervention produced a maximum torque of up to 3 Nm, which is less that that reached in our study[61].

This intervention aimed to maximise the produced muscle forces in order to increase bone stimulation while minimising limb movement to provide safety and convenience. However, this study had some limitations that can be avoided or tackled in the future research. First, the small number of the participants was the main limitation of this study, which was likely imposed by the Covid-19 pandemic. Secondly, the electrode configuration and electrical stimulation settings used in this study were assessed and optimised in able-bodied participants and not in people with SCI. The physiological changes caused by the injury may have effects on how their body respond to electrical stimulation. Thirdly, the knee torque signal recorded by the dynamometer was noisy and could not be filtered due to logistical and technical challenges. Finally, bone microstructure could have been further investigated from the MRI scans to investigate potential effects on trabecular bone.

This study introduced and tested a novel ES-intervention on people with SCI that has not been used before. RAMP-ES intervention showed positive effects on body composition by increasing muscle size and reducing fat content which are of clinical importance for these patients. Implications of these improvements could be investigated in the future. There was no evidence of positive effect on bone health, which could be, in part, attributed to the short training duration and the low muscle forces that was probably lower than that required to induce anabolic results in bone density.

6.5 Conclusions and Future Work

6.5.1 Conclusions

In this study, RAMP-ES training intervention was assessed in three people with chronic complete SCI. Participants underwent 4 weeks of muscle conditioning before starting 4 months of RAMP-ES training, which involved stimulating both knee extensors and flexors simultaneously. Effects of RAMP-ES intervention were assessed on muscles size, fat fraction and bone density.

The 4-month RAMP-ES training had positive effects on muscle size and fat fraction. Despite the weak statistical evidence, muscle size increased and the fat fraction decreased in the trained limb. However, the training did not show positive effects on BMD in these participants. That could be attributed to a number of factors such as the short training duration and the low force produced from atrophied muscles.

6.5.2 Future Work

Considering some of the current study outcomes and limitations, more work could be implemented in the future to further investigate the efficacy of this novel approach. Testing this intervention on larger number of people with SCI would give clearer indication on its effectiveness in improving bone health.

The positive effect of this intervention on body composition and its implications on body health could be further investigated. The increased muscle mass and reduced fat content reported in this study could be assessed clinically to understand and evaluate any potential clinical benefits in improving metabolic and/or cardiovascular function.

RAMP-ES protocol and setting, such as electrode placement, knee angle and stimulation parameters, could be further optimised by testing it on people with SCI instead of uninjured participants. Optimal protocol that suites this altered neuromuscular physiology could potentially help increase the produced muscle forces which remained far below those in uninjured population in this study. In future studies, training would be recommended to last at least 6 months excluding the muscle conditioning period, with sessions carried out 3-5 times per week.

Investigating RAMP-ES effects on bone microstructure, especially that of trabecular bone, could help understand whether the training have early positive effects at the micro level that are not detected when analysing total BMD.

All participants recruited in this study were more than 2-5 years postinjury. RAMP-ES could be tested on people with acute or sub-acute SCI to see if it could give better results in these individuals by preserving BMD and prevent it from further loss.

Chapter 7: Discussion and Conclusion

7.1 Discussion

The aims of this thesis were to develop a better characterisation of bone loss following SCI, and to develop and test the feasibility and effectiveness of a novel approach that stimulates antagonistic muscle pairs simultaneously to maximise bone loading in people with SCI. In the characterisation of bone loss in the first year after SCI, longitudinal bone loss in the fibula was found to be smaller compared to the tibia. This finding is in line with previous cross-sectional reports[258], although unlike these previous studies, here bone loss was observed in the shaft of the fibula. These findings might help explain the lower incidence of reported fibula fractures in individuals with chronic SCI[37]. Regional variation in bone loss following SCI across tibia cross-sections was also examined for the first time. In the tibia, bone loss was found to vary regionally in the diaphyses, with total (not regional) loss at four months being a strong predictor of total loss after twelve months postinjury. A novel RAMP-ES training protocol that was developed to tackle some of the limitations in the current training interventions, was tested in able-bodied participants. It was found to be feasible without resulting in increased knee torques. A four-month RAMP-ES training intervention was then tested in people with SCI. It improved muscle size and strength and reduced fat content in people with chronic SCI, but it had no clear effect on bone density.

