Bone Stress Injuries in Basic Military Training and Investigation of the Possible Role of Recombinant Parathyroid Hormone (1-34) in their Treatment

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A Thesis Presented for the Degree of Doctor of Philosophy at the University of East Anglia

Norwich Medical School

2024

Declaration

I verify that the work presented in this thesis is my own original work and has not been previously submitted for a degree at this or any other university. Where work by other authors has been included, their work has been fully cited and referenced.

In line with the regulations for submission for a degree of Doctor of Philosophy at the University of East Anglia, this thesis has a word count of 86,817 words, which includes all footnotes and references, but excludes appendices which consist of 8,374 words.

Abstract

Trainees in basic military training are susceptible to lower body bone stress injuries (BSIs). Current treatment is conservative and average healing times exceed 80 days. Improved treatment is required to accelerate healing and protect against re-injury. This thesis presents four studies that explored the risk factors for BSIs in infantry trainees, and the efficacy of recombinant parathyroid hormone (PTH(1-34)) for their treatment.

1. A prospective cohort study did not identify predictive intrinsic risk factors for lower body BSI in men during Infantry basic military training. 'Elite' Infantry were 9-times more likely than 'non-elite' to suffer a BSI (OR 9.3 [95%CI: 2.6 to 33.4], $P \leq 0.001$).
2. A meta-analysis found PTH(1-34) improved functional outcomes across a range of fracture types (MD −1.59, 95%CI: −1.97 to −1.21, $P \leq 0.00001$) but did not affect fracture healing rate (OR 0.96, 95%CI: 0.57 to 1.61, $P=0.87$) or reduce pain (MD −4.55, 95%CI: −7.47 to −1.63, $P=0.002$).
3. The pharmacokinetic response, to a single injection of PTH(1-34) persisted for up to 240 min with a significantly higher AUC for PTH(1-34) in males (AUC difference estimate 8306.6 pmol/mL, 95%CI: 1668.8 to 14944.0 $P=0.016$) than females. Adjusted calcium ($P=0.0079$) and cAMP ($P=0.0071$) were higher in males than females.
4. A clinical trial comparing daily PTH(1-34) to standard care ($n=34$ males, 1 female) did not demonstrate improved radiological healing at 8-weeks post randomisation (adjusted OR: 0.590, 95%CI: 0.071 to 4.350; $P=0.829$)) or time to complete radiological healing (adjusted difference: -1.022, 95%CI: -3.553 to 1.510; $P=0.411$) for lower body BSI in Infantry trainees.

Bone stress injury risk in 'elite' male trainees was more likely affected by training load. PTH(1-34) did not accelerate BSI healing in young healthy adults, but sex differences in response to PTH(1-34) suggest sensitivity to PTH(1-34) differs between males and females. Further research on the utility of PTH(1-34) in BSIs in a larger sample of males and females is warranted.

(311 Words)

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4 Abbreviations

1,25 dihydroxy vitamin D3 / 1,25 dihydroxy cholecalciferol	1,25 $(OH)_2$ D3
25 hydroxy vitamin D3	25 (OH) D3
Alanine transaminase	ALT
Alkaline phosphatase	ALP
Area Under the Curve	AUC
Army Recruitment and Training Division	ARTD
ARTD Lower Limb Injury Prevention Programme	ALLIPP
Bone mineral density	BMD
Bone morphogenic protein	BMP
Bone specific alkaline phosphatase	BSAP
Bone stress injury	BSI
Calcitonin	CT
Chronic kidney disease	CKD
Clinical Research Facility	CRF
Co-efficient of Variation	CV
Combined oral contraceptive pill	COCP
Computed tomography	CT
Consolidated standards of reporting trials	CONSORT
Cross-sectional moment of inertia	CSMI
Cyclic adenosine monophosphate	cAMP
Defence Primary Healthcare	DPHC
Deoxypyridinoline	DPD
Depo-medroxyprogesterone acetate	DMPA
Dickkopf WNT signaling pathway inhibitor 1	DKK1
Dual energy X-ray absorptiometry	DXA
Enzyme-linked immunosorbent assay	ELISA
European Medicines Agency	EMA
Fibroblast growth factor	FGF
Food and Drug Administration	FDA
Gastrointestinal	GI
GENEActiv wrist-mounted triaxial accelerometer	GA

Ground Close Combat	GCC
Ground Reaction Force	GRF
Haemoglobin	Hb
High Density Lipoprotein	HDL
High Resolution Peripheral Quantitative Computed Tomography	HRpQCT
Hormone Replacement Therapy	HRT
Indian hedgehog	Ihh
Interclass Correlation	ICC
Interleukin 6	IL-6
Infantry Training Centre (Catterick)	ITC(C)
Insulin-like growth factor 1	IGF-1
Liquid chromatography mass spectrometry	LCMS
Low density lipoprotein	LDL
Lower limit of quantification	LLQ
Macrophage- Colony Stimulating Factor	M-CSF
Magnetic Resonance Imaging	MRI
Matrix Metalloproteinases	MMP
Mantel-Haenszel test	M-H
Medicines and Healthcare Research Authority	MHRA
Ministry of Defence (UK) Research Ethics Committee	MODREC
Musculoskeletal	MSK
Non-steroidal anti-inflammatory drugs	NSAIDs
N-terminal propeptide of type I collagen	P1NP
C-terminal propeptide of type I collagen	P1CP
Osteocalcin	OC
Osteogenesis Imperfecta	OI
Osteoprotegerin	OPG
Paget's disease of bone	PDB
Parathyroid hormone	PTH
Parathyroid hormone receptor 1	PTHR1
Parathyroid hormone related peptide	PTHrP
Patient reported outcome measures	PROMs
Phosphate	PO_4

Principal Investigator	PI
Pyridinoline	PYD
Quantitative Computed Tomography	QCT
Receptor activator of nuclear factor kappa-b ligand	RANKL
Recombinant human parathyroid hormone	rhPTH
Red blood cell	RBC
Relative Energy Deficiency in Sport Syndrome	RED-S
Runt related transcription factor 2	RUNX2
Sclerostin	SOST
Sub-Cutaneous	SC
36-Item Short Form Health Survey	SF-36
Short musculoskeletal function assessment questionnaire	SMFA-D
Summary of Medicinal Product Characteristics	SmPC
Tartrate Specific Acid Phosphatase	TRAP
Transforming growth factor- β	TGF- β
Type I collagen c-telopeptide	CTX
Type I collagen n-telopeptide	NTX
Vertical loading rate	VLR
Vitamin D2 (ergocalciferol)	D2
White blood cell	WBC
Women in Ground Close Combat Research Group	WGCCRG

5 Acknowledgements

It has taken a ‘village’ to get me to the end of this programme of research which has seen me through a pandemic, three house moves, a wedding and the birth of my daughter Florence. I have made some lifelong friends and I am indebted to everyone who has made it possible.

Prof Bill Fraser, thank you for your guidance and encouragement throughout my time as a PhD student, your enthusiasm for research, honesty and high standards have motivated me and allowed me to develop as a clinician and scientist.

This body of work would not have been possible without four inspirational women;
Prof Julie Greeves, you have been there from the start and your mentorship has been invaluable. Thank you for being so generous with your incredibly limited time. Your passion for science and research within the Military is infectious.
Dr Dr Sarah Jackson, you set me out on this path, if I knew then what I know now, I would have asked more questions. Thank you for the support in those early days.
Dr Anna Casey, I learnt so much from your project management and determination to get a project done.
Dr Isabelle Piec, I can’t thank you enough for the help and support you have given me over the years, I will never forget (or return to) Halloween Horror Night at Universal Studios but what doesn’t kill us only makes us stronger. On a more serious note, I will always remember the hours and dedication you put in to help me deliver PHAB. I could not have done it without you.

To my ‘unofficial’ supervisors;
Dr Tom O’Leary, your mentorship, patience, seemingly encyclopaedic knowledge and enthusiasm for statistics have been a constant throughout my PhD process I can’t thank you enough. I look forward to simply being a participant in the future, just no more muscle biopsies please.
Dr Alex Carswell, you have been a great encouragement, I am a better researcher because of your mentorship in Catterick – thank you.

To all the participants volunteered for and took part in my studies, thank you. Without you, this work would simply not have been possible.

To my family, my husband Aaron who has lived the journey with me, thank you for your love, support and occasional nagging to 'get on with it'. I look forward to a time when we can just enjoy the evenings and weekends without either of us studying. Mum and Peter, thank you for your unconditional support, encouragement and endless hours of babysitting.
To my wider friends and family, thank you for always believing in me and I look forward to having more time to enjoy with you.

6 Publications

Eastman K, Gerlach M, Piec I, Greeves J, Fraser W. Effectiveness of parathyroid hormone (PTH) analogues on fracture healing: a meta-analysis. Osteoporos Int. 2021 Aug;32(8):1531-1546. doi: 10.1007/s00198-021-05847-0. Epub 2021 Feb 9. PMID: 33559713.

Carswell, A.T., Eastman, K.G., Casey, A. *et al.* Teriparatide and stress fracture healing in young adults (RETURN – Research on Efficacy of Teriparatide Use in the Return of recruits to Normal duty): study protocol for a randomised controlled trial. *Trials* **22,** 580 (2021). https://doi.org/10.1186/s13063-021-05556-3

Eastman, K., O'Leary, T.J., Carswell, A. *et al.* Distal Tibial Bone Properties and Bone Stress Injury Risk in Young Men Undergoing Arduous Physical Training. *Calcif Tissue Int* (2023). https://doi.org/10.1007/s00223-023-01111-1

6.1 Conference Proceedings

Piec I, Eastman K, Carswell A, Jethwa K, Tang JCY, Casey A, Greeves JP and Fraser WD. 2022. Sex differences in response to a single injection of teriparatide J Bone Miner Res 36 (Suppl 1). Available https://www.asbmr.org/meetings/annualmeeting/AbstractDetail?aid=cff716d5-b27c-47c1-aff0-07d275f01690 (Co-First Author)

6.2 Awards

3 Minute Thesis Competition. Norwich Medical School 1st Prize, 2019. 'Treatment of Bone Stress Injury in Women in Ground Close Combat'.

7 Introduction

7.1 Human Bone Physiology

7.1.1 Structure and Function of the Bone

The human skeleton provides a variety of essential functions. The bones of the skeleton provide structural support, enabling movement by providing levers for the muscles, protecting vital organs, maintaining mineral homeostasis and acid-base balance, serving as a reservoir of growth factors and cytokines and enabling haematopoiesis within the marrow spaces (Taichman, 2005; Clarke, 2008).

The density of bone varies throughout the body; dense bone, where the tissue is compact is called compact or cortical bone, less dense tissue is known as cancellous or trabecular bone. Cortical bone is the more highly calcified of the two, which mainly serves mechanical and protective functions. By contrast, trabecular bone contains a smaller proportion of calcified tissue but is rich in bone marrow, blood vessels and connective tissue.

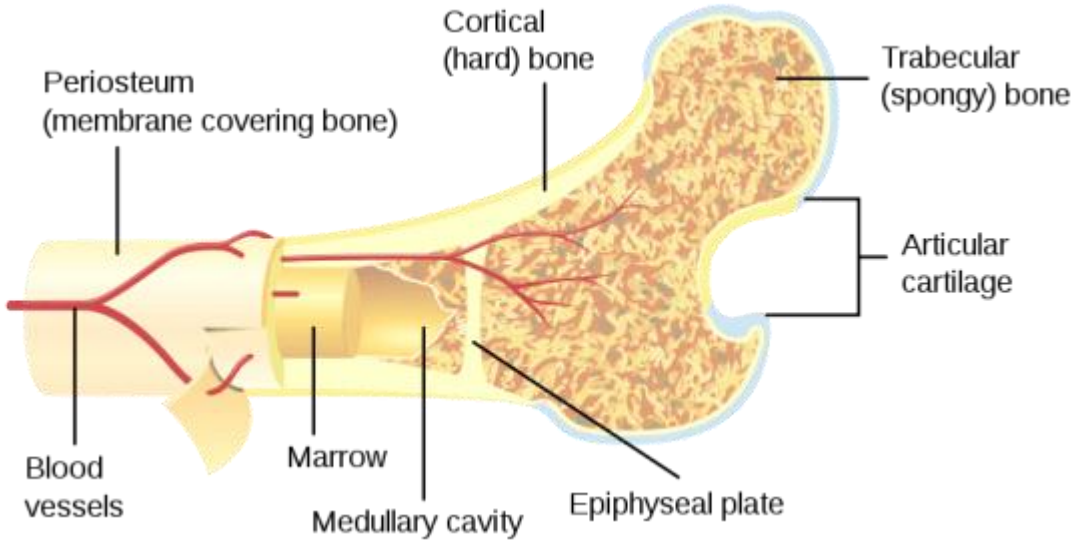


Figure 7-1: Schematic representation of the differences between cortical and trabecular bone. Available online at Difference Between Trabecular and Cortical Bone | Compare the Difference Between Similar Terms [Accessed 25/11/2022]

The general structure of both types of bone is the same, each having an external surface, periosteum and an internal surface, endosteum that lines the medullary cavity (Figure 7-1).

The tubular region of the bone is called the diaphysis, this flares at the end to form the metaphysis which is predominantly cancellous or spongy inside. The end of the bone is the epiphysis. In children, this is separated from metaphysis by the physis or growth plate.

The periosteum is a complex structure composed of an outer fibrous layer that lends structural integrity and an inner cambium layer than possesses osteogenic potential. During growth and development it contributes to bone elongation and modelling, and when bone is injured it participates in recovery (Dwek, 2010). The endosteum is a membrane lining the inner surface of the bony wall also identified as the lining membrane of the bone marrow cavity, it also lines the Haversian canal and all the internal cavities of the bone (Sims and Vrahnas, 2014). Trabecular bone is more commonly found around the endosteal surface, where the rich blood supply supports the majority of metabolic bone activity.

Human bone is composed of bone matrix and bone cells. Almost 90% of the bone matrix comprises type 1 collagen, the remainder is composed of non-collagenous proteins such as osteocalcin (OC), osteonectin, bone sialoproteins and proteoglycans (Chan and Duque, 2002; Raisz, 1999; Arvidson *et al.*, 2011). Bone tissue is mineralised by crystals of hydroxyapatite that are found within the collagen fibres. Collagen fibres lie parallel to each other in layered structure called a lamellar structure and form the circumferential lamellae. Lamellae are also arranged concentrically around channels containing capillaries, called a Haversian System. The Haversian canals provide structural support and enable the metabolism of bone as they also contain capillaries. Transverse branches from the Haversian canals are Volkmann canals.

In contrast, ‘woven’ bone is immature bone, composed of loose, randomly arranged collagen bundles containing osteocytes in lacunae that vary in size and shape. The wall of the lacunae is not well defined. Woven bone is formed by irregular and unpolarised extrusion of proto-collagen by osteoblasts, which bury themselves in the matrix produced. The matrix is disordered compared to lamellar bone. Woven bone can be found in the embryonic skeleton and in cortical and cancellous bone in states of rapid bone growth, bone replacement or high bone turnover. It is replaced in the normal skeleton by lamellar bone after completion of growth.

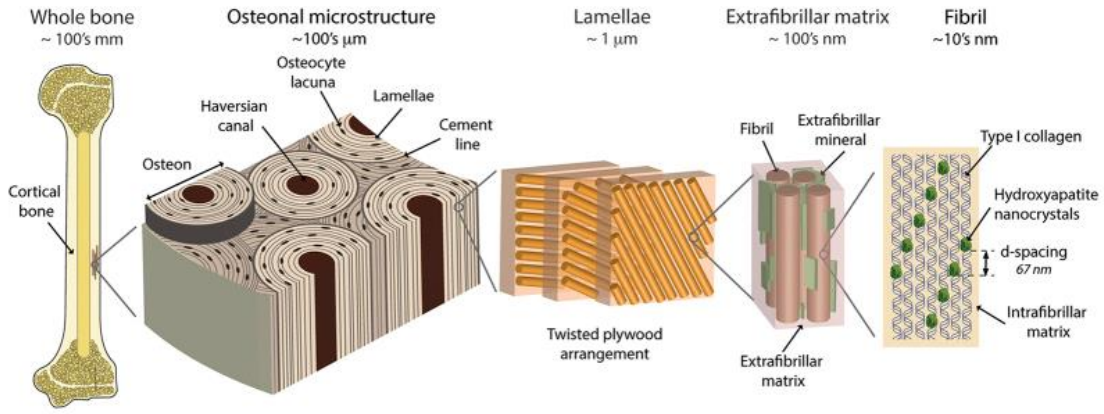


Figure 7-2: Schematic representation of the structure of bone. The structure of bone spans macroscopic whole-bone structures to nanoscale collagen and mineral components. At the microscale, the bone is composed of osteons that are 170-250 µm in diameter. Osteons have a central vascular channel (60-90 µm diameter), called haversian canals and a highly mineralised outer boundary called the cement line (<5 µm thickness) Inside the osteons the vascular channels are concentrically surrounded by lamellae (2-9 µm thickness). These lamellae are composed of bundles of collagen fibrils and have a twisted plywood arrangement. Where lamellae are less organised osteocytes reside in lacunae (5-10 µm diameter) and interconnect through canaliculi (100-400 nm in diameter). The fibrils (80-100 nm diameter) are surrounded by polycrystalline extrafibrillar mineral platelets (80-100 nm in diameter). The extra fibrillar as well as the intrafibrillar matrix may also contain molecular components, such as non-collagenous proteins or cross-links promoting the formation of sacrificial bonds. In the fibrils, type I collagen molecules and hydroxyapatite nanocrystals form a composite structure, where arrays of collagen molecules are embedded with nanoplatelets of hydroxyapatite mineral. Available online at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4562496/ [Accessed 31/12/2021]

The majority of bone turnover takes place on bone surfaces, but the calcified bone matrix is still metabolically active. Bone marrow is soft, gelatinous tissue that fills medullary cavities in the centre of bones. There are two types of bone marrow, red, known as myeloid tissue and yellow known as fatty tissue (Ishii and Kikuta, 2013).

7.1.2 Bone Cells

There are three main types of bone cells: osteoclasts, osteoblasts and osteocytes. In normal bone remodelling, osteoclasts remove calcified bone tissue, creating cavities in the bone in the process of bone resorption. Osteoblasts are the cells that form new bone in the process of bone formation (Marsell and Einhorn, 2011). The co-ordinated balance between bone resorption and formation maintains healthy bone. When this balance is disturbed and resorption outpaces formation, the fracture risk is increased as the structural integrity of the skeleton is compromised (Harris and Heaney, 1969).

7.1.3 Osteoclasts

Osteoclasts are multinucleated cells that originate from mononuclear macrophage / monocyte lineage haematopoietic precursor cells formed in red bone marrow (Boyce, Zuscik and Xing, 2013; Ishii and Kikuta, 2013). Mature osteoclasts are between 50 and 100 µm in diameter and normally contain between 10 and 20 nuclei (Roodman, 1991; Roodman, 1996). Defective formation of osteoclasts is described in a number of bone diseases: Paget's disease of bone (PDB) and osteogenesis imperfecta (OI) patients have larger than normal osteoclasts while osteopetrosis patients have smaller than normal osteoclasts (18 ± 3 µm in diameter) and fewer nuclei (2-3 in each cell) (Henriksen *et al.*, 2011; Cheung, Glorieux and Rauch, 2009; Madyastha *et al.*, 2000). Normal osteoclasts contain predominantly pleomorphic mitochondria, lysosomes, free ribosomes and cytoplasm containing large quantities of granules (A. and C, 2003; Roodman, 1996). Mature osteoclasts have a short life span of around 2 weeks and are mainly found on the s trabecular surface and endosteal boarder of cortical bone (Edwards and Mundy, 2011).

Mature osteoclasts are the only known cells in the body that are capable of resorption, removing old and damaged bone (Edwards and Mundy, 2011). Development and survival of osteoclasts are controlled and influenced by two key cytokines, receptor activation of nuclear factor kappa-B ligand (RANKL) and macrophage-colony stimulating factor (M-CSF). Osteoclast formation requires the presence of RANKL as it primes precursor cells and M-CSF, which contributes to the proliferation, survival and differentiation of osteoclast precursors (Boyle, Simonet and Lacey, 2003).

A number of proteins and hormones, including parathyroid hormone (PTH), calcitonin (CT), interleukin 6 (IL-6), oestrogen (E2) and osteoprotegerin (OPG), also affect osteoclast function. Over production of osteoclasts contributes to the pathology of several bone diseases, including osteoporosis (Boyce, Zuscik and Xing, 2013).

7.1.4 Osteoblasts

Osteoblasts are mononucleated cells that develop from bone marrow mesenchymal stem cells via the canonical Wnt signalling pathway (Logan and Nusse, 2004). Osteoblasts are single layer cuboidal cells located primarily on endosteal, but also on periosteal bone surfaces (Mackie, 2003). Osteoblasts are responsible for the regulation of bone formation and indirectly control bone resorption by secreting RANKL and OPG (Logan and Nusse, 2004; Mackie, 2003). RANKL / RANK signalling regulates osteoclast formation, activation and survival in normal bone modelling and remodelling, OPG protects bone from excessive resorption by binding to RANKL and preventing it binding to RANK (Boyce and Xing, 2008). Mature osteoblasts may be characterised by the presence of an extensive endoplasmic reticulum and a Golgi body.

As osteoblasts differentiate from mesenchymal cells, they begin to secrete bone matrix osteoid proteins. The major protein in the bone matrix is type I collagen (approximately 90% of the organic matrix), which provides the structure on which bone mineral is deposited. Non-collagenous proteins, including proteoglycans, glycoproteins and y carboxylated Gla proteins are also secreted by osteoblasts (Mackie, 2003). The major proteoglycans present in the bone matrix are decorin and biglycan, chondroitin sulphate proteoglycans. Decorin is co-distributed with type I collagen and regulates collagen fibrillogenesis (Mackie, 2003). Biglycan modulates osteoblast precursors to transforming growth factor- β (TGF- β). Glycoproteins produced by osteoblasts are involved in the regulation of osteoblast adhesion, migration, proliferation, and / or differentiation (Robey, 2002; Mackie, 2003; Harada and Rodan, 2003).

Osteoblasts possess a high concentration of alkaline phosphatase in their cell membranes, and express receptors for parathyroid hormone (PTH) and 1,25 dihydroxy vitamin D3 (1,25 $(OH)_2$ D3). A proportion of osteoblasts are trapped in the bone matrix and become osteocytes, which gradually stop secreting the bone matrix proteins. The remainder of the osteoblasts either become the lining cells on the surface of bones or undergo apoptosis (Jilka *et al.*, 1999).

The number of osteoblasts in humans decreases with increasing age, reducing bone formation and overall osteoblast activity, which is a major contributor to age-related osteoporosis (D'Ippolito *et al.*, 1999).

7.1.4.1 Osteoblast Differentiation

The differentiation of osteoblasts is controlled by Indian Hedgehogs (Ihh), Bone Morphogenic Protein (BMP), TGF–β, PTH and WNTs. The process of differentiation can be divided into four stages: proliferation, extracellular matrix deposition, matrix maturation and mineralisation (Stein and Lian, 1993).

Runt related transcription factor 2 (RUNX2), is a 'master switch' for osteoblast differentiation; RUNX2 knock out mice do not produce any osteoblasts, producing a solely cartilaginous skeleton (Otto *et al.*, 1997). RUNX2 interacts with transcriptional activators to either up or down regulate a number of osteoblast–specific genes (Harada and Rodan, 2003).

7.1.4.2 Bone Morphogenic Protein Signalling

BMPs belong to the TGF-β super family; they are expressed in skeletal tissue and are essential in skeletal development, maintenance of bone homeostasis, and fracture healing (Chen, Zhao and Mundy, 2004).

7.1.4.3 Transforming growth factor- β

Transforming growth factor-β(TGF-β) is involved in the regulation of proliferation, migration, differentiation and survival of many cell types. TGF-β is one of the most prolific cytokines in the bone matrix and plays a major role in the development and maintenance of cartilage and bone metabolism (Janssens *et al.*, 2005).

7.1.4.4 WNT Signalling

WNTs are secreted glycoproteins, they act in the canonical pathway and stimulate alkaline phosphatase activity and bone formation. Sclerostin antagonises WNT activity (Clevers and Nusse, 2012). See section 7.1.5.1 for further detail of the role of sclerostin.

7.1.4.5 Hedgehog Signalling

The Ihh protein co-ordinates a range of skeletal morphogenesis through PTHrP-dependent and independent processes. Ihh deficient mice show reduced chondrocyte proliferation and a failure of osteoblast development (St-Jacques, Hammerschmidt and McMahon, 1999).

7.1.4.6 Parathyroid Hormone Signalling

PTH and PTHrP can have both anabolic and catabolic effects on bone. Intermittent PTH administration induces bone formation and continuous administration induces bone resorption (Poole and Reeve, 2005; Reeve, Tregear and Parsons, 1976; Reeve *et al.*, 1980). PTH and PTHrP both have a critical role in bone development, demonstrated in both mice and humans. PTHrP-deficient mice have widespread skeletal abnormalities and die at birth (Karaplis *et al.*, 1994). PTH deficient mice exhibit reduced cartilage matrix mineralisation among other defects, but they did survive (Miao *et al.*, 2002). Overexpression of PTHrP causes transgenic mice to develop shortened limbed dwarfism and a delay in endochondrial ossification (Weir *et al.*, 1996).

Both PTH and PTHrP signal via the 7-transmembrane G protein coupled receptor, parathyroid hormone receptor 1 (PTHR1). PTHR1 is expressed on the surface of osteoblasts and osteocytes in bone, and tubular cells in the kidney (Datta and Abou-Samra, 2009; Fermor and Skerry, 1995). Stimulation of PTH1R by PTH activates the adenylyl cyclase, cyclic AMP (cAMP) and protein kinase A (PKA) signalling pathway (Gardella, 2015) and modulates key genes that control bone remodelling (Kramer *et al.*, 2010; Fu *et al.*, 2002). PTH1R also activates the phospho-lipase, protein kinase C (PKC) signalling which is inhibitory to the osteoanabolic actions of PTH (Kousteni and Bilezikian, 2008; Yang *et al.*, 2007; Ogata *et al.*, 2011). When PTH binds to the PTH1R, it down-regulates PTH-induced translocation of β-arrestins to the cell membrane downregulates cAMP activation and contributes to the anabolic action of PTH independent of G protein signalling (Gesty-Palmer *et al.*, 2009).

PTH-induced bone resorption is mediated indirectly through its actions on osteoblasts and osteocytes (Silva and Bilezikian, 2015). The OPG-RANKL-RANK pathway is essential for bone resorption induced by PTH. PTH modulates the expression of RANKL by osteoblasts and osteocytes and the decoy receptor osteo-protegerin (OPG) and regulates production of osteocytes (Lee and Lorenzo, 1999; Kanzawa *et al.*, 2000; O'Brien, Nakashima and Takayanagi,

2013). RANKL binds to the surface of osteoclast precursors, stimulating differentiation, survival and stimulating activity. OPG inhibits the binding of RANKL to RANK by acting as a decoy receptor for RANKL. (Simonet *et al.*, 1997; Fuller *et al.*, 1998; Khosla, 2001).

The bone resorption effects of continuous PTH administration are mediated by an increase in mRNA coding for RANKL and reduction of mRNA coding for OPG; the resulting imbalance is catabolic to bone (Lee and Lorenzo, 1999; Huang *et al.*, 2004; Ma *et al.*, 2001; Podbesek *et al.*, 1983)

7.1.4.7 Insulin-Like Growth Factor Signalling

Insulin-like growth factor 1 (IGF-1) is secreted by skeletal cells and is believed to be an auto or paracrine regulator of osteoblastic cell function (Canalis, 2009).

7.1.4.8 Fibroblast Growth Factor Signalling

Fibroblast Growth Factors (FGFs) are important regulators of endochondral and inter membranous bone formation, development and apoptosis, affecting both chondrogenesis and osteogenesis (Ornitz, 2005).

7.1.4.9 Notch Signalling

Notch proteins are transmembrane receptors that control cell fate decisions and inhibit osteoblastic differentiation. Disruption of Notch signalling in mice reduces the number of mesenchymal stem cells and increases trabecular bone mass; they also developed severe osteopenia (Hilton *et al.*, 2008).

7.1.5 Osteocytes

Osteocytes are the predominant cells in bone, making up 95% of bone cells (Dallas and Bonewald, 2010; Kini and Nandeesh, 2012; Mackie, 2003). Osteocytes are mature osteoblastic cells, the most active form of which lie within the calcified matrix of mature bone; they detect environmental changes that affect bone including changes in workload. The osteocytes signal osteoblasts and osteoclasts and direct them to undertake necessary repair and adaptation (Manolagas, 2000; Divieti Pajevic, 2013).

Osteocytes survive in the bone matrix for as long as the bone site they occupy exists. They signal nearby cells, distant tissues and organs though small channels called canaliculi, which

penetrate the surrounding bone. Osteocytes influence the activity of osteoclasts and osteoblasts via the canaliculi (Dallas and Bonewald, 2010).

Mature osteocytes are the only cells that produce sclerostin—a protein that inhibits the Wnt signalling pathway and decreases new bone formation (Bonewald, 2011; Brunkow *et al.*, 2001). Damage to the bone causes osteocytes to secrete IGF-1, which stimulates osteoblast activity potentiating new bone formation (Sheng, Lau and Baylink, 2014).

Osteocytes also act as endocrine cells through the production of Fibroblast Growth Factor 23 (FGF23) (Bonewald and Wacker, 2013; Ito *et al.*, 2021). FGF23 regulates serum phosphate homeostasis. Increasing circulating levels of phosphate and 1,25 $(OH)_2$ D3 result in an increase in FGF23. In the kidney, the FGF23 then binds to receptors and co-receptor α-Klotho to promote phosphaturia and reduce circulating 1,25 $(OH)_2$ D3 levels. FGF23 synthesis in bone is also affected by lipocalin-2, glycerol 3-phosphate, 1-acyl lysophosphatidic acid and erythropoietin, biomolecules produced in the kidney (Agoro and White, 2023; Noonan *et al.*, 2023). FGF23 expression is increased in various forms of rickets, tumour induced osteomalacia and chronic kidney disease (Bonewald and Wacker, 2013).

7.1.5.1 Sclerostin

Sclerostin, a signalling molecule synthesised by osteocytes, is involved in the regulation of bone modelling and remodelling by inhibiting bone formation. Osteoblast lineage cells are regulated by various molecules and signalling molecules including the WNT pathway. Activation of WNT signalling in bone stimulates osteoblastic activity and increases bone formation. WNT signalling is complex and there are many molecules involved in the controlling the various effects of WNT signalling on bone cells. Sclerostin binds to low density lipoprotein receptor-related protein, LRP 5 & LRP 6. These single transmembrane receptors inhibit WNT signalling, reducing osteoblastic activity and bone formation (Delgado-Calle, Sato and Bellido, 2017). This pathway is believed to play a key role in the skeletons ability to strengthen bone in response to mechanical stress. Osteocytes respond to stress by producing less sclerostin, which results in an increase in osteoblastic activity and bone formation (Drake and Khosla, 2017; Wang, Mazur and Wein, 2021).

Parathyroid hormone is a direct inhibitor of sclerostin, reducing the expression of sclerostin in pre-clinical and clinical studies. Although the molecular mechanism for the negative relationship between PTH and sclerostin remains unexplained, it is believed to mediate the anabolic effects of PTH (Li *et al.*, 2024).

7.1.6 Bone Lining Cells

Bone lining cells are quiescent osteoblasts that form a lining along bone surfaces that are not undergoing active remodelling and have a role in response to oestrogen (Nakamura, 2007). The full role of these lining cells is yet to be elucidated, but include maintenance of bone fluid, immediate release of calcium from bone to replenish blood calcium, and protecting bone from degradation factors (Nakamura, 2007).

Bone lining cells also seem to play an important role in coupling bone resorption to bone formation. They digest non-mineralised collagen protruding from the bone surface in segregated areas at the plasma membrane of bone lining cells and this depends on activity of matrix-metalloproteinases (MMPs). The presence of cysteine proteases and MMPs signal to osteoclasts to attach and resorb bone at these sites. Osteoclast resorption is incomplete, remnants of demineralised non-digested bone collagen remain. MMPs then prompt bone lining cells to enter the resorption lacunae and digest the remaining bone collagen. The bone lining cells then form a cement line and depositing a thin layer of fibrillar collagen (Everts *et al.*, 2002).

7.2 Endocrine Function of Bone

Physiological regulation of calcium metabolism and skeletal remodelling is controlled primarily by the calcium regulating hormones, PTH, 1,25 $(OH)_2$ D3 and calcitonin, which is produced from C-cells in the thyroid acting on osteoclasts. Figure 7-3 shows the key physiological regulatory mechanisms in the endocrine function of bone.

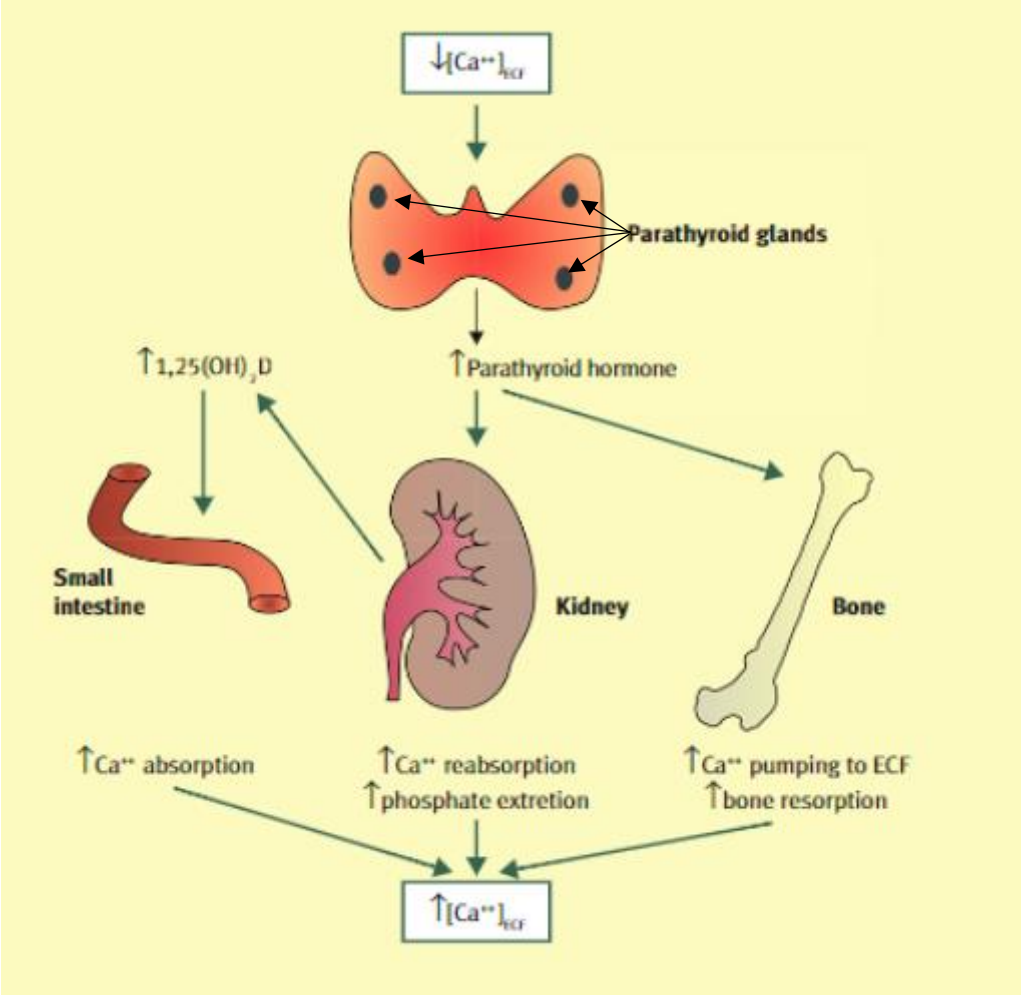


Figure 7-3: Key physiological regulatory mechanisms in the endocrine function of bone. In response to low levels of calcium in the extracellular fluid (ECF), Parathyroid hormone (PTH) acts on the bone and the kidney to release calcium into the extracellular fluid. Calcitriol acts on the intestines to increase resorption of calcium which increases extracellular fluid calcium. Available online at Normal bone physiology, remodelling and its hormonal regulation - ScienceDirect [Accessed 31/12/2021)

7.2.1 Parathyroid Hormone

A decrease in ionised calcium acting on the calcium serum receptor on the Chief cells of the parathyroid gland results in synthesis and secretion of PTH. PTH has several direct and indirect effects on the kidney, gastrointestinal (GI) tract and bone as shown in figure 7-4.

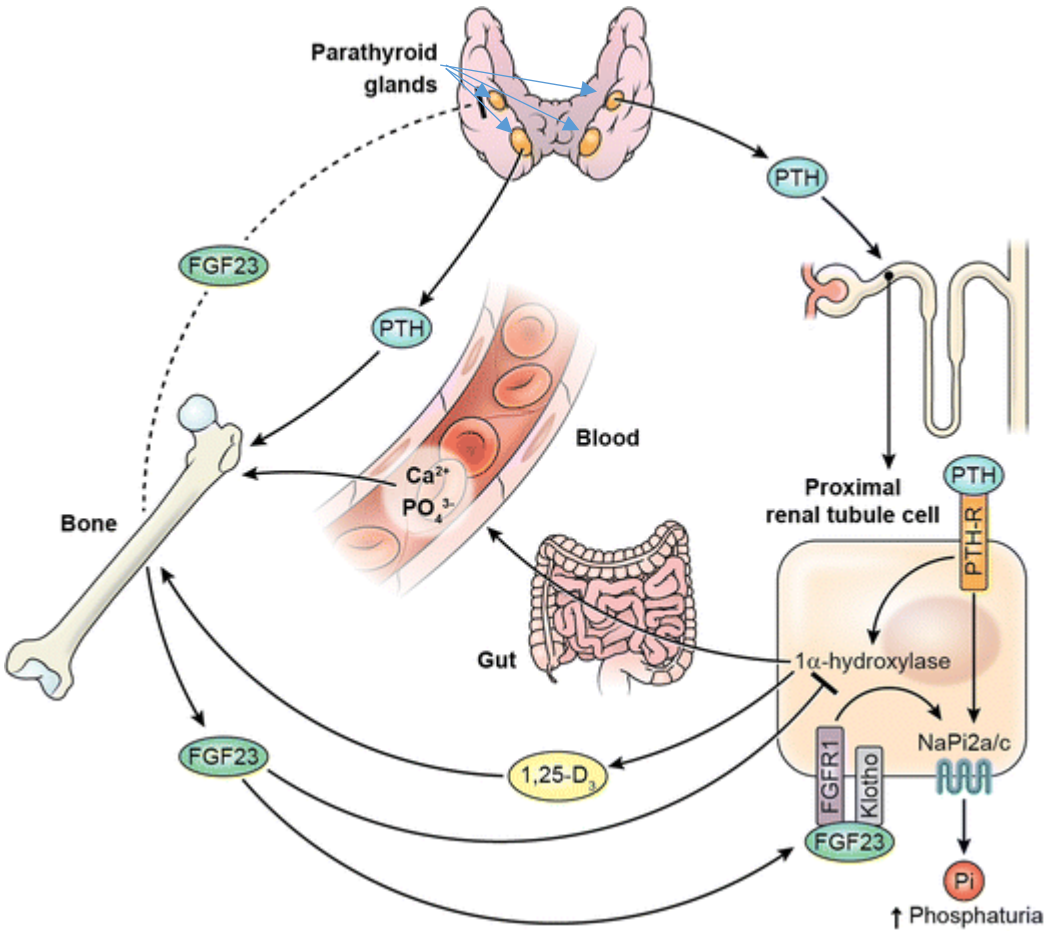


Figure 7-4: The PTH- Vitamin D- FGF23 axis. FGF23 secretion from bone osteocytes acts on the kidney to cause phosphaturia via NaPi 2a/c transporters similarly PTH acts via the PTH receptor also resulting in phosphaturia. PTH also induces 1α- hydroxylase, which hydroxylates 25 (OH) D to 1,25 $(OH)_2$ D. FGF23 supresses 1α- hydroxylase. 1,25 $(OH)_2$ D is responsible for increased calcium and phosphate absorption from the gut and promotes bone mineralisation. It has been reported that FGF23 acts on the parathyroid gland to stop PTH secretion in vitro and rodent models but this is yet to be shown in humans. Available online at https://link.springer.com/article/10.1007/s11154-015-9318-z. [Accessed 03/12/2022]

In the kidney, PTH stimulates the kidney cortex mitochondrial enzyme 1-α-hydroxylase (CYP27B1) (also stimulated by low levels of phosphate), which causes alpha hydroxylation of

25 hydroxy vitamin D3 (25 (OH) D3) to its active form 1,25 $(OH)_2$ D3. 1,25 $(OH)_2$ D3 enters the circulation and stimulates osteoclast activity and resorption.

In addition, PTH inhibits bicarbonate resorption in the kidney stimulating a metabolic acidosis, which favours calcium ionisation, resorption of calcium from bone and dissociation of calcium from plasma protein binding sites. PTH also increases urinary excretion of phosphate through direct action on the proximal tubules, where binding to PTH1R reduces surface exposure of the NaPi-IIa transporter preventing phosphate reabsorption and increasing excretion. PTH stimulates the uptake of calcium from the GI tracts as an indirect action of vitamin D metabolism, as increasing 1,25 $(OH)_2$ D3 augments intestinal calcium absorption (Goltzman, Mannstadt and Marcocci, 2018).

PTH acts directly on bone to release calcium, orthophosphate, magnesium citrate, hydroxyproline and OC. In response to increased concentrations of PTH, osteoblasts synthesise collagen on which calcium phosphate binds as hydroxyapatite crystals. Osteoclast cells then release the hyaluronic acid and acid phosphatase which solubilise the calcium phosphate. The subsequent relative hypercalcaemia prevents further production of PTH, limiting the synthesis of 1,25 $(OH)_2$ D3 by a classic feedback loop.

7.2.2 Vitamin D and Metabolites

Humans acquire vitamin D3 (cholecalciferol) from two sources, exposure of the skin to sunlight and ingestion in the diet, vitamin D2 (ergocalciferol) is acquired from plant sources in the diet. Cholecalciferol undergoes a two-step metabolism to convert to its active form, 1,25 $(OH)_2$ D3 (calcitriol) (DeLuca, 2004). The metabolic conversion starts in the liver where cytochrome P450 enzymes hydroxylate carbon 25 to produce 25 (OH) D3 (Cheng *et al.*, 2003; Ohyama and Yamasaki, 2004). 25 (OH) D3 is then transported in the blood bound to vitamin D binding protein (VDBP) to the kidney where it undergoes a further hydroxylation to 1,25 $(OH)_2$ D3 in the proximal tubular cells by the 1α-Hydroxylase enzyme. This second stage of the metabolism is stimulated by PTH and low phosphate concentrations, it is inhibited by high concentrations of 1,25 $(OH)_2$ D3 and high concentrations of phosphate. The cytochrome P450

activation and inactivation pathways of vitamin D3 (cholecalciferol) are shown in detail in figure 7-5.

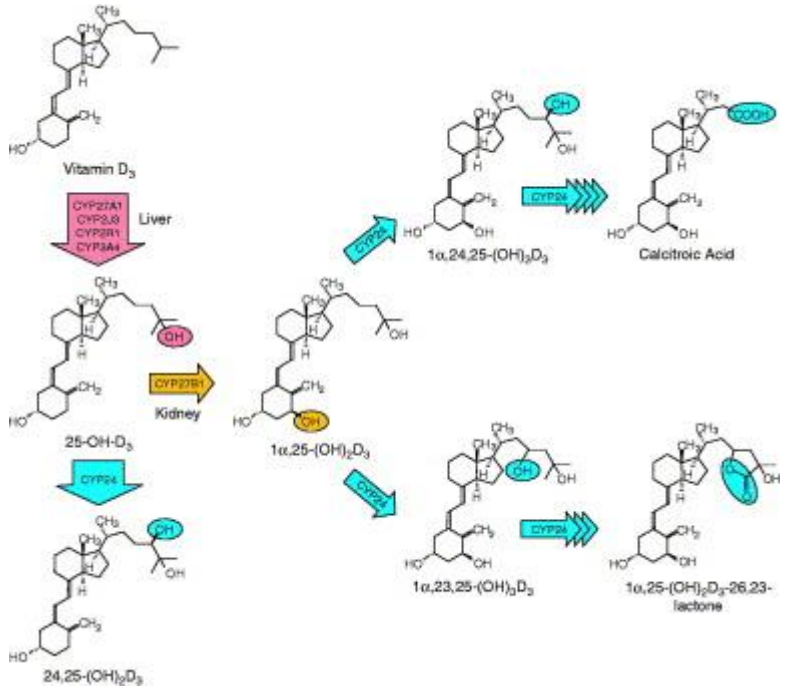


Figure 7-5: The three pathways of vitamin D metabolism. (1) The activation pathway from vitamin D3 to 1,25 $(OH)_2$ D3 by CYP enzymes 25- hydroxylase (pink) and 1α- hydroxylase (yellow). (2) The catabolic carbon- 24 oxidation pathway in which 24- hydroxylase (CYP 24) converts 1,25 $(OH)_2$ D3 to calcitroic acid. (3) The catabolic lactone pathway in which 24- hydroxylase (CYP 24) converts 1,25 $(OH)_2$ D3 to 1,25 $(OH)_2$ D3 – 26,23- lactone. Available online at https://www.sciencedirect.com/science/article/pii/S0968000404002701 [Accessed 03/12/2022].

Vitamin D binding protein is synthesised in the liver, production can be affected by liver disease. Individuals with liver, intestinal or renal diseases affecting VDBP levels may have low circulating concentrations of vitamin D metabolites without being vitamin D deficient (Christakos *et al.*, 2010).

High circulating concentrations of 1,25 $(OH)_2$ D3 up regulate the 24 hydroxylase (CYP24A1) enzyme which converts 1,25 $(OH)_2$ D3 to 1,24,25 $(OH)_3$ D3 and 25 OHD to 24,25 $(OH)_2$ D3 which are currently considered inactive excretory products of the vitamin D pathway. The transcription of the CYP24A1 enzyme is stimulated by fibroblast growth factor-23 (FGF23) and

suppressed by PTH (Tang *et al.*, 2017). High circulating concentrations of 1,25 $(OH)_2$ D3 also feedback to the parathyroid gland via internal nuclear receptors.

7.2.3 Calcitonin

Calcitonin is synthesised in the thyroid and brain. At normal serum calcium concentrations, CT release is low but an increase in calcium prompts a rapid increase in CT concentrations. CT influences both bone and kidney. CT inhibits bone resorption, whilst not affecting bone formation. In the kidney, CT is found in the renal cortex where receptors are present at the membranes of the tubule cells. In the presence of CT, excretion of calcium, sodium and potassium are increased whilst excretion of magnesium is reduced. CT affects vitamin D metabolism by lowering serum calcium, resulting in the release of PTH. The role of CT in the metabolism of calcium is less significant than that of PTH.

Calcitonin stimulates 1,25 $(OH)_2 D_3$ production, this is most significant during lactation, when CT concentrations as well as 1,25 $(OH)_2$ concentrations are elevated due to the increased calcium demand to protect the maternal skeleton (Christakos *et al.*, 2010).

7.3 Bone Turnover

Bone turnover consists of three processes, bone modelling, bone remodelling and a resting phase.

7.3.1 Bone Modelling

Bone modelling is the process whereby bones are shaped or reshaped by the independent action of osteoblasts and osteoclasts. Osteoblasts can deposit new bone matrix without prior bone resorption by osteoclasts. Bone modelling occurs typically during the early developmental stages of the skeleton when bone size and shape change. Mineralised tissue is deposited at developmentally determined sites, usually preceded by cartilage. In flat bones, such as the skull, bone is formed independently of cartilage as membranous bone adjacent to a cartilage template (Raisz, 1999). Once the skeleton is fully formed, as a person reaches puberty, hormonal changes cause fusing of the epiphyses, meaning bones can no longer 'model' by extending length and bone remodelling becomes the dominant process (Shim, 2015).

Bone modelling can be an adaptive response to the biomechanical force experienced by bone to prevent the occurrence of injury to the bone (Crockett *et al.*, 2011; Martin and Seeman, 2008; Haapasalo *et al.*, 2000; Hughes *et al.*, 2017). This adaptation is demonstrated in tennis players who have a thicker cortex and a larger external diameter in the radius of the dominant arm, however bone modelling is much less common than bone remodelling in later life; in humans, bone modelling is typically in those under the age of 20 years (Brandi, 2009).

7.3.2 Bone Remodelling

Bone remodelling describes the activity the bone undertakes to repair fatigue damage (Hughes *et al.*, 2017). The skeleton undergoes continuous remodelling throughout life. Bone remodelling can be triggered by mechanical forces or micro damage which result in a series of highly regulated sequential steps (Turner, 1998). Stable maintenance of bone mass during adult life is the result of the balance between bone formation by osteoblasts and bone resorption by osteoclasts (Bielby, Jones and McGonagle, 2007). In humans, bone remodelling

becomes the dominant process at around 21 years of age. In men from the age of 21 a slow steady decline in remodelling occurs. In women reduced oestrogen concentrations at the menopause (average age 51 in the UK) prompts bone loss in women as oestrogen inhibits osteoclast formation and activity by increasing OPG production and inducing osteoclast apoptosis by increasing transforming growth factor β (Demontiero, Vidal and Duque, 2012).

Aging also has a negative effect on osteoblast differentiation due to a shift from osteoblastogenesis to adipogenesis in the bone marrow resulting in a loss of bone mass in later life (D'Ippolito *et al.*, 1999; Demontiero, Vidal and Duque, 2012).