First, the literature was examined to study different aspects of osteoporosis in people with SCI, including aetiology, patterns and available treatments. Bone loss in people with SCI is rapid and site-specific, leading to fractures predominantly in the femoral and tibial epiphyses[33]. Longitudinal changes in bone mass have been widely described in the tibia and femur but not in the fibula[138][156]. Therefore, bone loss was assessed longitudinally in the fibula and compared to that of the neighbouring tibia to understand the importance and utility of fibula assessment following SCI. That bone loss found in the fibula was less pronounced compared to the tibia not only helps to explain the rarity of reported fibular fractures in individuals with SCI but may also indicate that bone loss is induced or influenced by different mechanisms in the two adjacent bones. Our current understanding is that the fibula

bears a decreasing proportion of total shank load with unloading[267]. In addition, it has a larger endocortical surface area: cortical area ratio, shown previously to be an important determinant of bone loss following SCI[144]. Therefore, we could have expected similar or larger bone loss in the fibula following SCI. Given the relatively minor bone loss and fracture incidence in the fibula, this corroborated the decision to focus the analyses on the tibia, given the clinical nature of this project. In future work, more detailed assessment of the patterns of bone loss in the fibula following SCI could help to identify determinants of inter-bone differences and understanding of the underlying mechanisms.

Regional assessment of SCI-induced bone loss was reported for the first time in this thesis. In the tibial diaphysis, bone loss within the first twelve months following the injury was found to vary regionally, with the posterior region showing the greatest loss compared to other regions of the bone cross-section. This may relate to regional variation in geometry, alternatively in loading across the cross-section prior to injury as identified in the femur. Further development of this work could be used to develop more effective interventions that target those areas with the largest loss. Specific patterns of muscle recruitment via electrical stimulation may stress those area of risk of greatest loss as demonstrated for voluntary physical activity in uninjured individuals in the proximal femur[313]. Furthermore, the total bone loss detected in the four-months postinjury scans, was found to be a strong predictor of the loss that occurred later at twelve months postinjury. Inter-individual variation in bone loss following SCI is pronounced, as in the longitudinal analysis in this thesis whereby decreases of 1-67% over 12 months were observed. Therefore, our findings represent a novel evidence of the importance of early bone scans, that could be utilised to identify fast bone losers. This evidence could also inform current medical practice and help develop a more consistent approach for medical professionals on the detection of SCI-induced bone loss to prevent further bone loss and, probably, prevent fractures in this patient group. Future studies should examine the predictive power of early bone assessments in identifying individuals at rapid bone loss in a larger cohort. Once established, the efficacy of pharmacological or rehabilitation-based interventions at this early stage can be assessed.

A thorough review of the currently available treatments for bone loss in people with SCI (Chapter 2) revealed a modest effect of electrically-stimulated rehabilitation

interventions on bone health. This effect is limited partly by the small muscle forces generated from the atrophied and weak muscles. Low muscle forces may have also been kept low in many interventions in response to safety considerations. This subsequently motivated the development of the novel RAMP-ES approach that was intended to maximise the produced muscle forces, and therefore, maximise bone stimulation, while keeping limb movement at minimal, to provide both safety and convenience.

RAMP-ES was found practical and safe to use after testing it in able-bodied individuals and before applying it in people with SCI. Unexpectedly, RAMP-ES did not reduce the net knee torque, although importantly (for participant safety) it did not increase it either. That net torque was not substantially reduced is likely due to the low knee flexion torque that was too small to balance out the extension torque. Increasing knee flexion torque was one of the challenges that was difficult to tackle due to the discomfort associated with increasing the ES levels. The discomfort was found to be higher during dual stimulation compared to single stimulation, which limited the number of torque readings obtained during dual stimulation that could be compared to those during single stimulation. Generally, the inter-individual variation in the muscular response to electrical stimulation and tolerance of the discomfort were some of the challenges encountered in this study.

Sine waveform was found to be more effective in producing high muscle forces while causing the lowest discomfort compared to the square wave used in our study and other waveforms tested in the literature[292]. However, electrical stimulators that produce square waveforms are the most widely available stimulators in the market for home use for muscle strengthening and pain relief purposes. Although testing sinewave stimulation would be potentially helpful in improving muscle output and minimising discomfort, it would not be relevant unless more affordable sinewave stimulators are made commercially available, and which can be used easily at home to provide convenience that is one of the main aims of RAMP-ES intervention. In the meantime, adjusting RAMP-ES stimulation currents to tolerable levels that maintain high muscle output in both antagonistic muscles could provide a convenient rehabilitation approach for those with intact sensation and limited mobility that puts them at risk of muscle atrophy or bone loss. These include those

undergoing prolonged bedrest, astronauts and individuals undergoing hip/knee surgery or trauma[69][314].