The bone remodelling process maybe prompted by, chemical signalling (PTH/PTHrP), by ischemic or microfractured bone, the drive being the requirement to maintain calcium homeostasis or substitution of primary bone (infantile bone) with secondary bone which is more mechanically robust. There are five phases to the bone remodelling process (Figure 7-6);

(i) Activation Phase

A stimulant such as microfracture causes an alteration of mechanical loading sensed by osteocytes or other factors in the bone micro-environment such as IGF-I, TNFα, PTH or IL-6, activate the lining cells which are quiescent osteoblasts (Rucci, 2008). The bone lining cells separate to expose the bone and a surface small cavity of bone tissue (∼150–200 μm in diameter) (CURREY, 1964) is made by osteoclastic bone resorption. The lining cells then differentiate into osteoblasts and begin to secrete RANKL, which binds to the RANKL receptor on the surface of osteoclast precursor cells, instigating the differentiation, migration, and fusion of osteoclasts.

(ii) Resorption Phase (Approximately 2 weeks in duration)

Once attached to the mineralised bone surface the osteoclasts initiate resorption by the secretion of hydrogen ions and lysosomal enzymes in the space below the ruffled border that lower the pH and degrade the components of the bone matrix, including collagen. The result is irregular cavities called Howship's Lacunae and Haversian canals on trabecular and cortical bone respectively (Raisz, 1999; Everts *et al.*, 2002).

Osteoclasts undergo apoptosis once they have fulfilled their role. Several factors trigger osteoclast apoptosis, including high concentrations of extra cellular calcium, oestrogens, pyrophosphates and pharmaceutical agents such as bisphosphonates (Boyce *et al.*, 2012). Osteoclast apoptosis results in the secretion of osteocyte apoptotic bodies, which promote osteogenesis through RANKL reverse signalling and induce vessels to improve local nutrient and metabolic waste transport (Ma *et al.*, 2021).

(iii) Reverse Phase (Approximately four to five weeks in duration)

The resorbed bone surface is then prepared for bone formation, as the debris produced by matrix degradation is removed in the 'reversal' phase, by mononuclear cells. It is uncertain if the lineage of these cells is osteoclastic / macrophagic or osteoblastic (Everts *et al.*, 2002).

(iv) Formation Phase (Approximately four months duration)

Growth factors including BMPs, FGFs and TFG β, released during bone resorption, recruit osteoblasts into the resorption space where they deposit osteoid which is predominantly collagen. Calcium and phosphate then crystallise around this collagen scaffold, providing a strong framework for the bone (Boyce *et al.*, 2012; Rucci, 2008)

(v) Termination

On completion of mineralisation osteoblasts undergo apoptosis, change into bone lining cells or become embedded within the bone matrix and terminally differentiate into osteocytes. Osteocytes signal the end of remodelling by secreting antagonists to osteogenesis in the Wnt signalling pathway such as sclerostin (Kenkre and Bassett, 2018).

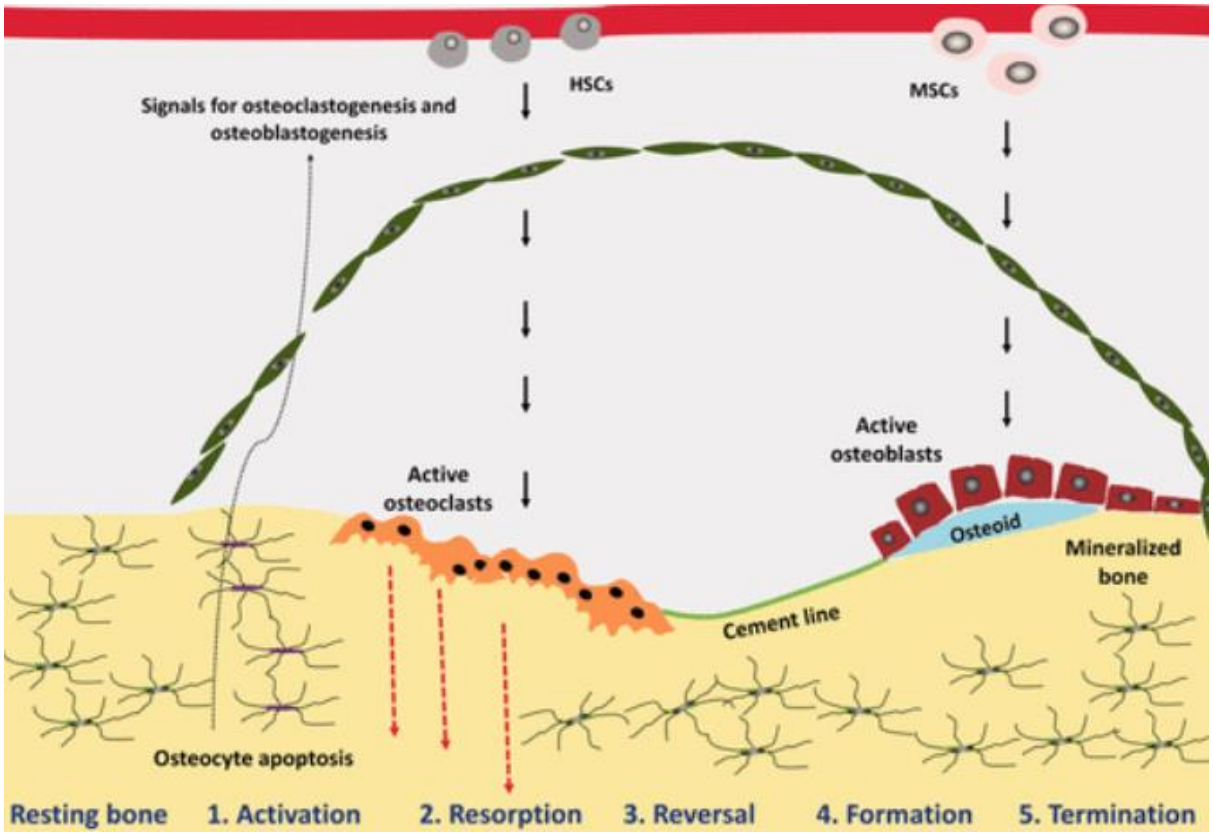


Figure 7-6: Phases in the bone remodelling process. Available online at https://journals.sagepub.com/doi/full/10.1177/0004563218759371 [Accessed 02/01/2022].

7.3.3 Bone Repair and Fracture Healing

Bone repair can be divided into 4 overlapping phases (Schindeler *et al.*, 2008);

1. Inflammatory;
2. Soft Callus Formation;
3. Hard Callus Formation;
4. Remodelling.

The inflammatory response is an almost instantaneous response to bone injury. It is characterised by pain and swelling and continues until bone formation begins. Bleeding at the bone injury site spreads into the surrounding tissue and develops into a haematoma. Degranulating platelets, macrophages, lymphocytes, monocytes and granulocytes enter the haematoma, these cells prevent infection and secrete growth factors and cytokines (Gerstenfeld *et al.*, 2003).

The swelling and pain reduce as the soft callus is formed. The soft callus provides mechanical support to the fracture and provide a template for the growth of the new bone. Hard callus formation or primary bone formation is the stage at which osteoblast activity and mineralisation of the bone matrix are at their highest levels. The soft callus is steadily removed and replaced with the hard callus. The hard callus formation is usually irregular, containing woven and under-remodelled bone.

The final stage, also called 'secondary bone formation' commences when the fracture has solidly fused with woven bone. The hard callus is slowly replaced with lamella bone during the remodelling process as described above, bone remodelling typically starts 6 weeks after the original fracture. Remodelling continues until the bone has completely returned to its original morphology (Gerstenfeld *et al.*, 2003; Mulari *et al.*, 2004).

7.3.4 Bone Markers

The key bone markers involved in the bone remodelling cycle are underpinned by the cellular and extracellular components of the skeletal matrix, they can be measured in serum, plasma or urine. Bone markers are used to monitor bone turnover and are categorised as either markers of bone formation or resorption (Wheater *et al.*, 2013; Ross and Knowlton, 1998; Ross, 1999). Bone markers of formation and resorption are listed in Table 7-1.

Table 7-1: Bone Marker of Bone Formation and Bone Resorption (Seibel, 2005; Marcus et al., 1999; Bhattoa, 2018).

Bone Formation	**Bone Resorption**
Amino-terminal propeptide of type I collagen (P1NP) *Specific product of proliferating osteoblast and fibroblasts; partly incorporated into bone extracellular matrix.*	Type I collagen c-telopeptides (CTX) *Collagen type I, with highest contribution from bone.*
Carboxy-terminal propeptide of type I collagen (P1CP) *Specific product of proliferating osteoblasts and fibroblasts.*	Type I collagen n-telopeptides (NTX) *Collagen type I, with highest contribution from bone.*
Osteocalcin (OC) *Specific product of osteoblasts; many immunoreactive forms in blood; some may be derived from bone resorption.*	Tartrate Specific Acid Phosphatase (TRAP) *Six isoenzymes found in human tissues (osteoclasts, platelets, erythrocytes). Band 5b predominant in bone (osteoclasts).*
Serum Alkaline Phosphatase (ALP) *Total ALP from several sources including liver, bone, intestines and kidney*	Free urinary deoxypyridinoline (DPD) and pyridinoline (PYD) *Collagens, with highest concentrations in cartilage and bone; absent from skin; present in mature collagen only.*
Bone Specific Alkaline Phosphatase (BSAP) *Specific product of osteoblasts.*	Urinary creatinine – corrected free DPD and PYD *Collagens, with highest concentration in bone; absent from cartilage or skin; present in mature collagen only.*

These biomarkers are widely used in research to assess bone remodelling and provide an understanding of the mechanisms underpinning the treatments that are used to modulate bone metabolism (NIH Consensus Development Panel on Osteoporosis Prevention, 2001). These markers can be analysed from blood and urine samples, but pre-analytic issues mean blood remains the sample of choice (Bhattoa, 2018). Urine PYD and urine NTX may be used in preference to plasma CTX, usually in oncology patients with tumours affecting the metabolism of collagen such that NTX anomalies occur in isolation. The advantage of analysing urine, it that it is non-invasive, which is preferable for needle phobic patients and those in remote locations as samples can be sent by post rather than the patient having to attend a medical appointment for the blood test (Bhattoa, 2018). In the USA, the NTX assay is licensed by the FDA and so tests are reimbursed by health insurance companies, other

markers including serum and plasma assays are not licensed by the FDA and so not reimbursed by health insurance companies resulting in very limited use.

A longitudinal study (over 13 years) of postmenopausal women (*n*=354) reported an association between decreases in bone density (measured by single-energy X-ray) at the calcaneus and increased formation markers, (bone specific alkaline phosphatase (BSAP) and OC) and increased resorption markers (free urinary pyridinoline, and free deoxypyridoline corrected for urinary creatinine) (Ross and Knowlton, 1998). These findings suggest that biochemical markers of bone formation and resorption provide information on bone turnover in postmenopausal women. Increased bone turnover, with a net increase in bone resorption relative to bone formation, results in decreased bone mass and, consequently, increased fracture risk (Misiorowski and Zgliczyński, 2012; Ross and Knowlton, 1998).

Biochemical markers of bone formation and resorption also provide an understanding of the fracture healing process, which vary according to the type and size of the fracture (Cox *et al.*, 2010). The utility of bone resorption and bone formation markers in fracture healing is discussed in more detail below in sections 7.3.5 and 7.3.6.

7.3.5 Bone Resorption Markers

7.3.5.1 C and N Terminal Telopeptide of Type I Collagen

During bone resorption, cathepsin K, a potent protease in the lysosomal cysteine family highly expressed in osteoclasts, degrades several components of the bone matrix including the triple helices of the mature type I collagen (which accounts for 90% of bone matrix), tartrate-resistant acid phosphatase (TRAP), osteopontin and osteonectin. Therefore, measuring the breakdown products of type I collagen such as the carboxy and nitrogen telopeptide containing fragments (CTX and NTX, respectively) is a useful measure of osteoclastic bone resorption activity. CTX is measured in plasma, a commonly used assay for CTX detects the relative concentration of the specific amino acid sequence of the telopeptide of Type I collagen, termed as Crosslaps, and those with β-aspartic acid (βCTX) (Hlaing and Compston, 2014). Plasma CTX has superseded all other bone turnover biomarkers including TRAP, PYD and DPD, hydroxyproline and bone sialoprotein, in the UK as the measure of choice for

osteoclastic bone resorption activity as it has been found to be the most specific analyte (Bhattoa, 2018).

The NTX assay is commonly seen in the USA as the CTX assay is not licensed by the Food and Drug Administration (FDA) and therefore not routinely reimbursed by insurance companies. Both CTX and NTX are renally excreted so their clinical utility in patients with impaired kidney function is limited.

Beta C-terminal telopeptide exhibits circadian variation, peak levels are seen in the early morning (approx. 0500 Hrs) and the nadir is early afternoon (approx. 1400 Hrs). (Hannon and Eastell, 2000; Qvist *et al.*, 2002; Ahmad *et al.*, 2003). Food intake can also effect CTX, postprandial levels are 20% reduced compared to the fasting state, in practice this variation can be reduced by collecting samples first thing in the morning after an overnight fast (Clowes *et al.*, 2002).

Urine NTX is thought to exhibit less circadian and postprandial variability as compared to CTX, however this may be due to the imprecision of the assay and the 24 h urine collection required is generally less palatable to patients (Baxter *et al.*, 2013).

7.3.6 Bone Formation Markers

7.3.6.1 N- and C- Terminal Propeptides

Type I collagen is secreted as a pro collagen by osteoblasts. Type I collagen forms a triple helix (containing an α- and β- chain) and contains a N- and a C- terminal propeptides—P1NP and P1CP, respectively. These propeptides are cleaved extracellularly and enter the blood circulation (Zimmermann, Busse and Ritchie, 2015) without being reutilised in further formation of collagen. As a result, P1NP and P1CP are used as bone formation markers (Wheater *et al.*, 2013). The cleaved products are initially excreted as a trimeric form by the liver and consecutively as a monomeric form by the kidneys. An accumulation of monomeric P1NP is common in chronic kidney disease (Koivula *et al.*, 2010; Vasikaran *et al.*, 2014). Assays are available to measure both the trimeric (total) and monomeric (intact) P1NP (Greenblatt, Tsai and Wein, 2017).

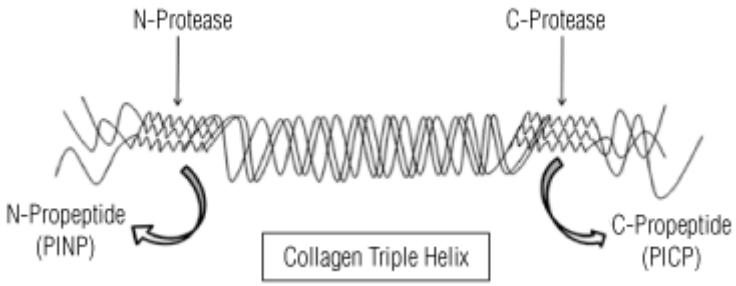


Figure 7-7: The catabolism of procollagen extension peptides by proteases during collagen formation leads to P1NP and P1CP production. Available online at https://www.researchgate.net/publication/44614586_Bone_turnover_assessment_A_good_surrogate_marker [Accessed 01/01/2022]

P1NP is the marker of formation best described in the literature (Bhattoa, 2018). Kurdy *et al.* showed concentrations of P1NP were significantly higher at 10 weeks post fracture in non-union fractures than in well healed fractures (Kurdy, 2000). Equally high concentrations or activity of serum bone specific alkaline phosphatase (BSAP) positively correlated with the successful healing of long bone fractures (Mukhopadhyay *et al.*, 2011).

Analysis and interpretation of some of these bone turnover markers are limited by their low specificity and presence in tissues outside of bone (Cox *et al.*, 2010; Seibel, 2005), but studying them has advanced the understanding of mechanisms of bone metabolism and bone diseases and therapeutic interventions (Seibel, 2005).

7.3.6.2 Osteocalcin

Osteocalcin (OC), also known as bone gamma-carboxyglutamic acid-containing protein, is a 49 amino acid non-collagenous protein. It is secreted by mature osteoblasts and incorporated into the extracellular matrix of bone. A fraction of the OC is released into the circulation and can be used as an indication of mineralisation (Maïmoun and Sultan, 2011). Whilst OC is chiefly a marker of formation, it can also reflect bone resorption as it is also released during degradation of the bone matrix and excreted by the kidney (Greenblatt, Tsai and Wein, 2017; Srivastava *et al.*, 2002).

Urinary OC is increased in post-menopausal osteoporotic patients and decreased by 27% after a month on alendronic acid treatment, suggesting that it could be used as a marker of bone resorption (Srivastava *et al.*, 2002).

OC concentration should be interpreted with caution. Metabolism into different fragments results in assay specific responses, in patients on vitamin K antagonists (such as warfarin) it undergoes vitamin dependent gamma carboxylation, leading to decreased osteocalcin concentrations (Price *et al.*, 1981). OC is renally excreted therefore of limited value in patients with impaired renal function.

Gundberg *et al.* reported a circadian rhythm in serum OC concentration production. They studied 10 healthy participants (6 men and 4 women) aged between 20-30 years. In 9 of the 10 participants, osteocalcin concentrations followed a standard pattern, being at their nadir in the morning increasing through the afternoon and evening to a peak at night. This pattern did not correlate with inorganic phosphate, ionised or total calcium (Gundberg *et al.*, 1985). This circadian variation highlights the importance of controlling the timing of OC measurements.

OC is particularly susceptible to degradation during freeze-thaw cycles (Gundberg *et al.*, 1985; Yang and Grey, 2006), although Hildebrand *et al.* investigated the effect of four freeze-thaw cycles on the stability of OC and reported no significant changes in its concentration (Hillebrand, Heijboer and Endert, 2016). This effect depends on the assay used and fragment measured.

7.3.6.3 Bone Specific Alkaline Phosphatase

In a healthy human, approximately half of the circulating total alkaline phosphatase (ALP) is produced from osteoblasts, the other half being a produced by in the liver, intestines and kidneys (Watts *et al.*, 2001; Kress *et al.*, 1999). Bone specific alkaline phosphase (BSAP) is a measure of the cellular activity of osteoblasts (although there is cross reactivity with liver enzymes); activity is highest during the early stages of development and declines as other markers such as osteocalcin are upregulated (Golub *et al.*, 1992). BSAP's major function is to inactivate pyrophosphate, a mineralisation inhibitor (Johnson *et al.*, 2000). BSAP has been used in a prospective study in pre- and post-menopausal women to show low radial bone

mineral density (BMD) and rapid bone loss is associated with high concentrations/activity of ALP (Sowers *et al.*, 1992). BSAP correlates with fracture risk in chronic kidney disease patients (CKD) patients (Bhattoa, 2018) and is a prognostic indicator in patients with Paget's disease (Bhattoa, 2018). High concentrations of serum BSAP positively correlated with the successful healing of long bone fractures (Mukhopadhyay *et al.*, 2011).

7.3.7 Analytical Considerations in the Determination of Bone Biomarkers

Blood sampling has become the preferred method of choice for measuring biochemical markers of bone metabolism because urine samples, both spot and 24 hour are less palatable to patient and need to be corrected for creatinine (Bhattoa, 2018). There are both endogenous and exogenous factors that must be considered when interpreting bone marker levels.

7.3.7.1 Age

Bone marker concentrations in blood usually decrease with age, being highest in children due to skeletal growth velocity and rapid bone modelling. Subsequently brief increases are also seen around the time of puberty (Saggese, Baroncelli and Bertelloni, 2002), where serum OC and BSAP are both key markers of high bone metabolism (Yang and Grey, 2006), and in women following the menopause (Jenkins *et al.*, 2013; Seibel, 2005). In men the age related decrease in bone turnover is associated decreasing free sex hormones and IGF concentrations (Fatayerji and Eastell, 1999).

7.3.7.2 Sex

Men have a greater BMD than women in total body and femoral neck ($P \leq 0.001$). There is no difference at the lumbar spine (Henry and Eastell, 2000). Consistent with greater body weights, men have a greater bone area than women at all sites (total body, femoral neck and lumbar spine) ($P \leq 0.0001$) (Henry and Eastell, 2000). Bone turnover markers (BSAP, OC and NTX) are also higher in men than women ($P \leq 0.05$) (Resch *et al.*, 1994; Vanderschueren *et al.*, 1990). In women, oestrogen deficiency is the primary cause of post-menopausal bone loss (Khosla *et al.*, 1997; McKane *et al.*, 1997; Riggs, Khosla and Melton, 1998). Men also have marked age-related decreases in both serum bioavailable testosterone and oestrogen levels

and oestrogen is a consistent independent predictor of BMD (Khosla *et al.*, 1998). The effect of other hormonal changes on bone is discussed below.

7.3.7.3 Female - Pregnancy and Lactation

Bone resorption markers, urine NTX and CTX and serum CTX, TRAP are low in the first trimester of pregnancy, increasing to twice pre-pregnancy levels in the third trimester (Kovacs, 2016), however correcting for serum albumin negates the reduction in CTX in the first trimester (Kaur *et al.*, 2003).

Bone formation marker OC is also low in the first trimester, with limited increases in the third trimester probably because of a dilutionary effect (Kovacs, 2016; Kaur *et al.*, 2003). OC increases post-partum following placental clearance (Rodin *et al.*, 1989).

P1NP and BSAP are also low in the first trimester, there is conflicting evidence about changes that occur as the pregnancy progresses with studies reporting they remain low, return to normal and rise above normal by term (Kovacs, 2016; Black *et al.*, 2000).

On balance, bone turnover appears relatively normal in early pregnancy, but the increases in the third trimester suggest a net resorptive state as foetal demand requires bone resorption from the maternal skeleton in the absence of sufficient intestinal mineral absorption (Kovacs, 2016; Black *et al.*, 2000). This is supported by a randomised trial of women in the second and third trimesters of pregnancy who consumed 2,300mg calcium per day had a 15% lower NTX level compared to women consuming 1,100mg calcium per day (Ettinger *et al.*, 2014).

During lactation bone resorption marker levels (NTX, CTX and TRAP) are increased above those of women in the third trimester and non-pregnant controls. P1NP is also usually increased during lactation compared to women in the third trimester and non-pregnant controls (Kovacs, 2016).

7.3.7.4 Female – Menstrual Cycle

In women, P1NP, BSAP and β-CTX concentrations remain the same through different phases of natural menstrual cycles. P1NP and BSAP concentrations remain the same through different phases on cycles during combined oral contraceptive (COC) use, however β-CTX

concentrations vary across a COC cycle. Nadir β-CTX concentrations occurred after two weeks of pill consumption when endogenous oestrogen is lowest and exogenous oestrogen is highest (Martin *et al.*, 2021). Data were obtained following a single COC preparation (Microgynon™, Bayer, UK) and the effect of other hormonal contraceptives is unknown, although in pre-menopausal women, timing of sample collection within a COC cycle should be considered as a confounding factor when assessing changes in bone metabolism during interventions.

7.3.7.5 Ethnicity

Ethnicity has an impact on bone markers, but the exact differences are not well described. Urinary bone marker of resorption PYD is 30% lower in in black children as compared to their age and sex matched white counterparts (P = 0.031) (Pratt, Manatunga and Peacock, 1996).

A review of studies in African populations assessing 25 (OH) D3 suggest a range levels from deficiency (in the rachitic range) to relatively high values. Within Africa, the health consequences of vitamin D deficiency including rickets and osteomalacia are well reported and there is emerging evidence of increased susceptibility to infectious disease. Compounding factors are thought to be low calcium intake and the burden of infectious disease (which results in increased utilisation of 25 (OH) D3). Underlying calcium nutrition should be considered when interpreting vitamin D status and considering the causes and health consequences in African populations (Prentice *et al.*, 2009).

7.3.7.6 Incapacitation

Bone formation markers (OC, BSAP, and P1NP) do not change significantly during bed rest. Both urinary markers of bone resorption (hydroxyproline, DPD, and N-telopeptide of type I collagen) and a serum marker of bone resorption (CTX) all demonstrated significant increases during bed rest and returned to normal during once normal ambulatory movement is restored (Zerwekh *et al.*, 1998).

7.3.7.7 Concomitant Comorbidities

Patients with concomitant comorbidities such as primary hyperparathyroidism, Paget's disease of bone, multiple myeloma, and metastatic prostate and breast cancer routinely have increased bone markers compared to healthy controls (Costa and Bilezikian, 2013; Brown and Sim, 2010; Cremers and Garnero, 2006; Leeming *et al.*, 2006; Seibel, 2008).

7.3.7.8 Seasonal Variation

Seasonal variation is observed in bone turnover, with a peak in bone remodelling occurring during winter months, though the degree of coupling varies with premenopausal women showing the greatest seasonal variation (Greenblatt, Tsai and Wein, 2017). 25 (OH) D, OC and βCTX demonstrate clear seasonal variation (Woitge *et al.*, 1998; Thiering *et al.*, 2015), where an increase in 25 (OH) D during the summer months, is associated with decreases in βCTX and OC. 25 (OH) D3 usually has highest concentrations in late summer and a nadir in the winter (Woitge *et al.*, 1998). PTH concentrations are also higher in the winter when 25 (OH) D is low (Woitge *et al.*, 1998; Thiering *et al.*, 2015). In females, serum BSAP, urinary PYD and DPD are higher in winter than summer whilst in males, serum total ALP and BSAP are higher in winter than summer, although there was no significant difference in any of the urinary bone markers. All other bone metabolic markers have similar but less distinct variation (Woitge *et al.*, 1998).

7.3.7.9 Lifestyle

Lifestyle habits affect bone metabolism. Woitge *et al* (1998) considered a number of lifestyle factors in multivariate analysis, whilst controlling for weight and height. Women who drink alcohol regularly (more than 2 times per week) and smoke have decreased markers of bone turnover compared to women who do not drink alcohol or smoke. In men, smoking was not found to alter bone markers, but alcohol intake did reduce them (Woitge *et al.*, 1998).

The effect of physical activity categorised as; no regular exercise, 2-hours or less of physical activity per week, more than 2-hours of physical activity per week, on bone markers was evaluated, in both men and women, urinary PYD, DPD and serum OC were higher in the groups reporting little or no regular exercise. There were no statistically significant differences in any other bone markers (Woitge *et al.*, 1998).

Exercise also affects bone markers, as shown following a single bout of exhaustive high impact exercise in 15 young physically active subjects. Blood samples for P1NP and CTX were taken at baseline, immediately after, 2 hours after and on days 1 and 2 following the exercise. CTX was increased on day 2 following the exercise (+32%, P = 0.0015), P1NP also increased but this was not significant (Rantalainen *et al.*, 2009).

Longer term high impact exercise has also been shown to increase bone markers, following the first 14 weeks of initial British Army training, samples were taken immediately prior to the first 'arduous' field exercise (Table 7-2) (Data from British Army 'Adapt' study, unpublished data).

Table 7-2: Mean ± SD of bone markers CTX and P1NP prior to starting and following 14 Weeks of initial British Army training compared to established reference ranges. Unpublished data from British Army study.

Bone Marker	Reference Range	Mean ± SD following prior to starting Initial British Army Training		Mean ± SD following 14 Weeks Initial British Army Training		Units
		Male	Female	Male	Female	
CTX	0.1-0.5	0.6 ± 0.2	0.8 ± 0.3	0.5 ± 0.2	0.7 ± 0.3	microg/L
P1NP	Male: 20-76 Female* *Pre-menopausal:* 30 - 78 *Post-menopausal:* 26 - 110	69 ± 28	50 ± 45	85 ± 29	104 ± 48	microg/L

*All participants were pre-menopausal. Post-menopausal reference range included for completeness only.

7.3.8 Intra-Individual Within Day Variation in Biochemical Bone Markers

C-terminal telopeptide concentration follow a circadian pattern, with a peak in the early morning. The peak to nadir ratio usually averages 1.5. This is mediated by feeding patterns, fasting blunts this circadian pattern (Clowes *et al.*, 2002). Blunting of the circadian rhythm in CTX has also been observed in post-menopausal women with osteoporosis, possibly due to the loss of circadian rhythm in PTH and phosphate that is also associated with osteoporosis in women (Eastell *et al.*, 2001). To minimise this preanalytical variability, CTX should be measured first thing in the morning, after an overnight fast (Shetty *et al.*, 2016).

Bone formation markers such as BSAP, P1NP and OC have longer half-lives than bone resorption markers and exhibit lower amplitudes (Clowes *et al.*, 2002).

The lack of circadian pattern in urinary bone markers is due to urinary voids representing an average over a time period rather than a true single point level. Less frequent sampling or longer pooling would also decrease the within day variation (Clowes *et al.*, 2002).

The diurnal rhythm is the same across the menstrual cycle, suggesting it is unaffected by oestrogen. Small differences the circadian pattern of bone markers are observed between men and women but children demonstrate a similar pattern to adults (Eastell *et al.*, 2001).

7.3.9 Intra-Individual Between Day Variation in Biochemical Markers

Bone turnover and its regulation vary with seasonal changes. Serum 25 (OH) D3 and urinary calcium are higher in summer months conversely PTH concentrations are higher in the winter months (Scharla *et al.*, 1996; Morgan, Rivlin and Davis, 1972). Other serum bone markers tend to exhibit less between day variation than urinary bone markers (Seibel, 2005). PYD has shown between day variation of 16% (range 12-21%) when measured weekly for 5 weeks, similarly DPD has shown between day variation of 17% (range 5-24%) when measured monthly for 5 months (Eastell *et al.*, 2001).

7.4 Effect of Exercise on Bone Metabolism Markers

Vincent and Braith *et al* (2002) examined the effect of 6 months progressive high- or low-intensity resistance exercise on BMD and biochemical markers of bone metabolism in adults aged between 60-83 years. BMD showed a slight significant increase (1.96%) in the high intensity group at the femoral neck, no other significant changes in BMD were seen. Osteocalcin significantly increased in both groups (25.1% and 39% in the low and high intensity groups respectively) (Vincent and Braith, 2002).

Markers of bone metabolism respond to acute bouts of weight-bearing aerobic (Scott *et al.*, 2011) and resistance exercise (Brooke-Wavell, Burns and Stensel, 2007). Rapid increases in P1NP have been reported during exercise, where concentrations increase by 10-31% ($P \leq 0.01$) (Scott *et al.*, 2011). P1NP concentrations return to normal within 30 mins following cessation of the exercise after which there were no further changes. CTX concentrations decreased by 16% following 60 mins of exercise ($P < 0.05$) and continued to decrease (to a max of 40%) during hour 3 of recovery, returning to normal within 24 hours of the exercise after which there were no further changes (Scott *et al.*, 2011). A previous study did show a significant increase in CTX for 4 days following an exhaustive run (Scott *et al.*, 2010). Changes in markers of bone metabolism are influenced by exercise intensity but not by pre-exercise feeding (Scott *et al.*, 2011). Concomitant increases in PTH have been observed, which return to, or below, basal concentrations on cessation of exercise (Scott *et al.*, 2011).

Changes in BMD occur at a slower rate than the changes in bone biomarkers and do not reflect minor changes in bone metabolism unless these occur over a long period of time, usually 1 to 2 years (Bennell *et al.*, 1997; Maïmoun and Sultan, 2011). Therefore, bone turnover markers provide a more sensitive way to detect acute changes in bone turnover than BMD (Maïmoun and Sultan, 2011).

7.5 Effect of Fractures on Bone Biomarkers

Ingle *et al* reported acute changes in bone biomarkers following traumatic forearm and ankle fractures (Ingle *et al.*, 1999a; Ingle *et al.*, 1999b). Significant increases of 11-78 % ($P \leq 0.01$) in biomarkers of formation (P1NP, OC and BSAP) were seen between 1-4 weeks of fracture. P1NP and OC were still increased compared to baseline at 52 weeks. No significant changes in resorption markers were identified. (Ingle *et al.*, 1999a; Ingle *et al.*, 1999b). Changes in bone markers following BSIs have not been reported.

7.6 Bone Strength

Bone mass in humans varies throughout life due to changes in relative rates of bone formation and resorption because of aging and extrinsic factors such as lifestyle. Bone density, mass and geometry can be assessed using a range of techniques, including Dual Energy X-Ray Absorptiometry (DXA), Quantitative Computed Tomography (QCT), High Resolution Peripheral Quantitative Computed Tomography (HRpQCT) and Magnetic Resonance Imaging (MRI) and finite element analysis which estimates bone strength (Banfi *et al.*, 2010).

DXA is the most frequently used bone densitometry technique and is considered the preferred method in the diagnosis of osteoporosis (Blake and Fogelman, 2007). DXA measures volumetric bone mass but lacks sensitivity to detect subtle changes in bone density and bone mineral content. DXA is two dimensional and cannot measure bone geometry, microarchitecture, and the intrinsic properties of the bone matrix. Glüer 1999 reported that because of the measurement coefficient of variation (CV), 18 to 24 months is required between DXA measurements to show any significant change unless large changes in BMD are expected perhaps due to aging or drug interventions (Glüer, 1999). Subsequently better instrumentation and smaller precision errors mean these measurements are now more sensitive and able to detect smaller changes in bone density, however clinical guidance in the UK for monitoring in osteoporosis remains that DXA scanning intervals should be based on a patient's clinical status, baseline BMD, expected change and the least significant change of the measurement. The least significant change is usually reported as a percentage change from baseline and / or the previous scan, it is calculated by the following equation; *2.77 x the coefficient of variance for the scanning equipment (Greenwald, Barajas and White-Greenwald, 2003; Peel and Griffin, 2019)*

DXA measurements have also been used to determine the risk of fracture in the general population—the lower the BMD, the higher the risk of suffering a fracture—which has been demonstrated particularly well in osteoporotic patients (Stewart, Kumar and Reid, 2009).

Software is available to estimate bone structure and regional BMD from DXA, and DXA has been used in other populations to determine bone health in relation to clinical risk of fracture. A study of 626 US Marine Corps showed BMD, cross-sectional areas, section moduli and widths to be significantly smaller at the tibia and femur in fracture cases compared to non-BSI cases (Beck *et al.*, 1996).

7.6.1 Bone Mass and Mechanical Loading

Bone mass increases with exercise and reduces with disuse. 'Loading' has an anabolic effect on the bone (Hughes *et al.*, 2017; Rubin, Rubin and Jacobs, 2006; Turner and Robling, 2004). Mechanical loading experienced by the skeleton can be caused by muscle contractions or the impact and ground reaction forces that result from weight bearing exercise, which can result in strain on the bone and tissue deformation of the matrix. Bone cells are subject to interstitial fluid flow from dynamic pressure changes and shear forces through the canaliculi (Han *et al.*, 2004; Qin *et al.*, 2003; Piekarski and Munro, 1977). The 'Mechanostat Theory' describes how bone remodelling is sensitive to the strain magnitude, number of loading cycles, the distribution of loading and the rate of strain (Frost, 1987). Bone mass increases only result from dynamic loading, the skeleton does not respond to static loads, supported by reduced BMDs of swimmers and cyclists compared to runners (Rubin and Lanyon, 1984). The mechanism by which mechanical loading results in increased BMD is yet to be fully explained (Rubin, Rubin and Jacobs, 2006), it is likely that no one mechanosensor or receptor mechanism is responsible for the response to the mechanical load. Bennell *et al* (1997) investigated bone mass and bone turnover in a longitudinal study amongst track and field athletes exercising 11.3 ± 6.1 hours / week. DXA scans were performed at baseline and at 12 months, the trial concluded that the differences in bone mass in different types of athletes were greatest at the site subject to mechanical loading, for example endurance runners had increased bone mass compared to controls at lower limb sites (Bennell *et al.*, 1997). Lower limb BMD did not change in athletes maintaining baseline fitness, possibly because bones in the lower limbs had already adapted to this level of mechanical loading. Gains in bone mass were attributed to increases in training load or intensity. Likewise, site specific-and regional-specific changes in vBMD and bone size have been demonstrated in military recruits undergoing basic military training. The adaptation threshold has yet to be described. Brahm

et al observed endurance trained athletes training 7 hours per week of running had increased total body and femoral neck BMD but not at the L1-4 vertebrae (Brahm *et al.*, 1997). A subsequent study found a threshold around 80-100 km per week above which lumbar spine BMD lowered by 1% per 10 km / week run (Burrows *et al.*, 2003).

Studies have also shown that sudden increases in training load also have a negative impact on bone health, with BSIs generally following a sudden change in training routine, as is experienced by Army recruits entering Phase 1 training (Duckham *et al.*, 2013; O'Leary, Rice and Greeves, 2021). Bone turnover plays important role in bone repair caused by repetitive loading resulting in 'targeted remodelling'. If loading is constant and/or excessive, accumulation of microdamage can consolidate, resulting in a BSI (Hughes *et al.*, 2021).

7.7 Bone Stress Injuries

Bone Stress Injuries (BSIs) result from repetitive sub maximal mechanical loading of the bone (Cosman *et al.*, 2013) which result in an imbalance. Bone remodelling is unable to keep pace with the demands placed on the bone as resorptive activity outpaces bone formation to an extent that the bone is weakened and liable to fracture. Bone Stress Injuries range from i) periostitis; to ii) periosteal, endosteal and bone tissue oedema; to iii) partial or complete stress fracture (Beck and Drysdale, 2021; Jones *et al.*, 2002; Nattiv *et al.*, 2013).

Bone Stress Injuries are a common and serious overuse bone injury, they were first reported as 'march fractures' in Prussian soldiers in the 19th Century and reported in other military populations in the mid-20th Century (Jones *et al.*, 2002). Repeated weight-bearing activity such as running or marching are the usual reported cause (Jones *et al.*, 1989; DeFroda *et al.*, 2017). Currently the most commonly reported BSIs, in both soldiers and athletes occur in the lower limbs, with tibia being the most commonly reported site (Matheson *et al.*, 1987; Brubaker and James, 1974; Gudas, 1980; Orava, Puranen and Ala-Ketola, 1978; DeFroda *et al.*, 2017). Bone stress injuries vary according to the sport and the intensity of the activity (Bennell *et al.*, 1996c; Bennell *et al.*, 1996b). The most common BSIs involve the lower extremities such as the tibia, metatarsals fibula, femur, and pelvis (Rauh *et al.*, 2006; Sallis and Jones, 1991). A review of BSIs in military populations found the distal tibia accounted for 71 to 85% of all BSIs (DeFroda *et al.*, 2017). The morbidity associated with BSIs can range from minor symptoms, which resolve quickly, to serious lifetime disabilities (Lappe, Stegman and Recker, 2001; Matheson *et al.*, 1987).

7.7.1 Epidemiology of Bone Stress Injuries

Bone Stress Injuries are more common in military personnel – notably recruits – and athletes. These injuries are usually seen in lower extremities with most common locations differing dependant on type of training the sufferer has been undertaking (Bishop *et al.*, 2020).

7.7.1.1 Athletes

The number of BSIs amongst civilian athletes are less well reported than in the military population, the annual incidence within US collegiate track athletes has been reported to be between 1.9 and 21% (Bennell *et al.*, 1996b; Goldberg and Pecora, 1994). More recently a study of collegiate athletes from a range of 25 sports found average BSI rates of 9.13/100,000 in females and 4.44/100,000 in males over the 9-year period of the study (Rizzone *et al.*, 2017) although the rates varied greatly between the different sports. Table 7-3 describes BSI rates of US collegiate athletes.

Table 7-3: Bone stress injury rates of US collegiate athletes across 25 sports. Adapted from Rizzone et al 2017. Data are injury rate per 100,000 athlete exposures (95% CI).

Sport	Injury Rate per 100,000 Athlete Exposures (95% CI)
American Football (men only)	3.01 (2.40, 3.62)
Wrestling (men only)	2.72 (0.71, 4.74)
Field Hockey (women only)	5.91 (2.42, 9.41)
Gymnastics (women only)	25.58 (15.75, 35.41)
Volleyball (women only)	6.03 (4.00, 8.06)
Baseball, Softball	
Men	1.74 (0.83, 2.65)
Women	2.59 (1.28, 3.90)
Basketball	
Men	8.29 (6.37, 10.20)
Women	14.04 (11.41, 16.66)
Cross Country	
Men	16.14 (9.40, 22.89)
Women	28.59 (19.73, 37.45)
Ice Hockey	
Men	0.36 (0.00, 0.86)
Women	0.43 (0.00, 1.28)
Lacrosse	
Men	4.10 (2.09, 6.11)
Women	7.64 (4.45, 10.84)
Soccer	
Men	4.37 (2.80, 5.93)
Women	7.38 (5.47, 9.30)
Swimming and Diving	
Men	0.58 (0.00, 1.71)
Women	1.66 (0.03, 3.30)
Tennis	
Men	1.51 (0.00, 4.47)
Women	9.66 (2.50, 16.81)
Indoor Track	
Men	4.60 (1.60, 7.61)
Women	11.63 (7.07, 16.19)
Outdoor Track	
Men	7.20 (3.29, 11.12)
Women	22.26 (15.18, 29.34)

7.7.1.2 Military

The incidence of BSIs in military personnel is widely reported in the published literature. The incidence of BSIs during 8 weeks of US Army basic training ranges from 0.9% to 5.0% in men (Brudvig, Gudger and Obermeyer, 1983; Jones *et al.*, 1993; Reinker and Ozburne, 1979; Lappe, Stegman and Recker, 2001) and 3.4% to 21.0% in women (Brudvig, Gudger and Obermeyer, 1983; Jones *et al.*, 1993; Reinker and Ozburne, 1979; Lappe, Stegman and Recker, 2001). Bone stress injury incidence in the US Marine Corp over 12 weeks of training is similar to the US Army, ranging from 0.8 to 4.0% in men (Almeida *et al.*, 1999; Beck *et al.*, 1996; Shaffer *et al.*, 1999a; Gardner *et al.*, 1988), and from 3.0% to 5.7 % in women (Beck *et al.*, 2000; Shaffer *et al.*, 1999b). Infantry trainees in the British Army (all males) BSI rates are reported to be 1.4 - 6.4% (Sharma *et al.*, 2015; O'Leary *et al.*, 2020). Women have only recently been permitted to undertake Infantry training in the British Army and numbers entering the training are small, as such BSI rates in women undertaking Infantry training are less well understood. As a comparator, women undertaking British Army officer training are over 5 times more likely to get a BSI than their male counterparts (Incidence; males 1.9%, women 11.4%) (O'Leary *et al.*, 2020). Bone stress injury rates for a range of nations militaries are listed in table 7-4.

Table 7-4: Incidence of bone stress injuries by Nation.

Nation	**Cohort (Duration of Course)**	**Bone Stress Incidence (%)**	**References**
US Army	Basic Training – Men (8 weeks)	0.9-5.0	(Brudvig, Gudger and Obermeyer, 1983; Jones *et al.*, 1993; Reinker and Ozburne, 1979; Lappe, Stegman and Recker, 2001)
	Basic Training – Women (8 weeks)	3.4-21.0	(Brudvig, Gudger and Obermeyer, 1983; Jones *et al.*, 1993; Reinker and Ozburne, 1979; Lappe, Stegman and Recker, 2001)
US Marine Corps	Basic Training - Male (12 weeks)	3.7	(Beck *et al.*, 1996)
	Basic Training – Female (12 weeks)	5.0-7.0	(Shaffer *et al.*, 1999b; Rauh *et al.*, 2006)

Nation	Cohort (Duration of Course)	Bone Stress Incidence (%)	References
British Army	Infantry Training (Phase 1 and 2) – Male (26 weeks)	6.4	(Sharma *et al.*, 2015)
	Infantry Training (Phase 1) – Male (14 weeks)	1.4	(O'Leary *et al.*, 2020)
	Standard Entry Basic Training – Male (14 weeks)	0.5	(O'Leary *et al.*, 2020)
	Officer Training (Phase 1) – Male (44 weeks)	1.9	(O'Leary *et al.*, 2020)
	Officer Training (Phase 1) – Female (44 weeks)	11.4	(O'Leary *et al.*, 2020)
Chinese Army	Infantry Training – Male (8 weeks)	13.5	(Zhao *et al.*, 2016a)
Israeli Army	Basic Infantry Training – Males (14 weeks) (1983 data)	31.0	(Milgrom *et al.*, 1985; Finestone and Milgrom, 2008)
Israeli Army	Basic Infantry Training - Males (14 weeks) (2003 data)	11.6	(Finestone and Milgrom, 2008)
Israeli Defence Force	Combat Warriors – Female (7 month, longitudinal study in trained soldiers)	11.2	(Schwartz *et al.*, 2018)
Israeli Defence Force	Combat Warriors – Male (7 month, longitudinal study in trained soldiers)	2.5	(Schwartz *et al.*, 2018)
Indian Army	Basic Training – Male (26 weeks)	6.9	(Kunte *et al.*, 2017)
Indian Army	Basic Training – Female (26 weeks)	15.8	(Kunte *et al.*, 2017)

7.7.2 Risk Factors for the Development of Bone Stress Injuries

Risk factors for the development of BSIs can be subdivided into intrinsic and extrinsic factors (Table 7-5). Intrinsic risk factors are those directly related to metabolic or anatomic

characteristics. Extrinsic risk factors are external and modifiable and include training habits, equipment, type of sport or activity.

Table 7-5: Risk factors for the development of bone stress injuries adapted from Jones *et al.* (Jones et al., 2002).

Intrinsic Risk Factors	Extrinsic Risk Factors
Demographic Characteristic • Female Sex • Increased Age • Ethnicity Menstrual function / hormonal status Anatomic Factors • High Foot Arches • Genu Valgus ('knock knees') • High Quadriceps ('Q') Angle • Leg Length Discrepancies Bone Characteristics • Lower Cross-Sectional Area • Decreased BMD Physical Fitness • Lower Aerobic Fitness • Lower Muscle Strength and Endurance • Lower Flexibility • Body Composition (low BMI, high lean mass) Health Risk Behaviours • History of Injury	Type of Activity or Sport Physical Training • High Amount Total • High Duration, Frequency, Intensity Equipment • Shoes • Boots • Insoles and Orthotic Inserts Environment (Roads, Trials, Tracks etc). Time of season Alcohol Intake Poor Diet (vitamin D deficiency, inadequate energy intake) Medications (glucocorticoid steroids and NSAIDs) Health Risk Behaviours • Sedentary Lifestyle • Smoking

7.7.2.1 Demographic Factors

7.7.2.1.1 Gender

Female sex is the most identified intrinsic risk factor for BSIs in military personnel. Multiple studies show that women performing the same physical activities as men incur BSIs at 2 to 10 times the rate of men (Brudvig, Gudger and Obermeyer, 1983; Jones *et al.*, 1993; Reinker and Ozburne, 1979; Kunte *et al.*, 2017; Nattiv and Armsey, 1997). The majority of studies in civilian athletes report a higher rate of BSIs in women (Goldberg and Pecora, 1994; Brunet *et al.*, 1990; Wentz *et al.*, 2011), but one study in track athletes found no difference (Bennell *et al.*, 1996b). This increased risk of bone stress injuries in women is caused by relatively higher physical activity levels and lower body mass indices which pre-dispose them to periods of low energy availability and insufficient nutrition (Chen, Tenforde and Fredericson, 2013; Wentz *et al.*, 2011; Wentz *et al.*, 2016). Over time, this energy deficit may lead to menstrual and hormonal irregularities that impair bone health and bone density (Wentz *et al.*, 2011; Wentz *et al.*, 2016; Chen, Tenforde and Fredericson, 2013; Zeni *et al.*, 2000). Smaller bone geometry and reduced muscle strength compared to males also places women at higher risk of BSI due to the additional relative stresses placed on bone by applied loads, or the reduced ability of the bone to resist deformation when loads are applied (Wentz *et al.*, 2011; Diehl, Best and Kaeding, 2006; Chen, Tenforde and Fredericson, 2013).

Amenorrhoea and irregular periods are associated with a greater risk of BSIs in women; a retrospective study of 207 found the BSI rate to be 3.3 times (95% CI 1.2-9.3) higher in female collegiate athletes with a history of menstrual irregularities compared to those with regular periods (Lloyd *et al.*, 1986). These findings are supported by survey of 241 female distance runners also collegiate athletes who had between 1.3 and 1.7 increased risk of BSI: these results were stratified by how irregular the menses were assessed to be (Barrow and Saha, 1988). The reliability of this report is limited by the low response rate and limited descriptions of methods used to make their conclusions (Barrow and Saha, 1988). The US Army conducted a survey of 1630 women and showed that those with a history of amenorrhoea greater than six months made them more likely to have at least one BSI in their lifetime (Friedl *et al.*, 1992). The 'Female Athlete Triad' has been used to describe the effect of amenorrhea on bone health, which describes the process whereby a female athletes' BMD can be measured over

time to reflect her cumulative history of energy availability and menstrual status as her BMD changes within the framework (Nattiv *et al.*, 2007). This concept was developed and the 'Relative Energy Deficiency in Sport' (RED-S) syndrome paradigm published, this takes account of the more complex interplay between, among others, metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular health that leads to impaired physiological function caused by relative energy deficiency. It also considers that male athletes can be affected and there is a psychological component which can either precede or result from RED-S. A full list of physiological effects of RED-S is described in figure 7-8a. This syndrome is caused when energy expenditure required for health, activities of daily living, growth and sporting activities are not met by energy intake a comprehensive list is described in figure 7-8b. The RED-S paradigm is seen in military personnel, particularly during combat training and field exercises although the multi-stressor environment makes it difficult to identify the size of the effect. The high energy expenditures in these environments accompanied by reduced energy intake impair several of the measures of health and performance in RED-S and studies supplementing energy to minimise the deficiency do suggest an independent effect of energy deficiency in metabolic, endocrine, immune disturbances and physical performance (O'Leary, Wardle and Greeves, 2020).

7.7.2.1.2 Age

Increasing age has also been identified as a risk factor for BSI in a large-scale study involving 15,994 male and 4,428 female Army trainees amongst whom 339 BSIs were radiologically diagnosed in 295 trainees (Brudvig, Gudger and Obermeyer, 1983). The average age of male trainees diagnosed with a BSI was 20.6 ± 4.5 years whereas the average age of females 22.5 ± 3.5 years. Bone stress injury incidence increased with increasing age between 17 and 34 years (Brudvig, Gudger and Obermeyer, 1983). The rate of incidence of BSIs in age categories are described in table 7-6.