The effect of RAMP-ES training on muscle and bone was assessed in a pilot study with people with chronic SCI. RAMP-ES was used for four months without negative side effects or adverse events and was well tolerated by the participants, who found using it at home easy and convenient. The 4-month training period resulted in non-significant improvements in muscle size and strength and a reduction in muscle fat content, but it had no effect on bone density. The lack of effect on bone health is thought to be caused by the relatively short training period and the relatively low muscle forces produced from the atrophied muscles in these individuals with chronic SCI. Despite this, the continuous improvement in muscle strength over the course of the training weeks, the muscle forces produced remained lower than those produced by ES in uninjured individuals. For example, at 60 mA, the mean knee extension and flexion torques were 44 Nm and 15 Nm in the uninjured participants, respectively, whereas they were only 4 Nm and 3 Nm in the participants with SCI, after the four months of RAMP-ES training (using the same stimulation settings in both studies). However, the positive effects on muscle and fat make it a potentially convenient intervention to prevent muscle atrophy and weakening.

Muscle atrophy is a rapid consequence of SCI that starts within the first few weeks following the injury[101] and continues throughout the chronic phase leading to 20-30% loss in lean mass in those with chronic SCI[315]. This loss in muscle mass causes further health consequences such as reduced basal metabolism (up to 29%), which can lead to up to 30% increase in fat mass when combined with immobility and an unhealthy diet[315]. These health complications put individuals with SCI at higher risk of diabetes and cardiovascular disease, which is the leading cause of death in people with SCI contributing to 50% greater mortality risk in this population[316]. Another health consequence of muscle mass loss (especially over the ischial tuberosities and greater trochanters) is pressure injuries or ulcers which occur due to chronic sitting [317] and impaired metabolism[318]. Risk of pressure injuries is more than two-fold greater in individuals with SCI[319], with more than 20% experiencing pressure ulcers[320] which result in around 25% of the incurred SCI-related healthcare costs[321]. Loss of muscle mass is also the primary cause of the extensive bone loss following SCI, which puts injured individuals at 2-fold

greater risk of sustaining fractures compared to uninjured individuals[58]. Therefore, it is clear that the loss of muscle mass presents major health challenge that causes serious consequences to individuals with chronic SCI.

To date, available approaches to attenuate or reverse muscle loss following SCI remain limited. Short-term (3-6 months) electrical stimulation interventions in acute SCI have been shown to mitigate muscle atrophy with modest effects on muscle mass[322][323]. However, their effects in chronic SCI or over the longer-term are unknown, and studies have not evaluated potential effects on other health complications such as metabolic activity. Of relevant note, these interventions require bulky equipment, supervision and substantial time commitment which may limit long-term commitment. The effects of other interventional modalities such as nutritional or pharmacological treatments remain relatively unexplored in this population[324], with no clear clinical guidelines for the prevention and management of muscle loss (and the secondary negative consequences) in SCI.

7.2 Strengths, limitations and future work

This thesis investigated and described for the first time the longitudinal changes in the fibula and the regional variation of bone loss in the tibia. It introduced new findings with regard to the characterisation of bone loss in people with SCI and produced novel evidence for the importance of early bone scans in identifying fast bone losers. It also proposed and tested a novel rehabilitation approach that targets muscle atrophy and bone loss in people with SCI. It confirmed its feasibility in able-bodied individuals and tested in detail its effect on muscle, fat and bone of individuals with chronic SCI.

The absence of a control group was the main limitation of both the fibula response and tibial regional analysis studies, which prevented an age-matched comparisons with uninjured individuals. However, the 18% bone loss observed in one year (at the distal tibia)[138][194] is far greater than that observed in uninjured individuals (up to 0.4% loss at the distal tibia)[325] hence results are unlikely to primarily reflect typical age-related losses. However, the primary focus of these analysis was on factors within the cohort including inter-and intra-bone variation in loss and longitudinal correlations which were not reliant on comparisons with uninjured

individuals. Furthermore, the pseudo-anonymisation code for fibula and tibia scans was not available for these studies, therefore, it was not possible to report age and sex of participants and to correlate them with bone loss outcomes.

The main limitation of both the able-bodied and the clinical studies was the low number of participants, which was probably caused by the stimulation-associated discomfort and the Covid-19 pandemic, respectively. Although most of the training sessions in the clinical study were conducted by the participants at home, all muscle and bone assessments were carried out at the hospital, which might have been a source of concern to some candidates. The multiple lockdowns imposed throughout 2020 added serious challenges to conducting the study in a clinical setting and restricted our access to the hospital causing about twelve months of delay. Even when restrictions were eventually relaxed, many individuals in this vulnerable population group did not feel comfortable participating in the study due to the requirements to attend the hospital on several occasions. Conducting the clinical study under these exceptional circumstances required amending the study protocols multiple times to comply with the government guidance and provide additional measures to ensure the safety of study participants, research team and hospital staff and patients.