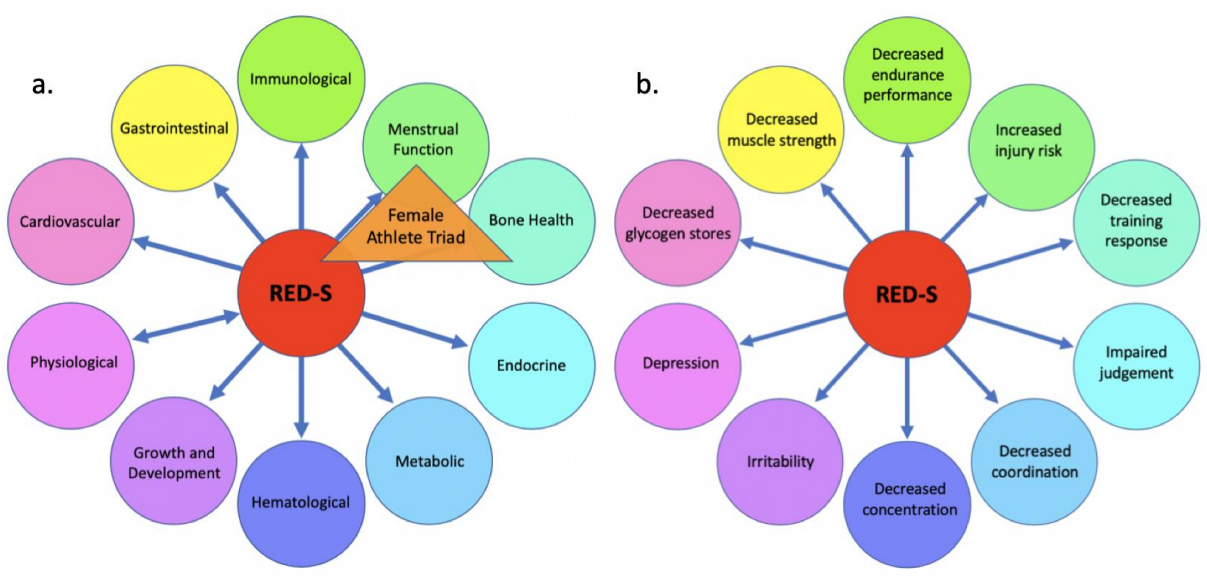


Figure 7-8: Health consequences of relative energy deficiency in sport syndrome (RED-S) (7-8a). The RED-S framework expands on the concept of the female athlete triad to include a wider range of outcomes and potential for male athletes to be affected. Potential performance effects of relative energy deficiency in sport (7-8b).

Table 7-6: Rate of incidence of bone stress injuries in different age groups. Adapted from (Brudvig, Gudger and Obermeyer, 1983).

Age (years)	**17-22**		**23-28**		**29-34**		**35 and over**	
Rate of incidence	1.27		2.32		5.01		2.36*	
Rate of incidence	Male 0.88	Female 2.73	Male 1.30	Female 4.70	Male 2.95	Female 8.18	Male 0.00	Female 7.69

*Non-significant finding due to the small number of trainees in this category.

A study in US Marines undergoing 12 weeks basic training showed similar results; in a cohort of 3,025 male recruits, men over the age of 21 had a BSI rate 1.7 times higher (95% CI 0.92-3.21, *p* value not reported) than those under the age of 21 (Gardner *et al.*, 1988). Conversely a newer study reported soldiers under the age of 20 were more likely to suffer a BSI compared to those aged 20 – 30 years (male: 5.98 [5.69, 6.28]; female: 4.71 [4.47, 4.96]), and soldiers over the age of 30 were less likely to suffer a BSI than those aged 20 – 30 years (male: 0.39 [0.37, 0.42]; female: 0.39 [0.37, 0.42]) (Bulathsinhala *et al.*, 2017). These results may differ as Bulathsinhala *et al,* (2017) included 1.3 million US army soldiers across their entire careers rather than specifically focussing on recruits in training. More BSIs occur during initial military

training than in other phases of a military career due to the sudden onset and increase in training load undertaken. Recruits undergoing initial military training are at the beginning of their military careers and so younger than the general military population. These factors are likely to bias the results of a whole population analysis such as Bulathsinhala *et al*, (2017) and account for the difference between this and earlier studies.

7.7.2.1.3 Ethnicity

Ethnicity has also been indicated as a risk factor for BSI. During US Army basic training, the incidence of BSIs was higher for white and Hispanic recruits (rates = 1.6-10.8%), followed by Asian recruits (rate = 5.8%) with the lowest rates in African American recruits (rates = - 0.7-4.9%) (Gardner *et al.*, 1988; Lappe, Stegman and Recker, 2001; Brudvig, Gudger and Obermeyer, 1983; Bulathsinhala *et al.*, 2017). These differences in BSI risk are attributed to differences in bone mass microarchitecture and strength (Pepper, Akuthota and McCarty, 2006). Tibial low aBMD, vBMD and cortical area are associated with increased risk of BSI (Tenforde, Kraus and Fredericson, 2016; Beck *et al.*, 2000; Jepsen *et al.*, 2013; Davey *et al.*, 2015) and studies in black individuals have routinely demonstrated higher BMD and more favourable bone microarchitecture (Putman *et al.*, 2013; Misra *et al.*, 2017). The 'Bone Mineral Density on Childhood' study examined healthy children between the ages of 5 and 20 years and found that BMD is higher at all skeletal sites in black compared to non-black children and young adults (Zemel *et al.*, 2011). pQCT data shows black and Hispanic children have 10-37% higher estimated bone strength at the tibia and radius compared to white children as a result of increased cortical BMD (2 – 5%) and bone area (7-18%) (Wetzsteon *et al.*, 2009). HRpQCT data confirms these findings in black and Asian adolescents who have more favourable bone microarchitecture at distal sites of the radius and tibia compared to non- Hispanic whites (Misra *et al.*, 2017; Kim *et al.*, 2013; Wang *et al.*, 2009).

Differences in bone metabolism may also contribute to ethnic differences in BSI. Low 25(OH) D3 is associated with increased risk of BSI (Lappe *et al.*, 2008; Burgi *et al.*, 2011; Ruohola *et al.*, 2006) yet 25(OH) D3 is usually lower in black men and women compared to white men and women. This is seen in both the general population (Kleerekoper *et al.*, 1994; Ginde, Liu and Camargo, 2009; Mitchell *et al.*, 2012; Nielson *et al.*, 2016) and in military recruits (Andersen *et al.*, 2010; Lutz *et al.*, 2012). During initial military training, 25(OH) D3

concentrations decrease in white and Hispanic recruits but are either maintained or increased in black recruits while PTH concentrations increase approximately 30% independently of ethnicity (Lutz *et al.*, 2012; Andersen *et al.*, 2010). Black women are reported to have lower skeletal sensitivity to the resorptive action of PTH (Cosman *et al.*, 1997) which may be contributing to the resistance to bone resorption whilst PTH is increased during the intense physical training period.

Conversely, Shaffer *et al* conducted an analysis of BSIs in 1,296 male US Marines undergoing an 11-week basic training programme, controlling for age, physical fitness, and physical activity levels. The authors reported no significant difference in BSI risk between Caucasian and non-Caucasian groups (relative risk 1.04, 95% CI 0.58-1.88) (Shaffer *et al.*, 1999a). This finding may be a result of the smaller sample size, which was the smallest of all the studies considering ethnicity.

A survey of female collegiate distance runners found no significant differences in BSI incidence between athletes of different ethnicities (Barrow and Saha, 1988). Studies in Korean and Japanese athletes have shown increased BSI risk when compared to white populations, but these studies did not control for activity type (Iwamoto and Takeda, 2003; Ohta-Fukushima *et al.*, 2002; Ha *et al.*, 1991).

7.7.2.2 History of Bone Stress Injury

A history of BSI has been consistently associated with increased risk of future BSI (Kelsey *et al.*, 2007; Nattiv *et al.*, 2013; Nattiv *et al.*, 2007).

7.7.2.3 Genetics

Genetic pre-disposition to BSIs is supported by a case study of monozygotic twins, with multiple BSIs at the same anatomic sites after 6 weeks of Army training (Singer *et al.*, 1990). Studies comparing twins and immediate families found that differences in bone size, shape and BMD result from genetic rather than environmental influences (Nguyen and Eisman, 2000) and a Finnish military study identified genetic patterns in eight genes, involved in bone metabolism and pathology associated with increased risk of BSI (Korvala *et al.*, 2010). More recently, a study considering both military recruits in the Israeli Defence Force and elite

athletes found that two functional polymorphisms within the P2X7R gene are independently associated with BSI injury (Varley *et al.*, 2016).

7.7.2.4 Anatomic Factors

Foot morphology has been identified as a potential indicator of BSI risk, but the evidence is inconclusive. One study in the Israeli Defence Force suggested high foot arches are associated with a higher risk of BSI, but two further studies in the US Naval Special Welfare Training Centre found no significant correlation between foot morphology and BSI (Jones *et al.*, 2002; Kaufman *et al.*, 1999; Montgomery *et al.*, 1989). This difference may be accounted for by the different types of footwear issued to recruits in the different studies, which are not described in any of the reports. Boot selection has been identified as an extrinsic risk of BSI (Jones *et al.*, 2002; Bensel and Kish, 1983)

Leg length discrepancy has been associated with increased BSI risk (Brunet *et al.*, 1990; Bennell *et al.*, 1996a; Korpelainen *et al.*, 2001; Friberg, 1982). This finding is not supported by two further military studies which found no correlation (Pepper, Akuthota and McCarty, 2006; Cowan *et al.*, 1996). The reason for the inconsistency is not clear.

Valgus knee alignment and increased quadricep angle are risk factors for lower leg BSIs (Cowan *et al.*, 1996; Finestone *et al.*, 1991). In a prospective study of 294 male infantry trainees risk of lower extremity overuse injuries was increased by valgus knee alignment (RR = 1.9) and increased quadricep angle (RR = 5.4) (Cowan *et al.*, 1996). These findings have not been reproduced in more recent studies investigating injury in runners (Hespanhol Junior *et al.*, 2016; Christopher *et al.*, 2019). This may be because infantry trainees are a more homogenous group, following a standardised training programme which is more intense compared to the training undertaken by 'recreational runners' included in the later studies. This makes the causes of injury easier to elucidate.

7.7.2.5 Bone Characteristics

The ability of bone to resist external loads applied during weight bearing activities depends on several factors including bone geometry and bone material properties (Crossley *et al.*, 1999). Bone characteristics have been investigated as a contributing factor for BSIs. Beck *et*

al investigated 626 male Marine recruits during 12 weeks of basic training. 3.7% (n=23) developed BSIs, the mean values for the cross-sectional area, the section modulus, and the width of the tibia were significantly lower in the trainees who developed BSIs than those who did not. A further prospective study of 286 male recruits within the Israeli Defence Force reported a BSI incidence of 31% (Milgrom *et al.*, 1985), biomechanical investigation identified the tibial, femoral and total BSIs to be significantly lower in the low- anterior to posterior axis of the cross-sectional moment of inertia (CSMI) subgroup. The CSMI is a property of a bone cross-sectional area that represents the magnitude of the greatest bending rigidity of the section (cm^4). CSMI is derived from the integral of the bone mass profile across the bone together with its center of mass. Controlling for height and weight did not change this finding (Milgrom *et al.*, 1985; Milgrom *et al.*, 1988).

The relationship between BSI risk, BMD and bone width has been investigated in several studies. A prospective study of 693 female US Marine recruits reported a 5.3% BSI incidence, with the mean BMD and cortical bone thickness of the tibia being significantly lower in those who suffered a BSI than those who did not. Similar results were reported in male US Marine recruits (Beck *et al.*, 2000) and Israeli Defence force soldiers (Giladi *et al.*, 1987). A subgroup analysis reported that the low tibia width prior to basic training was associated with increased risk of BSIs however bone density was not (Giladi *et al.*, 1991).

7.7.2.6 Physical Fitness

Multiple studies in the military populations have shown significant associations between low aerobic fitness levels and increased risk of BSI (Jones *et al.*, 1993; Shaffer *et al.*, 1999a). In 1,078 male US Marine recruits, lower aerobic fitness (measured by 1.5 mile run time) was associated with higher incidence of BSI (Shaffer *et al.*, 1999a). The same was shown in a female cohort, where a 1 mile run time of greater than 9.75 mins was associated with an increased risk of BSI (Jones *et al.*, 1993). Conversely, a study in the Israeli Defence Force reported no significant correlation between aerobic fitness (measured by the sub maximal bicycle test) and BSI rates (Giladi *et al.*, 1991; Swissa *et al.*, 1989). These contrasting findings may be due to using a bicycle measure for fitness, while all the other studies used run times and inclusion of asymptomatic fractures in the Israeli Defence Force.

7.7.2.7 Health Behaviours and Medical History

Sedentary lifestyle prior to joining the Military has been associated with an increased risk of BSIs. Gardner *et al* reported a survey of 3,025 Marine recruits relating to their past health and health behaviours, rating their previous physical activity using five categories between inactive and very active. The study reported previously inactive trainees were 10 times more likely than the average of the other four activity categories to suffer a BSI. Previously inactive or below average activity levels had a relative risk of fracture of 2.40 (95% CI 4.58, 1.26) (Gardner *et al.*, 1988). Shaffer *et al* also showed that the more physical activity, particularly running, a recruit undertakes prior to commencing basic training the less likely they are to suffer a BSI (Shaffer *et al.*, 1999a). This protective effect has also been reported in collegiate athletes, amongst whom over a three-year period 67% of BSIs occurred among freshmen with 7% in sophomores, 9% in juniors and 7% in seniors[1] (Goldberg and Pecora, 1994).

In a survey of 915 female US Army recruits those who smoked one or more cigarettes in total in the month prior to starting their 8-week basic training programme developed more BSIs than those who did not (RR = 2.2 95% CI 1.4-3.6) when demographics, physical fitness and health variables were controlled for (Altarac *et al.*, 2000). The same survey in 1,087 male recruits also found smoking to be associated with a higher risk of fracture (RR = 1.4 95% CI 0.7-2.9) (Altarac *et al.*, 2000). Friedl *et al*. also found smokers to have an increased likelihood of BSIs in 1630 female recruits (RR = 1.7 95% CI 1.2-2.1)(Friedl *et al.*, 1992).

7.7.2.8 Type of Sport or Activity

Goldberg *et al.* quantified BSI incidence amongst collegiate athletes and reported the highest incidence in softball (6.3 %) and track athletes (3.7%). The lowest incidence was amongst rowers and hockey players (both 2.2%). Military studies have indicated that different units and different training regimes are associated with different levels of BSI risk; for example, of 120 Finnish male military recruits, the incidence of BSIs was highest in paratroopers compared with regular or light role infantry recruits (Kuusela, 1984).

[1] In the US College System years are named, in order, Freshman, Sophomore, Junior, and Senior.

7.7.2.9 Medication Use

Hormonal contraception affects bone health. A study performed in 45 female British Army recruits comparing no hormonal contraception use, the combined oral contraceptive pill (COCP) and the depo-medroxyprogesterone acetate (DMPA) injections showed no difference in aBMD, but a higher tibial speed of sound in women taking no hormonal contraception compared to the DMPA injection (P = 0.014). CTX was higher in the women taking no hormonal contraception (P = 0.037) and the DMPA injections (P = 0.003) compared to COCP users. P1NP was higher in DMPA injections compared with both no hormonal contraception (P = 0.045) and COCP users (P = 0.014). BSAP was higher in DMPA injections users than COCP users (P = 0.044) (Coombs *et al.*, 2021).

The relationship between oral contraceptive pills (OCPs) and BSIs is unclear. A number of studies have reported a protective effect (Barrow and Saha, 1988; Bennell *et al.*, 1995; Myburgh *et al.*, 1990; Jones *et al.*, 2002) while others have reported no association (Tenforde *et al.*, 2013; Wright *et al.*, 2015; Cline, Jansen and Melby, 1998; Kelsey *et al.*, 2007; Bennell *et al.*, 1996a; Shaffer *et al.*, 2006; Cobb *et al.*, 2007). The reason for the inconsistency in results is not clear but this may be due to studies not differentiating between OCP's in terms of estrogenicity, progesterone potency and androgenicity, as a number of the studies did not specify any more detail other than 'OCP use'. OCP's can also mask menstrual irregularities which in themselves are a risk factor for BSI and this may contribute to lack of association in some patients (Cheng *et al.*, 2021). Depo medroxyprogesterone injections have shown to reduce BMD (Berenson *et al.*, 2001) and increase BSI risk (Lappe, Stegman and Recker, 2001).

Long-term oral corticosteroid use is associated with an increased risk of fractures within the first 2-3 months of treatment and BMD reductions of 5-15% in the first year, both of which are reversible once the corticosteroid is stopped (Briot and Roux, 2015). Short-term use has also been associated with an increased risk of fracture in the Military but minimum duration of treatment and reversibility remains unclear (Lappe, Stegman and Recker, 2001).

Non-steroidal anti-inflammatory drugs (NSAID's) reduce adaptive bone formation responses to mechanical loading and weight bearing exercise programmes (Jankowski *et al.*, 2015; Kohrt *et al.*, 2010; Duff *et al.*, 2016; Duff *et al.*, 2017; Brewer *et al.*, 2015). These effects have been

reported in both military and athlete populations (Bulathsinhala *et al.*, 2017; Hughes *et al.*, 2019; Warden, 2010). There is also evidence that NSAIDs impair BSI healing (Aisa *et al.*, 2018; Wheeler and Batt, 2005) although further study is required before treatment recommendations could be made (Staab *et al.*, 2021).

Other medications associated with an increased risk of fracture are levothyroxine (Pepper, Akuthota and McCarty, 2006), antiepileptics (Pack, 2003), antidepressants (Rizzoli *et al.*, 2012), aluminium containing antacids (Spencer and Kramer, 1985) and proton pump inhibitors (Targownik *et al.*, 2008). Each of these has a different mode of action contributing to the risk of BSI.

7.7.3 Prevention of Bone Stress Injuries

The majority of evidence supporting interventions to prevent BSIs are in military populations that focus on two lines of investigation: modifying training regimes and modifying footwear.

7.7.3.1 Strategies to Promote Adaptive Bone Formation

Mechanical loading can cause microdamage to bone and potentiate BSIs (Beck *et al.*, 2000). Osteogenic exercise (exercise which is unaccustomed, dynamic, high-impact, multidirectional, intermittent, and includes extended rest periods) can be used to promote adaptive bone formation and build bone whilst minimising the risk of BSIs (Hughes *et al.*, 2021). The bone adaptations can take up to 12 months to mineralise (Fuchs *et al.*, 2008).

One strategy to prevent BSI is to initiate long term progressive training programmes well in advance of activities with a high risk of BSI such as initial military training (Hughes *et al.*, 2021). Bone mechanosensitivity is at its peak in early adolescence and increasing physical activity during this period promotes skeletal health in later life (Hughes *et al.*, 2021). Intense periods of physical activity in later life also prompt adaptive bone formation including initial military training (Rice *et al.*, 2018; Hughes, Dickin and Wang, 2019) although the activities are usually unique to the military and prior training is often insufficient hence the increased risk of BSI (Hughes *et al.*, 2021). During intense periods of exercise, adaptive bone formation can be aided by adequate sleep, vitamin D, calcium, energy availability and avoidance of drugs such as non-steroidal anti-inflammatories which supress adaptive bone formation (Hughes *et al.*,

2021). Figure 7-9 describes strategies to promote adaptive bone formation and prevent BSIs in military personnel (Hughes *et al.*, 2021).

7.7.3.2 Modifying Training to Prevent Bone Stress Injuries

Bone stress injuries in military personnel are typically reported in recruits undertaking basic military training rather than in trained personnel suggesting that bone adapts to the requirements of military training and load carrying (Finestone and Milgrom, 2008; Hughes *et al.*, 2017; Hughes *et al.*, 2021).

Two studies have investigated the effect of rest periods following weight bearing training in early weeks of Army Basic Training (Scully and Besterman, 1982; Popovich *et al.*, 2000). Scully and Besterman divided 880 male US Army trainees into two groups. Normal training was compared with training with a recovery week (no running, marching or jumping) in the third of the 8-week training programme. Trainees on the training programme with the recovery week had an absolute reduction in BSI incidence of 3.2%.

Ben-Sasson *et al* studied the effect of sleep deprivation on Israeli infantry recruits to investigate the influence of gravitational forces on bone metabolism as sleep deprivation is common during military training. There were three arms to the study; (1) soldiers who were sleep deprived for 63 hours, (2) soldiers who slept in a vertical position for 6 hours for three consecutive nights; (3) soldiers who slept 6 hours a night horizontally for three consecutive nights. In the sleep deprived arm, urinary calcium excretion increased by 170%, those who slept in the vertical position showed an increase of 68% and the recruits who slept horizontally showed no change in urinary calcium compared to their basal level. A comparison of pre- and post- test BMD showed reduced BMD in the sleep deprived arm suggesting a pre-disposition to bone resorption in response to changes in gravitational forces (Ben-Sasson *et al.*, 1994). Circadian rhythm changes resulting from sleep changes may have been a compounding factor, but these were not measured in this study.

Bone stress injuries are also observed in racehorses, where increased galloping distances were associated with a higher incidence of injury. Nunamaker *et al* identified that intense exercise reduced bone remodelling and rest stimulated it in a horse model, when training was

modified to include more short periods of fast running it reduced BSI rates (Nunamaker, Butterweck and Provost, 1990).

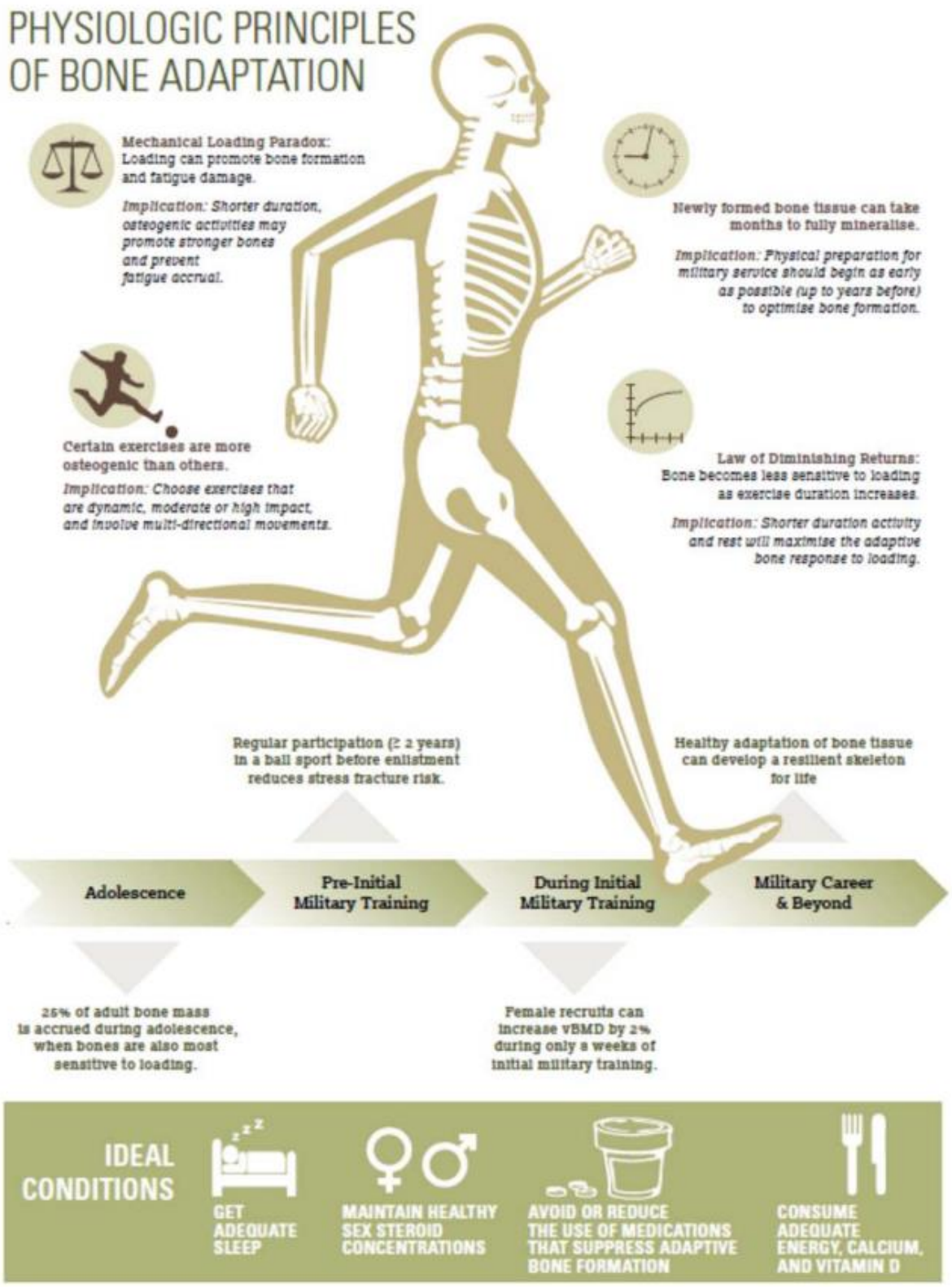


Figure 7-9: Strategies to promote adaptive bone formation and prevent bone stress injuries in military personnel. Reproduced with permission from Hughes *et al*., 2021.

7.7.4 Diagnosis of Bone Stress Injuries

Radiological diagnosis of bone stress injuries (BSI's) may be determined by plain film radiographs, magnetic resonance imaging (MRI), computed tomography (CT) or bone scintigraphy. MRI has been described as the gold standard technique for BSI diagnosis (Schneiders *et al.*, 2012), and is used by the British military.

Whilst all imaging modalities are in clinical use, studies comparing their efficacy are limited. Computed tomography and MRI are able to detect BSI-related lesions in bones (Gaeta *et al.*, 2005). Both CT and MRI are more sensitive than plain film radiography to detect BSIs (Gaeta *et al.*, 2013; Spitz and Newberg, 2002; Daffner and Pavlov, 1992; Greaney *et al.*, 1983). MRI detects periosteal and endosteal marrow oedema, which are markers for BSI with both T2-weighted and STIR images demonstrating good reliability for detecting the presence of cortical stress injury (Shearman *et al.*, 1998; Feydy *et al.*, 1998; Fredericson *et al.*, 1995).

Trabecular BSI's result in non-specific bone marrow changes, again these injuries are detected with both T2- weighted and STIR images as increased marrow signal intensity (Anderson and Greenspan, 1996). These changes correspond to microfractures that cause oedema and haemorrhage. Plain film radiographs and CT do not detect these changes.

Bone scintigraphy has a higher sensitivity but lower specificity to detect BSI's compared to MRI and whilst some consider it the reference standard for evaluating BSI's (Pozderac, 2002; Ammann *et al.*, 1988; Roub *et al.*, 1979), others have described the modalities failure to identify BSIs (Milgrom *et al.*, 1988; Keene and Lash, 1992; Sterling *et al.*, 1993; Bal and Sandow, 1996). In one case series, bone scintigraphy failed to depict eight cortical stress injuries identified by MRI, CT or both (Gaeta *et al.*, 2013). This lack of sensitivity may be due to the lack of significant osteoblastic response during the early stages of a BSI, resulting in a lack of uptake of the radionucleotide. Consequently, MRI is now considered more sensitive than bone scintigraphy.

Both CT and MRI identify early changes associated with BSI's, MRI is superior to CT when assessing early BSIs due to its sensitivity to detect these injuries. CT may be used in preference in the detection of osteopenia, the earliest sign of fatigue damage to the bone, but this is transient and as BSIs develop, MRI becomes more sensitive. MRI also has the advantage that it is the only modality for which the validated grading classification has been found to be significantly related to the clinical severity of BSIs (Fredericson *et al.*, 1995; Beck *et al.*, 2012).

CT scans expose patients to ionising radiation, which increases the risk of cancer in later life and birth defects in unborn children. This is a consideration when deciding the most appropriate scan modality for patients as MRI does not carry this risk.

Other radiographic modalities discussed in this thesis include DEXA and HRpQCT, however these are of no value in the diagnosis of BSI's. DXA provides a more general measure of bone health but does not have sufficient resolution to detect and grade BSIs. HRpQCT can only measure very specific distal bone sites. To capture a BSI using HRpQCT, the injury would have to be in a very specific location on the bone so would only be applicable to a very small cohort of patients.

7.7.5 Classification of Bone Stress Injuries

Bone stress injuries may be classified as being at high or low risk of non-union, those which are high risk are in zones of high tension or have poor blood supply. Low risk fractures are in regions of good blood supply and along lines of compression. The classification of fractures by site are described in Table 7-7 (Boden, Osbahr and Jimenez, 2001).

Table 7-7: Classification of bone stress injuries by site as high risk and low risk. Adapted from Boden et al 2001.

Low Risk Bone Stress Injuries	High Risk Bone Stress Injuries
Femoral neck of the medial cortex	Femoral neck fractures of the superior cortex
Tibial shaft fractures of the posteromedial cortex	Tibial shaft fractures of the anterior cortex
Fractures of the distal second to fifth metatarsals	Fifth metatarsal, at the diaphyseal-metaphyseal junction
Calcaneal fractures	Navicular fractures
Fractures of the fibula	Proximal fractures of the second metatarsal

Fractures of the pubic ramus	Fractures of the talus
Cuboid fractures	Fractures of the medial malleolus
Cuneiform fractures	Sesamoid

Treatment for BSIs is dependent on the site and grade of the fracture. Most BSIs are managed non-operatively by off-loading and stopping the causative activity (Tuan, Wu and Sennett, 2004). The general principle of managing low risk BSIs is to slowly increase the impact loading once ambulation and day-to-day activity is pain free, this resumption of activity depends on the individual and should be modified dependant on symptoms (Brukner, Bradshaw and Bennell, 1998). Treatment of high risk fractures are treated with more aggressive non-weight bearing, casting and / or bone simulation for extended periods (Brewer and Gregory, 2012). The evidence for the use of pneumatic braces in tibial BSIs is mixed; whilst they may reduce the time to healing the benefit is limited due to the discomfort and inconvenience, indicated in trials of athletes and military personnel (Allen *et al.*, 2004; Moen *et al.*, 2010; Patel, Roth and Kapil, 2011; Swenson *et al.*, 1997).

Classification scales for BSI grading have been developed over several years. Zwas *et al.* developed the first comprehensive scintigraphic classification based on bone dimension, bone extension and tracer concentration in the lesions ranging from 'early and mild' (Grade 1) to 'advanced and severe' (Grade 4) (Zwas, Elkanovitch and Frank, 1987). More recently, studies have shown that whilst scintigraphy may be reliable for the detection of stress injuries, this method lacks specificity (Dobrindt *et al.*, 2012; Gaeta *et al.*, 2005). Gaeta *et al.* reviewed 50 patients with tibial BSIs by bone scintigraphy, computed tomography (CT) and MRI. The different modalities were shown to have a sensitivity of 42%, 74%, and 88% respectively for the identification of BSIs. MR imaging had specificity of 100% and an accuracy of 90% (Gaeta *et al.*, 2005). This study also showed MRI to be significantly more effective than both CT and bone scintigraphy in the early detection of BSIs (McNemar test; $P \leq 0.001$ and $P = 0.008$, respectively). Consequently, MRI has been established as the preferred tool to assess diagnosis of stress injuries and fractures.

Classification and quantification of the severity of BSIs are important for providing appropriate and effective treatment recommendations. Fredericson *et al.* developed a classification and adapted a method to identify any correlation between clinical symptoms

and severity of stress injury (Fredericson *et al.*, 1995). Table 7-8 describes the criteria observed on MRI scan for each of the grades on the scale developed by Fredericson *et al.*

Table 7-8: Grading criteria for bone stress injuries on MRI scan. Adapted from Fredericson et al., 1995.

Grade	**Description**
Grade 0	No appreciable lesions of the bone.
Grade 1	Mild – moderate periosteal oedema only.
Grade 2	Mild - moderate periosteal oedema and possible bone marrow oedema.
Grade 3	Moderate – severe oedema of both the periosteum and marrow.
Grade 4	Moderate – severe oedema of both the periosteum and marrow. A low signal fracture line would be visible only in grade 4 bone stress injuries.

The severity of the fracture influences the healing time, but this is also dependent on the location. DeFroda *et al.* proposed a predictor of BSI healing times following a review of studies of military and civilian athlete BSI sufferers by location (DeFroda *et al.*, 2017) outlined in table 7-9.

Table 7-9: Prediction of fracture healing time by fracture site proposed by DeFroda et al. 2017.

Site	**Healing Time**	**Comments**
Pelvis	8 – 12 weeks	Non- operative treatment, main treatment activity modification.
Femur	12 – 16 weeks (to light activity)	Femoral shaft more common in military populations.
Tibia	3 –11 weeks (rising to 4 months if surgery required)	
Metatarsals	6 weeks	

7.7.6 Histology and Mechanism of Healing of Bone Stress Injuries

Bone stress injury healing is not well described. Bone modelling and bone remodelling processes play a role in the occurrence and healing of bone stress injuries. Whilst bone modelling is primarily associated with growth during childhood and adolescence, it can also occur as a compensatory response to bone stress injuries as a means of stabilising the injury site (Shim, 2015). Healing may begin by reinforcing the bone with a callus in response to a periosteal inflammatory reaction (Warden, Edwards and Willy, 2021).

Concurrently or subsequently, bone remodelling takes place along the fracture line. Bone remodelling is characterised by the temporally and spatially coordinated actions of osteoclasts and osteoblasts acting sequentially on the same surface, unlike modelling, remodelling occurs continuously throughout life but does not typically affect bone size and shape (Zaidi, 2007). Following microdamage formation, targeted remodelling removes and replaces specific packets of bone (Burr *et al.*, 1985; Burr and Martin, 1993). Microdamage induces apoptosis of osteocytes in the bone matrix (Verborgt, Gibson and Schaffler, 2000), this alters the RANKL/OPG ration in favour of RANKL resulting in bone resorption (Parfitt, 2002; O'Brien, Taylor and Clive Lee, 2005) and removal of the microdamage. Subsequently,

layers of osteoid are deposited on the newly exposed bone surface by osteoblasts. Some osteoblasts are embedded in the osteoid to become osteocytes and the newly exposed bone surface is mineralised over time. In athletic populations remodelling is difficult to detect before the formation of a complete stress fracture (Winters *et al.*, 2019). Biopsy samples of bone with chronic tibial BSI's have shown the fracture line to be filled with low-density bone undergoing intense remodelling, rather than a void filled with unmineralized tissue (Schilcher, Bernhardsson and Aspenberg, 2019). There is also a suggestion that the healing mechanism is specific to the type of bone (cortical or trabecular) and the stage of bone injury, with microfractures being repaired by classical fracture healing (inflammation, callus formation and remodelling) and microcracks being repaired by targeted bone remodelling (Avin *et al.*, 2015; Hoenig *et al.*, 2022).

7.7.7 Assessing Fracture Healing

There is no 'gold standard' technique for assessing fracture healing (Cook *et al.*, 2015; Fisher *et al.*, 2018). Corrales *et al.* confirmed this lack of consensus; they conducted a systematic review of assessment of fracture healing in orthopaedic trauma trials published in three journals: The Journal of Bone and Joint Surgery (American Volume), The Journal of Bone and Joint Surgery (British Volume) and the Journal of Orthopaedic Trauma, over an 11- year period. 62 % of trials used a composite measure of clinical and radiographic healing, 37 % used radiographic outcome only, and 1 % used purely clinical criteria. There were 12 different clinical criteria used in the trials, the most common being the absence of pain or tenderness at the fracture site during weight bearing exercise, and no validated method of clinical assessment was identified. Lack of standardisation was also identified among radiological assessment of healing, even among the studies using a plain film; 11 different criteria for confirmation of healing were identified (Corrales *et al.*, 2008). A patient's own assessment of restoration of function and pain level is increasingly becoming a popular approach in the management and assessment of fracture healing (Beaton and Schemitsch, 2003).

Different components of fracture healing such as radiological imaging and pain do not always align. Patients' age, occupation, function level and biochemical demands on the bone (a weight bearing bone such as a femur requires a higher level of bone strength than a non-

weight bearing bone such as the ulna) influence the fracture union assessment (Axelrad and Einhorn, 2011).

7.7.7.1 Clinical Assessments of Fracture Healing

A systematic review of Clinical Criteria used for clinical assessment of fracture healing in orthopaedic studies concluded that the most commonly included criteria were (Axelrad and Einhorn, 2011):

1. Ability to weight-bear;
2. Absence of pain/tenderness on weight-bearing;
3. Absence of pain/tenderness on palpation.

A number of scales and indices have been used for assessing fracture healing but their validation and reliability are uncertain (Morshed *et al.*, 2008). There has been a move away from Clinical Assessment of fracture healing to patient reported outcome measures (PROMs) such as SF-36 and SMFA-D. (Axelrad and Einhorn, 2011; Cunningham *et al.*, 2017).

There are two clinical scoring systems described in the literature in sufficient detail to enable clinical reproduction. Beck *et al.* have scored 7 clinical signs or symptoms (Table 7-10) from grades 0 to 3, totalling a maximum of 21, and validated these scores against different imaging methods. Pain with daily activities was negatively associated with imaging severity on MRI scan ($P \leq 0.001$) no other clinical sign or symptom was associated with image severity. There is insufficient information in the literature to employ this score as the details of the criteria that define each score from 0–3 are not provided (Beck *et al.*, 2012).

Table 7-10: The 7 clinical signs and symptoms which are scored from 0-3 using the clinical assessment of bone stress injury healing as described by Beck *at al*., 2012.

Sign or Symptom
Pain during daily activities
Pain when running
Night pain
Local tenderness
Local swelling
Pain during percussion
Pain when hopping

Bhandari *et al.* have published preliminary findings of a clinical assessment index, which scores participants from 0 - 3 for a single leg stand, ambulation and pain in terms of palpation and stress. The lower the overall score, the more disability the participant is experiencing from the BSI. A score of 12 gives an indication that normal function has been restored. Instructions for administering the assessment are in figure 7-10. The authors assessed the reliability and validity of the scale in 50 patients with lower extremity fracture. Each patient was assessed by 2 orthopaedic surgeons, 1 orthopaedic fellow, 2 orthopaedic residents and 2 research co-ordinators. The overall interrater reliability was 0.879 (95% confidence interval 0.828 – 0.921). This high score interrater agreement across the multiple examiners suggests this index is a reliable adjunctive clinician measure, however, the authors identified the need for further research to fully validate the index (Bhandari *et al.*, 2013). No further validation has yet been published, so the clinical efficacy remains uncertain.

WEIGHTBEARING				
Score	Single leg stand score	Actual Score	Ambulation score	Actual Score
0	Unable to bear full body weight on affected leg (includes subjects requiring assistive device or support to stand)		Unable to bear any body weight on affected leg as evidenced by requirement for wheelchair or 2 crutches to ambulate	
1	Able to bear full body weight on affected leg as evidenced by ability to stand on affected leg momentarily (< 10 seconds)		Able to bear modest body weight on affected leg as evidenced by ability to ambulate with one crutch, cane, or walker	
2	Able to bear full body weight on affected leg as evidenced by ability to stand on affected leg momentarily (10 to 30 seconds)		Able to bear modest body weight on affected leg as evidenced by ability to ambulate without assistive device, but with clear difficulty (eg, abnormal gait, limp)	
3	Able to bear full body weight on affected leg as evidenced by ability to stand on affected leg > 30 seconds		Able to bear full body weight on affected leg as evidenced by ability to ambulate without assistive device and restoration of prefracture gait	
Total single leg stand score		/3	Total Ambulation Score	/3
Total score for ability to bear weight on fractured limb (A)				/6
PAIN				
Score	Palpation score	Actual Score	Stress score	Actual Score
0	Pain without palpation (light touch)		Pain without stress	
1	Pain with light palpation		Pain with mild stress	
2	Pain with deep palpation		Pain with strong stress	
3	No pain with deep palpation		No pain with strong stress	
Total palpation score		/3	Total Stress Score	/3
Total score for pain at fracture site (B)				/6
TOTAL SCORE (Maximum 12 points)				

Instructions for Administering the FIX-IT measure

I. Ability to bear weight on the fractured limb

Subject ability to bear weight on the fractured limb will be evaluated using the two procedures described below.

1. Ability to stand on affected leg without assistive device (single-leg standing)

a) Subjects will be asked to stand on the affected leg with the non-affected leg raised at least 1 inch or 3 cm off the ground. No assistive device (e.g., cane, crutches) or support (e.g., table, chair) are permitted. Subjects will be assessed for their ability to bear their full body weight on the fractured limb in a standing position.

2. Ability to walk without assistive device (ambulation)

a) Subjects will be asked to walk 10 feet or 3 m, turn around, and return (total of 20 feet or 6 m). No support (eg, table, chair, family member) is permitted and treating physicians will request that subjects make their best attempt to minimize use of assistive devices (e.g., canes, crutches). Subjects will be assessed for their ability to ambulate while bearing their full body weight on the fractured limb.

II. Absence of Pain at the Fracture Site

Subjects will be assessed for the absence of pain at the fracture site by both pressing directly over the fracture site and applying stress to the fractured limb. Subjects will be asked to sit upright on the examination table.

1. Pain elicited by directly pressing on the fracture site:

a) The examiner will place one hand above the fracture site and one below the fracture site. The examiner will apply light pressure with his/her thumb to determine if patient has pain. If no pain is elicited, the examiner will apply deep pressure with his/her thumb to elicit pain. If no pain is elicited with deep pressure, the examiner will give a score of 3.

2. Pain elicited by applying stress to the fracture site

a) Subjects will be asked to extend their lower extremity so that the leg is straight. The examiner will place one hand proximal to the site of fracture (which will act as a fulcrum) and the other hand distal to the fracture site. An anteroposterior and medial-lateral stress will then be applied to the distal leg.

b) To determine the presence and severity of pain, assessors will directly ask subjects if pain is present and will observe the physical response to the pressing and stressing procedures.

Figure 7-10: Appendix 1 to Bhandari M, Wasserman SM, Yurgin N, et al. Development and preliminary validation of a function index for trauma (FIX-IT). Can J Surg 2013;56(5): Instructions for Administering the FIX-IT measure.

7.7.7.2 Radiological Assessments of Fracture Healing

Radiological assessment does not provide sufficient information to be the sole basis on which clinical management decisions for fracture treatment are made, but they can inform decisions on whether a fracture is healing and rehabilitation programmes. Radiographs, computerised tomography (CT) and, in BSIs, MRI are the most commonly used techniques (Fisher *et al.*, 2018).

Historically, radiological assessment relied on plain film radiographs with the criteria based around bridging of the fracture by bone, callus or trabeculae, bridging of the fracture at three of the four cortices and absence of fracture line and/or cortical continuity (Dijkman *et al.*, 2010).

Inconsistency between the methods of radiological assessment has been well described in the literature. A survey of 444 orthopaedic surgeons highlighted the lack of consensus. There is very little consensus on the schedule for follow-up imaging even within the same type of fractures (Bhandari *et al.*, 2002). Bohl *et al.* conducted a survey of 40 hand surgeons and the radiographic follow-up following an intervention, this ranged from 1 to 6 series per patient, with the mean number of radiographic series 2.6 (±1.0) (Bohl *et al.*, 2014). Within BSI healing there is the same lack of consensus between clinicians, with serial imaging only usually taking place if there is suspected fracture non-union (Brukner and Bennell, 1997; Brukner, Bradshaw and Bennell, 1998).

Different modes of radiographic assessment for BSIs are discussed in the literature; plain film, CT and MRI are the most commonly used (Fisher *et al.*, 2018) and primarily as a diagnostic tool. MRI is the imaging modality of choice partly because it does not expose the patient to radiation, and the criteria for diagnosing a BSI are well defined by an American College of Radiology (ACR) expert panel (Bencardino *et al.*, 2017). The Kijowski-modified Fredericson grading of BSIs on MRI is now accepted as the grading system of choice for BSIs (Mandell, Khurana and Smith, 2017). Figure 7-11 shows the Kijowski-modified Fredericson grading scale by description and illustration.

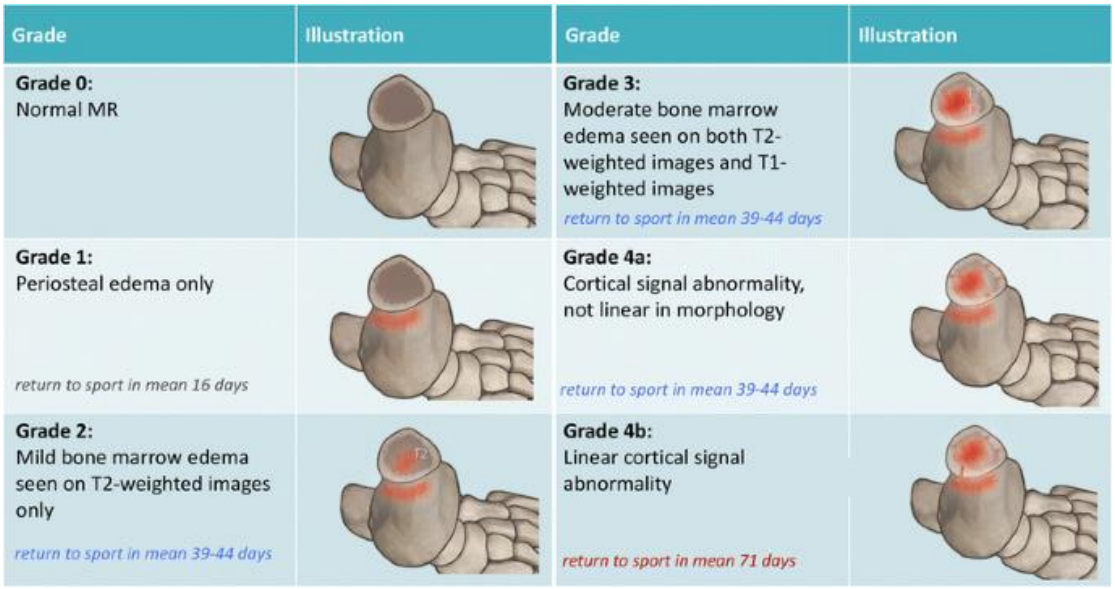


Figure 7-11: Summary of the Fredericson grading of distal tibial bone stress injuries, with modifications by Kijowski to reflect the 4a and 4b subclasses (Mandell, Khurana and Smith, 2017).

The criteria for assessing serial MRIs to monitor fracture healing is less well described. Almirol *et al.* used a modified version of the Fredericson scale to assess fracture healing by MRI at 8 weeks using their modified criteria shown in table 7-11 (Almirol *et al.*, 2016).

Table 7-11: Bone stress injury grading system by MRI and high-signal short TI inversion recovery (STIR) images. Modified from Fredericson, 1999 used by Almirol 2013 (Almirol et al., 2016).

Grade	MRI Changes	
Grade 0	Normal	Normal
Grade 1	Positive STIR image	Irregular and/or a poorly defined high-signal area
Grade 2	Positive STIR image plus positive T2	Similar to grade 1, more intense yet still poorly defined
Grade 3	Positive T1 and T2 but without cortical break	Sharply marginated high signal area, usually focal or fusiform in shape
Grade 4	Positive T1 and T2 fracture line	Similar to grade 3 but more intense high signal

There is one published peer-reviewed paper on the use of Hounsfield units for quantifying fracture healing, but this is in distal radial fractures where there is a discrete fracture line. There is no published evidence validating Hounsfield units to assess BSI healing. Grigoryan *et al.,* have compared the quantitative measure of the Hounsfield unit and qualitative definitions of CT features of fracture healing which, if were to be employed to grade BSI healing

specifically, would require the development of a descriptive scoring system (Grigoryan *et al.*, 2003).

7.7.7.3 Routine Treatments for Bone Stress injuries

Treatment for BSIs varies depending on the location, severity and population. Low risk fractures (Table 7-7) are treated conservatively with ice, pain medication, a walking boot, cross training and limiting high impact activities (Brewer and Gregory, 2012; Patel, Roth and Kapil, 2011). High risk fractures (Table 7-7) should be treated with aggressive non-weight bearing methods, casting and / or bone stimulation for extended periods (Brewer and Gregory, 2012). Pneumatic braces have been investigated to expedite tibia BSI healing; however, studies suggest any additional benefit is limited by discomfort and inconvenience to the patient (Allen *et al.*, 2004; Moen *et al.*, 2010; Patel, Roth and Kapil, 2011; Swenson *et al.*, 1997).

7.8 Potential Targets in the Treatment of Bone Stress Injuries

There are several osteoporosis medications which may have utility in the treatment of BSI's. An overview of their different functional mechanisms and activity is described in figure 7-12.