Moreover, testing RAMP-ES protocols on able-bodied individuals first was important to check its feasibility and safety, but it was also important to test it in individuals with SCI over a couple of sessions/days before being tested in the four months training period. Testing the protocols on able-bodied participants only was less relevant due to the different physiological environment resulting from the neurological impairment. It also limited the levels of electrical current that could be used, and the possibility of getting higher muscle forces due to the discomfort caused by the stimulation.

The only available technique to measure muscle output in this thesis was the dynamometer, which measures it indirectly by measuring knee torques. Dynamometers are designed to measure voluntary muscle output in able-bodied individuals and might not be ideal for measuring involuntary (electrically-stimulated) output in paralysed individuals, especially small contractions that do not lead to readable torques or limb movement. In fact, the majority of available

techniques that assess muscle strength mimic day to day activities like walking (or sit-to-stand), which cannot be used for people with paralysis.

More work could be carried out in the future to confirm the outcomes of this thesis and to improve the effectiveness of RAMP-ES intervention. First, fibular and regional tibial bone loss should be investigated in SCI alongside an age-matched control group. Investigating regional bone loss at other bone sites that are prone to fracture in people with SCI such as the proximal tibia and distal femur, would be crucial to optimise rehabilitation interventions. Regional bone mass distribution could also be investigated in athletes and non-athlete individuals and compare it with these in people with SCI, to further investigate any role of mechanical loading on the heterogeneous bone loss reported in this study across bone diaphysis.

The RAMP-ES protocol and settings might be further improved by using sine waveform and longer pulse widths to help increase the elicited muscle forces (and minimise discomfort in those with intact sensation), but the effects of these settings should be tested on a larger groups of uninjured and injured individuals. Another factor that might improve RAMP-ES effects on bone health could be increasing training duration to be 6 months or more. Finally, the positive effects of RAMP-ES on muscles and fat reported here could be further assessed on different patient groups, alongside assessing any potential cardiovascular or metabolic effects.

This thesis introduced new insights on the characterisation of bone loss in people with SCI, filling some of the gaps in the examined literature. It described for the first time the longitudinal changes in the fibula bone and the regional variation of the bone loss in the tibia. It also highlighted the importance of early bone scans in predicting those with high rates of bone loss. Finally, it assessed the feasibility and effectiveness of a novel training approach(RAMP-ES), that tackles some of the limitations in the current approaches, in able-bodied and individuals with SCI.

Appendix A: Torque results recorded during single stimulation of quadriceps and hamstrings and dual stimulation

Intensity(mA) / Participant/ Torque (Nm)	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**
AB1																	
Quads	5	6	9	20	20	20	42	55	71	95	90	103	113	117	115		
Hams	0	2	3	2	7	12	19	26	26	45	35	39	47	40	49		
Dual	0	5	7	10	0	11	12	19	30								
AB2																	
Quads	0	0	0	0	59	73	72										
Hams	0	1	2	4	6	8	11	13	19	17	20	22	25	25	26	26	26
Dual	0	0	0	2	56	61											
AB3																	
Quads	0	0	1	1	4	6	9	11	14	15	19	20					
Hams	0	0	1	2	5	7	10	11	12	9							
Dual	1	4	8	8	9	7											
AB4																	
Quads	0	0	4	7	14	20	31	40	48								
Hams	0	0	1	2	3	4	7	9	12								
Dual	2	3	9	12	20	27	32	37	45								

AB5																	
Quads	0	2	4	8	27	38	48	56	64	70	78						
Hams	0	1	2	4	6	8	9	10	11	13	14	14	15				
Dual	0	3	7	25	45	56	63	71	76	80							
AB6																	
Quads	0	0	1	4	4	5	7	9									
Hams	0	0	0	1	3	6	11	14	17	16	19						
Dual	0	0	2	5	7	9	9										
AB7																	
Quads	0	1	1	3	7	17	22	24	25	29	33	37	44				
Hams	0	0	1	1	3	8	9	12	16	13	14						
dual	0	1	1	4	7	6	8	10	11	14							

Appendix B: Scans of one candidate showing heterotopic ossification in both limbs

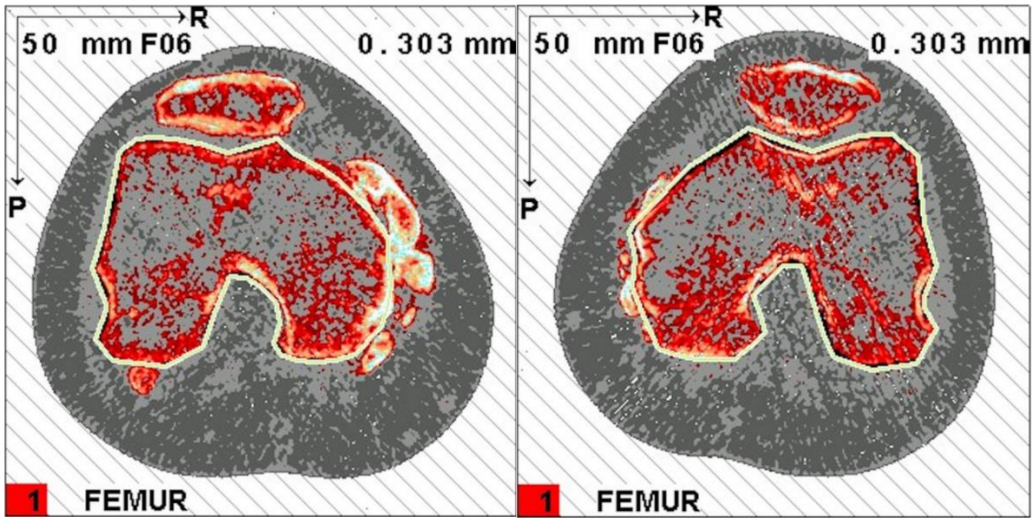


Figure B.1: pQCT scans of the DF showing Heterotopic Ossification and the resulting calcified soft tissues adjacent to the bone at both limbs

Appendix C: Muscle cross-sectional area (CSA) and fat fraction (FF) measured at 50% femur length for Participants 1,2 and 3

Table C.1: CSA and FF results for Participant before and after completing the RAMP-ES intervention

Limb / Participant/ROI	Right				Left			
	Pre-Area (mm^2)	**Post-Area (mm^2)**	**Pre-Mean FF (%)**	**Post-Mean (%)**	**Pre-Area (mm^2)**	**Post-Area (mm^2)**	**Pre-Mean FF (%)**	**Post-Mean (%)**
P1								
Quads	2701.6	2895.5	18.4	15.4	2246.4	2168.8	26.4	22.5
Hamstrings	1880.8	2001.6	25.9	21.1	1265	1364.7	30.2	30.9
Muscle 3	685.6	712.2	25.1	28.1	581.5	447.5	23.2	26.9
Muscle 4	202.7	235.9	16.4	12.9	158.4	158.4	20.6	21.1
Muscle 6	75.3	106.4	33.8	30.9	101.9	96.4	37.1	37.7
Average FF in all ROIs:	-	-	23.9	21.7	-	-	27.5	27.8
Total area in all ROIs =	5546.1	5951.5	-	-	4353.2	4235.8	-	-
Average area in all ROIs =	1109.3	1190.3	-	-	870.6	847.2	-	-
P2								
Quads	2749.3	3459.3	27.5	22.5	2683.9	2703.8	27.5	28.0
Hamstrings	2097.9	2256.3	37.1	33.3	1823.2	1946.2	40.3	39.3

Muscle 3	1245.0	1575.1	26.5	23.0	1107.7	1009.1	29.2	27.8
Muscle 4	157.3	176.1	24.9	52.8	187.2	216	18.5	18.6
Muscle 6	165.1	216.0	56.7	22.5	130.7	151.8	48.5	48.5
Average FF in all ROIs:	-	-	34.6	30.8	-	-	32.8	32.4
Total area in all ROIs =	6414.5	7682.8	-	-	5932.7	6026.9	-	-
Average area in all ROIs =	1282.9	1536.6	-	-	1186.5	1205.4	-	-
P3								
Quads	4494.2	4959.1	10.3	10.0	4229.2	4362	10.5	12.3
Hamstrings	3303.4	3292	17.1	17.1	3136.3	3023.9	20.2	20.1
Muscle 3	1705.3	2193.2	11.4	9.7	1800.6	1979.4	13.6	13.0
Average FF in all ROIs:	-	-	12.9	12.3	-	-	14.8	15.1
Total area in all ROIs =	9502.9	10444.3	-	-	9166.0	9365.4	-	-
Average area in all ROIs =	3167.6	3481.4	-	-	3055.3	3121.8	-	-

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