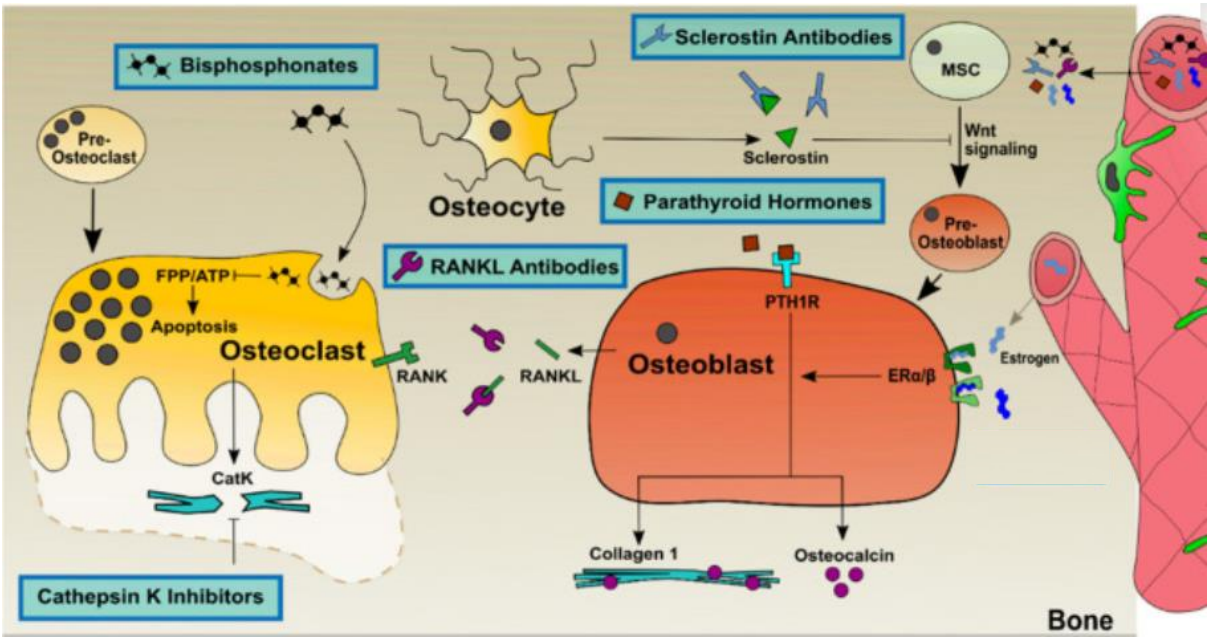


Figure 7-12: An overview of the mechanisms of action of drugs currently used in the treatment of osteoporosis and which may have utility in the treatment of BSI. Bisphosphonates bind to adenosine triphosphate and farnesyl pyrophosphate resulting in osteoclast apoptosis. RANKL antibodies bind to RANKL preventing it binding to osteoclasts preventing their activation and differentiation. Cathepsin K inhibitors inactivate mature cysteine protease cathepsin K and prevent bone resorption. Sclerostin antibodies bind to sclerostin, inhibiting Wnt signalling and osteoblast differentiation. In the presence of sclerostin antibodies osteoblastneogenesis is active (Tonk *et al* 2022).

7.8.1 Anti-Resorptive Treatments

7.8.1.1 Bisphosphonates

Bisphosphonates, inhibit bone resorption and are routinely used in the treatment of osteoporosis, hypercalcaemia, metastatic bone disease and Paget's disease of bone. Bisphosphonates have a P-C-P structure which mimics the P-O-P structure of endogenous pyrophosphate but is resistant to degradation by alkaline phosphatase (Drake, Clarke and Khosla, 2008), they inhibit osteoclastic bone resorption by attaching to the hydroxyapatite binding sites on bone surfaces undergoing active resorption. The bisphosphonate then prevents the osteoclast forming the ruffled border and adhering to the bone so resorption of the bone is prevented (Sato *et al.*, 1991; Rodan, 1998; Colucci *et al.*, 1998).

The suppression of bone remodelling by bisphosphonates makes them unlikely candidates to promote stress fracture healing—which occurs by endogenous remodelling processes—although some small studies have reported improvements in functional outcomes and reductions in pain. A case series of five athletes with BSI reported that intravenous pamidronate reduced pain and accelerated return to training (Stewart *et al.*, 2005), however this study was not controlled, and did not demonstrate any effect on fracture healing. A retrospective study of 25 athletes reported intravenous ibandronate and high dose vitamin D reduced pain and improved mobility (Simon *et al.*, 2014), but again no controls were included.

The efficacy of risedronate in the prevention of stress fractures was investigated in 324 infantry recruits in a randomised, double-blind trial. Recruits were treated with 30mg risedronate or placebo daily for 10 doses during the first two weeks of basic training followed by the same dose weekly dose for the next 12-weeks. The study found no difference in tibial, femoral, metatarsal, or total stress fracture incidence between the treatment group and the placebo, concluding that prophylactic treatment with risedronate in a military training population does not lower stress fracture risk (Milgrom *et al.*, 2004).

Concerns regarding the safety of bisphosphonates limits the palatability of their further investigation in populations which include women of childbearing potential. Bisphosphonates given to pregnant rats crossed the placenta and resulted in reduced bone growth and foetal weight (Patlas *et al.*, 1999). A review of the use of bisphosphonates in women prior to or during pregnancy found that whilst the majority of women did not demonstrate any adverse events, there were cases of shortened gestational age, low neonatal birthweight and transient hypocalcaemia of newborns (Stathopoulos *et al.*, 2011).

7.8.1.2 Denosumab

Denosumab is a fully human monoclonal antibody of RANKL (receptor activator of nuclear factor kappa-B ligand) an osteoclast differentiating factor. Denosumab inhibits osteoclast formation, by binding to RANKL inhibiting its interaction with RANK resulting in decreased osteoclast generation and stimulation (Iqbal, Sun and Zaidi, 2010). The net result is a decrease bone formation, and increase bone mineral density at the lumbar spine (+3.0% to +6.7% compared to -0.8%, $P<0.001$), total hip (+1.9% to +3.6% compared to -0.6%, $P<0.001$), distal

radius (+0.4% to +1.3% compared to -2.0%, *P*<0.001) and total body BMD (+0.6% to +2.8% compared to -0.2%, *P*<0.01) following 12 months treatment (McClung *et al.*, 2006)

There are no reports of the use of denosumab in the treatment of BSI, this may be in part due to the lack of safety data in young people whilst anabolic agents such as teriparatide have shown more promise in preliminary studies.

7.8.2 Anabolic Treatments

7.8.2.1 Human Recombinant Parathyroid Hormone (PTH) (1-34)

Intermittent administration of human recombinant PTH (1-34) (teriparatide) is an FDA- and European Medicines Agency (EMA)-approved anabolic treatment for patients with osteoporosis that stimulates bone formation to surpass bone resorption (Hutton, 2003). Prior to its approval, osteoporosis treatment included hormone replacement therapy (HRT), and antiresorptive drugs that inhibit resorption and prevent further bone loss. The primary indication for teriparatide is the treatment of postmenopausal and steroid-related osteoporosis in women and men (Orwoll *et al.*, 2000; Orwoll *et al.*, 2003; Rubin and Bilezikian, 2003b). In a study of 101 osteoporotic postmenopausal women, teriparatide treatment of daily 20 or 40 µg sub-cutaneous injection (SC) reduced the risk of non-vertebral fractures and increased trabecular BMD at the non-dominant distal radius compared with placebo (Zanchetta *et al.*, 2003). The pivotal licensing study of 1637 postmenopausal women by Neer *et al*. showed daily teriparatide (20 or 40 µg SC daily) increased vertebral (20 µg SC daily +9.7 ± 7.4% , 40 µg SC daily +13.7 ± 9.7%), femoral (20 µg SC daily +2.8 ± 5.7% , 40 µg SC daily +5.1 ± 6.7%) and total body (20 µg SC daily +3.1 ± 4.3% , 40 µg SC daily +4.5 ± 5.7%) BMD over a median duration of observation of 21 months (Neer *et al.*, 2001).

The effects of PTH administration in both normal subjects and hypoparathyroid patients were first described by Albright *et al* and Bauer *et al,* respectively. The similar changes in both cohorts included; (1) immediate increased phosphate excretion, peaking within 2 hours; (2) increased serum calcium and urine phosphate excretion, and decreased serum phosphate; (3) a critical serum calcium value identified at 2.1 mmol/L at which a negligible urine calcium excretion suddenly changed to a measurable one (Albright and Ellsworth, 1929; Bauer, Albright and Aub, 1930).

Intact PTH is a single-chain polypeptide with 84 amino acids and molecular weight of 9423 Da, with the first 34 amino acids being the biologically active component (Quattrocchi and Kourlas, 2004). Teriparatide has a molecular weight of 4117.8 Da, its amino acid sequence is shown in figure 7-12.

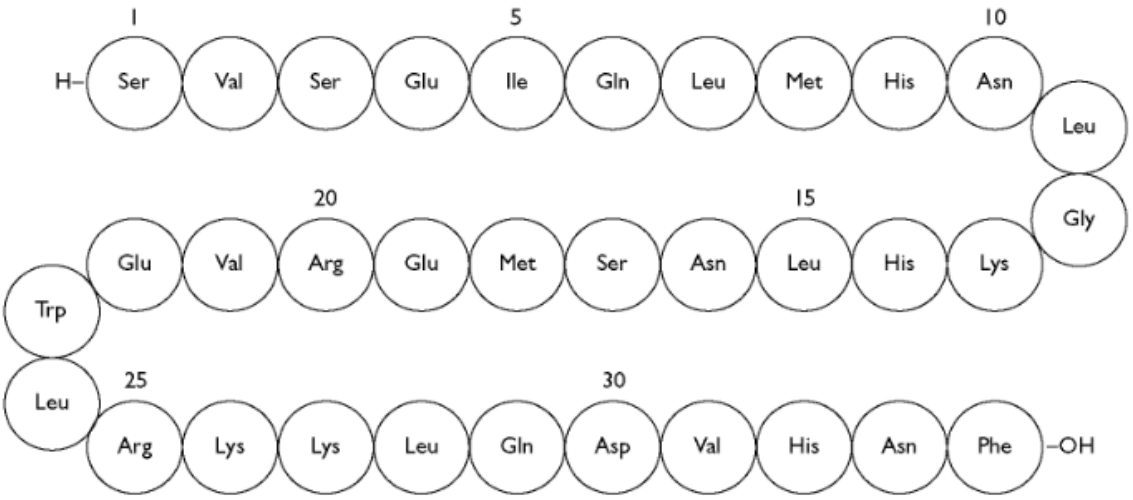


Figure 7-13: Amino acid sequence of teriparatide (Quattrocchi and Kourlas, 2004).

Reeve *et al* described the use of PTH (1-34) given as a once daily 100µg SC injection for up to 24 months in osteoporotic patients. Increased iliac trabecular bone volume of 70% from baseline were reported. On examination, the new bone was histologically normal and the authors concluded that treatment with PTH (1-34) caused a disassociation between bone formation and resorption in trabecular bone (Reeve, Tregear and Parsons, 1976; Reeve *et al.*, 1980).

The osteoanabolic effects of PTH were observed in rats following the administration of low dose intermittent PTH (1-84) (Sato, Zeng and Turner, 1997; Tam *et al.*, 1982). These findings were replicated with teriparatide, which increased the number of osteoblasts and osteoclasts in in the spine of rabbits after surgical fusions, and improved early callus formation and mechanical strength in rodents with tibial fractures (Andreassen, Ejersted and Oxlund, 1999; O'Loughlin *et al.*, 2009).

These osteoanabolic effects have been shown in humans in a paired biopsy study (n=16). Treatment with teriparatide 400U/day for 36 months increased cortical width of the lumbar spine in women and increased connectivity density of cancellous bone (Dempster *et al.*, 2001). A similar study in post-menopausal women treated with 20 or 40 µg daily of SC teriparatide, or placebo showed teriparatide treatment enhanced trabecular thickness, wider cortex and increased bone connectivity and volume at the iliac crest (Jiang *et al.*, 2003). A double-blind randomised trial with 712 participants (81 % women, age range 22 – 89 years old) compared the effects of teriparatide (20 µg SC daily) to alendronate (10 mg orally daily) in long term glucocorticoid users. The teriparatide treatment group had a significantly greater increase in lumbar spine and total hip BMD (Saag *et al.*, 2007). Teriparatide showed similar efficacy in premenopausal women with glucocorticoid-induced osteoporosis, suggesting its effects are unconnected to gender and menopausal status (Langdahl *et al.*, 2009; Carpinteri *et al.*, 2010) but more research is required to conclude this definitively. These findings suggest that teriparatide acts by directly stimulating bone formation, counteracting the mechanism by which glucocorticoids promote bone loss (Mazziotti *et al.*, 2006).

7.8.2.2 Teriparatide and Fracture Healing

Teriparatide improves bone mineral content, structure and microarchitecture in trabecular bone in human studies (Rubin and Bilezikian, 2003b; Zanchetta *et al.*, 2003). These effects have prompted further investigation into the use of teriparatide in delayed or non-union fractures (Hodsman *et al.*, 2005; Zhang *et al.*, 2014). A placebo-controlled trial in CD1 female mice with closed fractures showed an increase in anabolic activity and improved mineralisation during callus formation after daily teriparatide (40 µg/kg) (Mognetti *et al.*, 2011). The mice models were pre-treated with tetracycline fluorescent label that concentrated at sites of new bone formation. The intensity of fluorescence was then quantified in luminance arbitrary units. The teriparatide treated mice showed a greater increase in fluorescence than those in the placebo arm, with a maximal level at 13 days in the teriparatide treatment group and 18 days in the control group. Mognetti *et al.* concluded that teriparatide considerably improved callus consolidation in the very early stages of bone healing.

Following the successful animal studies, retrospective studies and case reports supported the positive effects of teriparatide in fracture healing (see Appendix 4 for details). Subsequently,

trials that are more scientifically robust have taken place. Chapter Eleven details a meta-analysis conducted of the randomised controlled trials exploring the use of PTH analogues in the healing of all fracture types.

7.8.2.3 Teriparatide and Bone Stress Injury Healing

The quality of evidence to support the use of teriparatide in BSI healing is limited, the only randomised controlled trial currently published was not sufficiently powered to draw any significant conclusions (Almirol *et al.*, 2016). Short term daily teriparatide treatment (20 µg / day by SC injection) in pre-menopausal women with BSIs was shown to increase bone formation makers (P1NP and OC), result in an anabolic window (P = 0.05) and increase tibial cortical area and thickness when compared to placebo at 8 weeks. MRI review also showed a trend towards improved BSI healing over the same time period (Almirol *et al.*, 2016). The remainder of the evidence to support teriparatide in this indication is case reports (Raghavan and Christofides, 2012).

7.8.2.4 Teriparatide Dosing Regimen

In the UK, the licensed indication of teriparatide is for the treatment of osteoporosis in post-menopausal women at increased risk of fracture and treatment for osteoporosis associated with sustained glucocorticoid therapy in men and women at increased risk of fracture. The only licensed dose is 20 µg / day by SC injection. In Japan there are two licensed doses, 20 µg / day and 56.5 µg / week for the same indications. A retrospective study in patients with atypical femur fractures showed teriparatide treatment significantly shortened fracture healing times (5.4 ± 1.5 months) compared with the non-teriparatide control group (8.6 ± 4.7 months; P = 0.012) (Miyakoshi *et al.*, 2015).

Tsuchie *et al.* retrospectively compared the effectiveness of a daily *versus* a weekly dose in 43 patients with atypical femur fractures; the fracture healing time in the daily group (7.1 ± 5.2 months) was not significantly shorter compared to the fracture healing time in the weekly group (9.3 ± 4.5 months) (P = 0.179). Sub group analysis of complete and incomplete fracture healing times found daily administration of teriparatide to be more effective than weekly administration (6.1 ± 4.0 months compared to 10.1 ± 4.2 months (P = 0.021)) at healing complete fractures but there were no significant differences in the healing times compared with incomplete fractures (7.5 ± 5.4 months versus 8.8 ± 6.7 months (P = 0.731)) (Tsuchie *et*

al., 2018). This may have been because the more frequent teriparatide stimulus prompted a greater anabolic response.

7.8.2.5 Effects of Teriparatide on Biochemical Markers of Bone Metabolism

Early increases in biochemical markers of bone formation correlated with improved aBMD at both the lumbar spine and femoral neck following 24 months treatment with teriparatide 20µg SC daily (Blumsohn *et al.*, 2011). Osteoporotic women treated with teriparatide over a 3-year period experienced a 55% increase in OC from baseline compared with a delayed 20% increase in bone resorption marker β-CTX; the resulting net gain in bone formation created an anabolic window (Lindsay *et al.*, 1997).

There is a significant relationship between increases in P1NP within the first month of teriparatide treatment and subsequent increases in lumber spine BMD with teriparatide treatment. The effect of teriparatide *versus* placebo on bone biomarkers (PINP, BSAP and CTX) was evaluated in a double-blind placebo-controlled trial (Tsujimoto *et al.*, 2011). The study analysed correlations between changes in bone biomarkers and increases in lumbar spine BMD at 12 months. Increased PINP correlated most strongly with increased lumbar spine aBMD (r=0.56; $P \leq 0.01$). Increases in aBMD were also reported at the total hip (r=0.21; $P < 0.05$) after 12 months teriparatide treatment compared to placebo. There were no changes in femoral neck aBMD at the same timepoint (Tsujimoto *et al.*, 2011). 92 % of participants in the treated group experienced an increase of > 10 µg/L in PINP after 1 and 3 months of treatment, and a 3 % increase in lumbar spine BMD. No changes in either P1NP or LS BMD were observed in the placebo group. Increases in CTX were slower than those of P1NP, increases were only reported following 3 months of treatment, but did remain higher that placebo thereafter (Tsujimoto *et al.*, 2011). As such, P1NP can be used to monitor response to teriparatide. In a study of combined therapy of antiresorptive and anabolic treatment in postmenopausal women, P1NP concentrations increased after 1 and 6 months treatment with teriparatide independent of previous types of antiresorptive therapy, but response was poorer in patients who had received previous bisphosphonate treatment or had a poor response to previous treatments (Obermayer-Pietsch *et al.*, 2008).

7.8.2.6 Effects of Teriparatide on Vitamin D Metabolites

Endogenous PTH plays a pivotal role in the conversion of 25(OH)D to $1,25(OH)_2D$. The effect of exogenous PTH in the form of teriparatide (20 µg / day SC) has been investigated in a cohort

of older men (n=287) and postmenopausal women with osteoporosis (n=336) (Cosman *et al.*, 2012; Rubin and Bilezikian, 2003a). Participants received supplementation of calcium 1000mg and vitamin D 400-1200IU daily. In women, median 1,25 $(OH)_2D$ concentrations increased by 27% from baseline to one month ($P \leq 0.0001$) in the teriparatide treatment group but did not change in the placebo group (-3% $P = 0.23$); similar results were also seen at 12 months. In men, median 1,25$(OH)_2D$ concentrations increased from baseline by 22% ($P \leq 0.0001$) in the teriparatide treatment group *versus* 0% ($P = 0.99$) in the placebo group at one month ($P = 0.0001$). At 12 months, the median 25(OH)D concentrations decreased in men by -11% in the teriparatide treatment group versus 1% ($P \leq 0.05$) in the placebo group, and in women by -19% in the teriparatide treatment group *versus* 0% change ($P \leq 0.05$) in the placebo group respectively. These findings support the effect of teriparatide treatment on vitamin D metabolism (Cosman *et al.*, 2012; Rubin and Bilezikian, 2003a; Rubin and Bilezikian, 2003b).

7.8.2.7 Pre-Menopausal Women and Teriparatide

Few studies have examined the effect of teriparatide on bone in pre-menopausal women. Cohen *et al* examined the use of teriparatide in pre-menopausal women with idiopathic osteoporosis with unexplained fragility fractures or low aBMD (Cohen *et al.*, 2013). Compared to baseline aBMD increased after 6 months of treatment at the lumbar spine and total hip ($P \leq 0.001$ and $P \leq 0.01$ respectively (percentage change not published)); after 12 months of treatment at the lumbar spine , total hip and femoral neck ($P \leq 0.001$, $P \leq 0.001$ and $P \leq 0.05$ respectively (percentage change not published)); after 18 months of treatment BMD increased at the lumbar spine (9.8 ± 5.2%; $P \leq 0.001$), total hip (2.9 ± 3.6%; $P \leq 0.001$) and femoral neck (3.5 ± 5.3%; $P \leq 0.01$)(Cohen *et al.*, 2013). Bone compartments were not separated in this study as DXA was used for the analysis.

Serum P1NP doubled following one month of treatment, peaked at 6 months, and had returned to baseline by 18 to 24 months of teriparatide treatment. Serum CTX also showed a small increase, peaking at 75% above baseline at six months and returning to baseline by 18 months. Significant increases in cortical width, porosity and trabecular bone volume were demonstrated with trans-iliac biopsies. Adipocyte area, perimeter, and volume/marrow volume decreased, with no change in the adipocyte number. Four of the 24 women did not respond to treatment, and showed no increase in aBMD and a blunted, delayed increase in

serum P1NP. The reason for non-responses were not elucidated, however, it was noted that these non-responders had significantly lower baseline bone formation rates and higher serum IGF-1 than responders (Cohen *et al.*, 2013).

Teriparatide has been used in a placebo-controlled trial of premenopausal women with endometriosis as an adjunct to nafarelin therapy - a drug used to induce oestrogen deficiency in endometriosis and other oestrogen-dependent conditions (Finkelstein *et al.*, 1994; Finkelstein *et al.*, 1998). Significantly higher formation and resorption bone turnover markers were recorded in the teriparatide treatment group. Women who received nafarelin alone for 12 months had a reduction in mean BMDs at the anterior-posterior spine -4.9% (0.6%) ($P \leq 0.001$), lateral spine -4.9% (0.8%) ($P \leq 0.001$), femoral neck -4.7% (1.1%) ($P \leq 0.001$), trochanter -4.3% (0.9%) ($P \leq 0.001$), and total body -2.0% (0.6%) ($P = 0.003$). In contrast women who received nafarelin and teriparatide for 12 months had increased BMD at the anterior-posterior spine +2.1% (1.1%) ($P = 0.09$) and lateral spine +7.5% (1.9%) ($P = 0.002$) whilst bone loss at the femoral neck, trochanter and total body was maintained supporting teriparatide use in the oestrogen-deficient premenopausal women to prevent bone loss (Finkelstein *et al.*, 1998).

7.8.2.7.1 Pharmacokinetics

Sex differences in pharmacokinetics (PK) should be considered in four stages: absorption, distribution, metabolism and excretion. Absorption is primarily relevant to drugs administered orally so would not have a significant effect on the PK of teriparatide, which is currently only licensed as a SC preparation (Soldin and Mattison, 2009). Distribution of a drug is affected by body composition (Table 7-12), which may account for differences in the concentration of a drug at the target site and result in varying responses (Schwartz, 2003; Schwartz, 2007; Soldin and Mattison, 2009).

Table 7-12: Body composition parameters affecting the distribution of drugs in-vivo. M=men; W=women adapted from (Soldin and Mattison, 2009; Farkouh et al., 2020; Frey, Schaad and Frey, 1984)

Parameter	Physiologic Difference	Pharmacokinetic Impact
Plasma Volume	M > W	None
Body Mass Index	M > W	None
Average Blood Organ Flow	M > W	None

Total Body Water	M > W	Decreased concentration of water-soluble drug in men. Smaller volume of distribution in women.
Plasma Proteins	M = W	Proportions of the binding proteins differ, resulting in variations in concentrations of plasma binding and subsequently alter the free or active fraction of the drug. NB. Oestrogen containing hormonal contraceptives reduce the activity of liver enzymes and concentrations of plasma proteins.
Body Fat	W > M	Increased lipid soluble drug in women.
Cardiac Output	M > W	Increased rate of drug distribution in men.

Proteins influence drug distribution volumes; albumin is a major plasma protein responsible for reversible drug binding that is not greatly affected by sex (Verbeeck, Cardinal and Wallace, 1984). Differences in α1-acid glycoprotein concentrations, sex-dependent stereospecific binding and oestrogen-mediated α1-acid glycoprotein have been observed (Verbeeck, Cardinal and Wallace, 1984)

Drug metabolism occurs primarily in the liver, and despite large variations between sexes in drug metabolism, correcting for height, body mass, surface area and body composition negates most sex differences (Lilly, 2017). Teriparatide has a volume of distribution of approximately 1.7L/kg body mass with a half-life of approximately 1 hour when administered by SC injection (Lilly, 2017). To date, no metabolism studies have been performed with teriparatide but the peripheral metabolism is believed to occur in the liver and kidney (Lilly, 2017).

7.8.2.7.2 Pharmacodynamics

Pharmacodynamics (PD) is the study of the biochemical and physiological effects of drugs. Differences in PD responses to drugs are largely influenced by circulating sex steroid hormones in women (Spoletini *et al.*, 2012). To date, there has been no investigation into sex differences in the PD of teriparatide. This is probably because commercially, the drug is aimed at post-menopausal women in whom circamensal fluctuations are no longer a consideration.

In a younger population, circamensal fluctuations and use of hormonal contraception may affect PD of teriparatide and requires further investigation.

7.8.2.8 Sex Differences in Response to Drug Treatment

Sex refers to a person's physical characteristics at birth, as opposed to gender which considers a person's identity, expressions and societal roles. Physiological differences between men and women can alter their response to drug treatment, probably due to endogenous hormones influencing CYP isoenzyme expression (Soldin and Mattison, 2009). An understanding of the pharmacokinetics and pharmacodynamics of teriparatide is required to determine the impact of sex differences in clinical therapeutics to ensure safe and effective treatment of both sexes.

Classification of an organism as male or female based on their reproductive organs determines sex as opposed to gender, which is expressed in terms of masculinity and femininity. Guidance and regulations are in place to ensure that both sexes are represented in all phases of drug development and testing (Bhandari *et al.*, 2016; FDA, 1998). There is very little published data on the sex differences in response to teriparatide, which, contrary to most drugs, underwent pre-licensing clinical testing almost exclusively in women (Neer *et al.*, 2001).

A CD-1 mouse study has investigated the effect of sex differences in the response of bone to PTH. Wang *et al*, found that the same 2-week course of PTH is anabolic on cortical bone in this mouse strain in both male and female mice, but the effect was accentuated in male mice (Wang *et al.*, 2006). These findings have not been repeated in human models.

Haden *et al.* used citrate and calcium infusions to characterise the impact of age and sex on PTH dynamics in 48 healthy subjects split into 4 cohorts; (1) 'young' women with mean age ± SD of 26.4 ± 1.6 years, (2) 'young' men with mean age of 26.6 ± 1.3 years, (3) 'older' women with mean age of 68.6 ± 1.3 years and (4) 'older' men with mean age of 67.2 ±1.6 years. No differences in baseline serum PTH concentrations were reported between any of the cohorts. Intact PTH in response to the calcium and citrate infusions did not differ between men and women in the 'young' or 'older' cohorts. Differences were reported between 'young' and 'older' women for both citrate and calcium infusion; intact PTH responses calculated for area under the curves were higher in the 'older' women. In men, the intact PTH response

calculated for area under the curves was higher following the citrate infusion only (Haden *et al.*, 2000), but the study was not designed to investigate the clinical significance of this.

Rosen and Bilezikian published a review of the evidence supporting the licensed indications of PTH use. They present a number of early trials demonstrating the efficacy of this drug in the treatment of osteoporosis in both male and female humans, concluding that teriparatide treatment increases spine BMD with equal efficacy in both sexes (Rosen and Bilezikian, 2001). The evidence cited in this review is primarily based on post-menopausal women, in line with the licensed indication of PTH.

The principal finding, common to all studies in both men and women is a marked increase in spine BMD with PTH. This increase in BMD is substantially greater than the increase commonly observed after 1 year of antiresorptive therapy. Changes at cortical sites were less consistent.

7.8.2.9 Parathyroid Hormone (1-84)

Parathyroid hormone (rDNA 1-84) is currently licensed for the adjunctive treatment of adult patients with chronic hypoparathyroidism who cannot be adequately controlled with standard therapy alone (calcium, vitamin D and magnesium supplementation) (TakedaUKLtd, 2024). Parathyroid (1-84) has previously been licensed for the treatment of osteoporosis but the evidence only supports benefit for the prevention of vertebral fractures (Greenspan *et al.*, 2007) compared with PTH (1-34) which demonstrated a reduction in vertebral and non-vertebral fractures excluding hip fractures (Lilly, 2017). Parathyroid hormone (1-84) has been shown to be effective in fracture healing in a small study (n=65), improving fracture healing and pain at 8-weeks post treatment (both P <0.001), and functional outcomes at 12-weeks (P <0.001) (Peichl *et al.*, 2011). The formulation of PTH (1-84) is more thermo-stable than PTH (1-34) and can be stored at 25°C for a maximum of 7 days.

The biological activity of the carboxyl-terminal region of PTH (1-84) compared to PTH (1-34) has been investigated in a few studies. In a chick model, PTH (1-84) was approximately 3X more potent than PTH (1-34) on a molar basis although there was no difference in the rate of clearance of the 1-34 metabolite. Plasma calcium concentrations were higher after PTH (1-

84) administration compared to PTH (1-34), an effect seen between 30 and 120 mins after administration (Yamamoto *et al.*, 1994).

The difference between the biological activity of PTH (1-34) and PTH (1-84) is not well described in humans, the lack of directly comparable studies is a key limitation. A review comparing the effects did identify higher incidences adverse events associated with PTH (1-84), compared to PTH (1-34), namely hypercalcaemia (identified in 28% of patients treated with PTH (1-84) and 11% of patients treated with PTH (1-34) and no further statistical comparison undertaken) and nausea (identified in 22.6% of patients treated with PTH (1-84) and 8% of patients treated with PTH (1-34) (Verhaar and Lems, 2009). It is likely these findings are biased by the relatively high dose used as the PTH (1-84) dose corresponded to 43mg of PTH (1-34), and the inclusion of patients with high normal or elevated serum calcium levels in the PTH (1-84) study.

The lack of a licensed bone indication remains the key limitation for further investigation of PTH (1-84) in fracture healing indications in a healthy population, this is compounded by the current lack of commercial availability.

7.8.2.10 Romosozumab

Romosozumab is a humanised monoclonal antibody (IgG2) that binds and inhibits sclerostin, resulting in an increase in bone formation due to the activations of bone lining cells, increasing osteoblast production and recruitment of osteoprogenitor cells. In addition, romosozumab prompts a change in the expression of osteoclast mediators decreasing bone resorption. Romosozumab was first licensed in 2019 in the treatment of severe osteoporosis in postmenopausal women at high risk of fracture and is administered as two 105mg sub-cutaneous injections once monthly for 12 months, after which transition to an antiresorptive therapy is recommended to extend the benefit achieved beyond 12 months. There is no data on romosozumab in pregnant women, skeletal malformations were observed in animal (rat) studies (UCBPharmaLimited, 2023; Markham, 2019).

The clinical efficacy and safety of romosozumab has been assessed in two key clinical trials, the ARCH study which was alendronate controlled and the FRAME study which was placebo controlled.

The ARCH study was a multicentre randomised controlled double-blind trial which recruited 4,093 postmenopausal women with previous osteoporotic fractures. The primary endpoint(s) were the incidence of new vertebral fractures at 24 months and the incidence of clinical fracture, A lower risk of vertebral fractures was observed in the romosozumab group compared to the alendronate group (6.2% vs 11.9% p <0.001), similarly a lower risk of clinical fractures was observed in the romosozumab group compared to the alendronate group (9.7% vs 13.0% p <0.001) (Saag *et al.*, 2017).

The FRAME study was also a multicentre randomised controlled double-blind trial, it recruited 7180 postmenopausal women with a T score of -2.5 to -3.5 at the total hip or femoral neck. The primary endpoints of the trial were incidence of new vertebral fracture at 12 and 24 months (after transition to denosumab). Romosozumab was found to reduce the incidence of new vertebral fractures compared to placebo at months 12 (absolute risk reduction 1.3% [95%CI: 0.79; 1.80]) (Cosman *et al.*, 2016).

Evidence for the use of romosozumab in men is more limited, the BRIDGE study was a randomised placebo-controlled double-blind study which recruited 245 male participants (randomised 2:1 to receive romosozumab or placebo for 12 months). The primary endpoint was percentage change from baseline in the lumbar spine BMD at 12 months. Romosozumab was found to increase lumbar spine BMD at 12 months (12.1% vs 1.2% p <0.001), it was however associated with an increased number of serious cardiovascular events (8 (4.9%) vs 2 (2.5%) (Lewiecki *et al.*, 2018).

Mechanistically romosozumab has the potential to be of benefit in the treatment of osteoporotic and BSI, its monthly administration would also be of benefit as it would reduce the treatment burden but there is no evidence to support these indications at present. Ultimately, the lack of license in men limits its use and suggestion of increase serious cardiac events require further investigation before it can be investigated in BSI in young fit healthy population.

7.8.3 Sequential and Combination Therapy

There are no clinical studies reviewing the use of combination or sequential therapy in fracture healing or the treatment of BSIs. The consequences of discontinuing drugs affecting bone metabolism without transition to subsequent therapy differ, with some, especially denosumab prompting a rapid rate of bone loss if not followed up with further therapy (Cosman, Langdahl and Leder, 2024). As a result, there is growing evidence to support the use of sequential and combination therapies in osteoporosis. Possible treatment sequences include (1) an antiresorptive agent followed by an anabolic agent, (2) an anabolic agent followed by an antiresorptive agent and (3) an antiresorptive agent followed by another antiresorptive agent these are discussed in more detail below.

7.8.3.1 An Antiresorptive Agent Followed by an Anabolic Agent

This sequence of treatment has the greatest body of evidence because of the relatively higher cost of anabolic agents they are not usually funded by some health authorities, unless there is a treatment failure with one or more antiresorptive agents. Bisphosphonates, particularly those with the longest skeletal half-lives limit the BMD responses to anabolic agents, particularly teriparatide (Boonen *et al.*, 2008; Miller *et al.*, 2008). The same effect is not observed when raloxifene is used prior to teriparatide (Ettinger *et al.*, 2004). In subgroup analysis, there VERO study concluded BMD responses to teriparatide with prior bisphosphonate use do alter vertebral or clinical fracture risk reduction. They also concluded the interval between bisphosphonate cessation and teriparatide initiation did not alter treatment outcomes (Kendler *et al.*, 2018).

The DATA-SWITCH study, which investigated 2 years of denosumab, followed by 2 years of teriparatide in post-menopausal women, found a reduction in spine, total hip and femoral neck BMD at 6 months following the change in therapy. After 48 months, the primary outcome of mean spine BMD increased (14.0% [10.9–17.2], *P*=0.30) in the denosumab to teriparatide group (n=27). At the same timepoint the total hip BMD increased (2.8% [1.3–4.2], *P*=0.0002) as did femoral neck BMD (4.9% [2.2–7.5]; *P*=0.0447), but radius BMD decreased (−1·8% [−5·0 to 1·3] *P*=0·0099) (Leder *et al.*, 2015). These outcomes were all worse than the outcomes for patients in the other arms of the study (teriparatide to denosumab or

teriparatide and denosumab to denosumab), which are discussed in more detail below. It is hypothesised that this is a result of the simulation of osteoclast precursors, that have been quiescent during the initial denosumab therapy (Leder *et al.*, 2015).

In the STRUCTURE study, post-menopausal women pre-treated with bisphosphonates for an average of 6 years were randomised to teriparatide or romosozumab for 1 year. Bone mineral density increases in the romosozumab arm were greater than seen in the teriparatide treated arm, but increases in both arms were less than usually seen in treatment naïve women (Ettinger *et al.*, 2004).

7.8.3.2 An Anabolic Agent Followed by an Antiresorptive Agent.

Bone mineral density increases (both aBMD by DXA and trabecular vBMD by HRpQCT) and fracture risk reductions obtained when the patient is on PTH are lost when anabolic therapy is stopped (Leder *et al.*, 2009; Black *et al.*, 2005) as a result there is a substantial body of evidence supporting this treatment sequence. In the European Study of Forsteo (EUROFORS) women with post-menopausal osteoporosis were randomised to further teriparatide, raloxifene or calcium and vitamin D. Participants in the raloxifene arm maintained gains in lumbar spine BMD and had further increased in BMD at the total hip (Eastell *et al.*, 2009).

Bisphosphonates have proved effective for preventing bone loss following discontinuation of PTH. The PaTH study demonstrated alendronate has suitability in this indication following discontinuation of PTH 1-84, during the alendronate treatment phase, further increases in hip and neck of femur aBMD were observed(Black *et al.*, 2005).

The DATA-Switch study evaluated the efficacy of teriparatide for 2-years, followed by denosumab for 2-years, it concluded patients had increases of aBMD at the lumbar spine and hip (18.3% [95% CI 14.9 to 21.8] and 6.6% [95% CI 5.3 to 7.9] respectively) (Leder *et al.*, 2015). Further sub-group analysis of this study found teriparatide followed by denosumab to improve total and cortical vBMD, cortical thickness and estimated strength on HRpQCT (Tsai *et al.*, 2016; Tsai *et al.*, 2017).

In the ACTIVExtend study, post-menopausal women who were randomised to abaloparatide, or placebo for 18-months in the ACTIVE study were offered a further 2-years of open label alendronate 70mg weekly. At the end of this treatment, there was a reduction in vertebral fractures, non-vertebral fractures, all clinical fractures and major osteoporotic fractures (relative risk reductions of 84%, 39% , 34% and 50% respectively (all $P \leq 0.05$) in the abaloparatide to alendronate group vs the placebo to alendronate group (Bone *et al.*, 2018; Cosman *et al.*, 2023). The FRAME study reported a reduction in vertebral fracture risk 2-years after switching from romosozumab to denosumab after 1-year compared to denosumab for 2-years (0.62% versus 1.26% [odds ratio = 0.45; p=0.003]). There was no reduction in rates of clinical, non-vertebral and hip fractures (Cosman *et al.*, 2024; Cosman *et al.*, 2016).

In the ARCH study, post-menopausal women with a fragility fracture were randomised to romosozumab or alendronate for 1-year, followed by 2-years of alendronate. The romosozumab to alendronate group had reduced non-vertebral fractures compared to the alendronate-to-alendronate group, but there was no difference in vertebral fracture rate between the two groups, (Saag *et al.*, 2017) and McMclung *et al. 2020,* have recently reported that a single dose of zoledronate maintains BMD gains following romosozumab treatment albeit they do not report the effects on fracture risk (McClung *et al.*, 2020).

7.8.3.3 An Antiresorptive Agent Followed by an Antiresorptive Agent

In 2012 the International Osteoporosis Foundation recommended that in the event of treatment failure with a weak anti-resorptive agent (e.g. raloxifene or ibandronate), further treatment with a more potent oral agent (e.g. alendronate or risedronate) should follow. If treatment failure occurs on a potent oral antiresorptive further treatment with a parental antiresorptive (e.g. denosumab or zoledronate) is appropriate (Diez-Perez *et al.*, 2012). Subsequently this guidance has been superseded by the UK National Osteoporosis Guideline Group (NOGG) Guideline for the Prevention and Treatment of Osteoporosis, which has been endorsed by NICE (Gregson *et al.*, 2022). This guideline recommends the following treatment pathway an antiresorptive as first line therapy stating oral alendronate, oral risedronate and IV zoledronate represent the most cost-effective options. Denosumab is the most potent antiresorptive (Cummings *et al.*, 2009) but is considered a second line antiresorptive, due to the risk of rebound vertebral fracture and requirement for ongoing bisphosphonate

treatment following discontinuation (Gregson *et al.*, 2022; Tsourdi *et al.*, 2020; Sølling *et al.*, 2023; Kendler *et al.*, 2020).

7.8.3.4 Combination Therapy

The NOGG guideline for the Prevention and Treatment of Osteoporosis does not consider combination therapy for the treatment of osteoporosis (Gregson *et al.*, 2022). There are however a small number of studies evaluating this approach, although findings are mixed and participant numbers small. In a study assessing adding vs. switching to teriparatide in patients on alendronate or raloxifene, alendronate was found to increase bone turnover markers more in the 'switch' group [6-month PINP (64 vs. 401%); bone ALP (15 vs. 71%); βCTX (27 vs. 250%); all $P<0.001$] however BMD gains at the total hip were greater in the 'add' group (6-month BMD +1.4 vs. -0.8%; $P=0.002$) (Cosman *et al.*, 2009). The PaTH trial compared the effect of adding PTH 1-84 and alendronate to monotherapy with either agent, there was no difference in the aBMD increase at the spine between any of the groups. Trabecular BMD at the spine and bone formation markers increased in the PTH 1-84 treated group compared to both the combination therapy group and the alendronate monotherapy group (Black *et al.*, 2003). The combination of PTH and bisphosphonate in the treatment of bisphosphonate naïve patients is less effective at increasing aBMD and vBMD compared to PTH alone (Cosman *et al.*, 2011; Black *et al.*, 2003).

Parathyroid hormone analogues in combination with zoledronate, a more potent antiresorptive have been compared to monotherapy with each of the drugs for 1-year. Lumbar spine BMD increased 7.5%, 7.0%, and 4.4% in the combination, teriparatide, and zoledronic acid groups, respectively ($P<0.001$ for combination and teriparatide versus zoledronic acid). Total hip BMD increased 7.5%, 7.0%, and 4.4% in the combination, teriparatide, and zoledronic acid groups, respectively ($P<0.001$ for combination and teriparatide versus zoledronic acid) (Cosman *et al.*, 2011). These findings suggest that the combination of PTH with zoledronate does not negatively affect the anabolic activity of PTH at trabecular bone sites and is beneficial in preventing excessive cortical remodelling at the hip. The DATA trial evaluated the combination of teriparatide and denosumab following a per-protocol analysis of 84-patients, over 24-months total hip BMD increased by >3% in 36%, 53%, and 92% of women in the teriparatide, denosumab, and combination groups, respectively,

and by >6% in 11%, 17%, and 50% in the teriparatide, denosumab, and combination groups, respectively (*P*<0.01 for all comparisons vs combination), similar results were seen at the femoral neck. At the lumbar spine, there was no difference in the number of patients with a >3% BMD but >6% increase in BMD were seen in 63%, 78%, and 100% of women in the teriparatide, denosumab, and combination groups, respectively (combination vs teriparatide, *P*=0.001; combination vs denosumab, *P*=0.016) (Leder *et al.*, 2016; Tsai *et al.*, 2017). Overall, greater increases in BMD were seen in the combination group compared to either therapy alone and whilst this is a small trial in osteoporotic patients it supports continued investigation in this patient cohort. Future studies would be more clinically meaningful if they considered fracture reduction as endpoints.

It remains to be seen if sequential or combination therapy have a place in the treatment of BSIs. If PTH were to prove effective in the treatment of BSI's this is a potential area for future investigation.

7.9 Thesis Objectives

This thesis has four key aims:

Aim 1: To explore the association between properties of the ultradistal tibia and lower body bone stress injuries in young adult men undergoing arduous military training.

Hypothesis 1: Trabecular microarchitecture and estimated mechanical strength would be associated with lower body stress fracture injury sustained during basic military training.

Aim 2: To identify the level and quality of evidence for the efficacy of PTH analogues on fracture healing in the published literature.

Hypothesis 2: PTH Analogues are effective is for fracture healing in all cause fracture types.

Aim 3: To investigate the pharmacokinetic and pharmacodynamic equivalence of teriparatide between men and women.

Hypothesis 3: There is no difference in the pharmacokinetic and pharmacodynamic profiles of teriparatide between men and women.

Aim 4: To investigate the efficacy of teriparatide on BSI healing in military personnel undergoing arduous basic military training.

Hypothesis 4: Treatment with a teriparatide SC injection accelerates: i) the time to healing of a BSI injury; ii) the cessation of pain associated with the injury, and; iii) the return to training time, compared to standard care.

8 General Methods

Experimental methods which were common to all chapters of this thesis are described below. The remaining experimental methods are described within the chapter to which they relate due to the diverse experiments included in this thesis.

8.1 Ethics

Ethical approval was obtained from the Ministry of Defence (UK) Research Ethics Committee (MODREC) (Chapters 9, 10, 12, 13) and Medicines and Healthcare Research Authority (MHRA) (Chapters 12 and 13). All protocols were conducted in accordance with the Declaration of Helsinki (2013). The nature and purpose of each study was fully explained in writing and verbally to each participant. All participants voluntarily agreed to participate and provided fully informed written consent (appendix 1).

8.2 Anthropometry

At baseline in all studies (Chapters 9, 10, 12, 13), height was measured using a stadiometer and body mass was determined using a digital platform scale (Seca, Hamburg, Germany).

8.3 Blood Collection and Handling

Whole blood samples were collected by venepuncture (Chapters 10 and 13) and cannulation (Chapter 12) from an antecubital vein into plain containers containing 1) a serum separator gel and 2) Ethylenediaminetetraacetic acid (EDTA) vacutainers (Becton Dickinson, Oxford, UK). Plain (serum) samples were left to clot at room temperature for 60 minutes before centrifugation. EDTA (plasma) samples were spun within 30 minutes of collection. Samples were centrifuged for 10 minutes at 4,000 rotation per minutes (rpm) and the acellular fluid aliquoted, frozen and stored in 24/7 temperature monitored -80°C freezers (t-scan monitoring solutions, London, UK).

8.4 Blood Analysis

In chapters 10 and 12, biochemical and haematology analyses were undertaken at the Norfolk and Norwich University Hospital NHS Trust (NNUH) (Norwich, UK). Biochemical analyses performed on the Abbott Alinity c platform (Illinois, USA). The methodologies were as follows; i) creatinine, total cholesterol and alanine transferase - enzymatic, ii) urea - urease, iii) sodium & potassium - ion-selective electrode diluted (integrated chip technology method), iv) phosphate – phosphomolybdate, v) albumin - colorimetric (bromocresol purple), vi) alkaline phosphatase - para-nitrophenyl phosphate, vii) calcium - Arsenazo III, viii) triglycerides - glycerol phosphate oxidase, ix) high density lipids - accelerator selective detergent, x) total bilirubin - diazonium salt, x) total protein – biuret.

Adjusted calcium (Adj Ca) was calculated using the equation;

$$\text{Adjusted Calcium} = \text{Total Calcium (mmol/L)} + 0.018\ (\text{Albumin (g/L)} - 41)$$

Globulins were calculated using the equation;

$$\text{Globulins (g/L)} = \text{Total protein (g/L)} - \text{Albumin (g/L)}$$

Haematological analyses on the Abbott Alinity Hq platform. Counts of white blood cells, their differentials (lymphocytes, neutrophils, monocytes, eosinophils and basophils) and red blood cells, were determined by optical counting and fluorescence. Platelets were measured by optical counting. Haemoglobin concentration was measured by photometry.

In chapters 10, 12 & 13 all research biochemical analyses were undertaken by the Bio-Analytical Facility at the University of East Anglia (Norwich, UK) following GCP guidelines and their respective analytical plan. Analytical plan, in-life experiments and analytical reports were audited by an external quality assurance consultant (Buckingham QA Consultancy Ltd, Lowestoft, UK). The laboratory participates in external QA schemes for LCMS 25(OH)D and 1,25$(OH)_2$D (Vitamin D External Quality Assessment Scheme, DEQAS) and in a sample swap for CTX and PINP (NNUH based scheme).

C-terminal telopeptide (CTX), amino-terminal propeptide of type 1 procollagen (P1NP) and PTH were measured using a Roche Diagnostics Limited (Burgess Hill, UK- Roche thereafter) immunoassay on a COBAS 6000 analyser. PTH (1-34) was measured by liquid chromatography mass spectrometry (LCMS) on a Waters Limited (Wilmslow, UK -thereafter Waters) Xevo using an in-house method (Al Riyami, 2017). Cyclic adenosine monophosphate (cAMP), 25(OH)D2, 25(OH)D3, 24,25$(OH)_2$D2 and 24,25$(OH)_2$D3 were measured by LCMS on a Waters Ultima Quattro using an in-house method. Lower limit of quantification and imprecision for the assays can be found in table 8-1.

Table 8-1: Lower limit of quantification and impression for 25(OH)D2, 25(OH)D3, 24,25$(OH)_2$D2 and 24,25$(OH)_2$D3, P1NP CTX, PTH, PTH (1-34) and cAMP.

Assay Analyte	Mean CV (across a range of concentrations)		Lower Limit of Quantification
	Intra-assay (%)	Inter-assay (%)	
25(OH)D2	4.9	7.4	0.1 $nmol.L^{-1}$
25(OH)D3	8.3	9.6	0.1 $nmol.L^{-1}$
24,25$(OH)_2$D2	7.7	10.6	0.8 $nmol.L^{-1}$
24,25$(OH)_2$D3	9.0	8.9	0.8 $nmol.L^{-1}$
CTX	2.5	2.7	0.01 $\mu g.L^{-1}$
P1NP	1.6	7.3	5 $\mu g.L^{-1}$
PTH	3.2	7.4	0.13 $pmol.L^{-1}$
PTH (1-34)	6.3	10.0	5 $pg.L^{-1}$
cAMP	7.1	6.5	3 $nmol.L^{-1}$

Sclerostin, Osteoprotegerin, sRANKL and Dickkopf WNT signaling pathway inhibitor 1 (DKK1) were measured by enzyme-linked immunosorbent assay (ELISA) using a Biomedica assay kit (Vienna, Austria). FGF-23 c-terminal was measured by ELISA (Quidel, San Diego, USA). sRANKL, OPG and c-FGF23 assays were performed using an Agility ELISA automated system (Dynex Technologies Inc, Chantilly, VA USA - Dynex thereafter).

8.5 Imaging

8.5.1 Dual X-Ray Absorptiometry

DXA (Lunar iDXA™, GE Healthcare) equipped with Encore 13 software was used to assess whole body (Chapters 9 and 10), spine, and dual femoral neck (Chapter 13) composition. Daily quality control checks with a standardised control phantom (GE Healthcare) were conducted according to the manufacturers guidelines to validate the device prior to scanning. BMD was expressed in terms of absolute BMD values (g/cm^3). Participants were asked to wear shorts and t-shirt, and to remove shoes and jewellery. The CV for leg aBMD in our laboratory is 0.6% (chapter 9).

A total body scan was performed with participants lying supine on the scanning table. Velcro straps were placed around the knees and ankles. Whole body and regional aBMD, BMC, fat mass and lean mass were analysed from the scan.

A spine scan was performed with participants lying supine on the scanning table, a foam block was placed under the participants legs with their knees bent and the lower leg parallel to the bed. The spine was aligned to the centre line of the bed. Regional aBMD of L1-4 was analysed from the scan. A laser indicator was used to position the x-ray arm prior to scanning.

A dual femur scan was performed with participants lying supine on the scanning table, a triangle frame was placed between the participants legs. The participant was asked to rotate their legs inward towards the frame ensuring that the twist came from the hip rather than the knee. Ankles were secured with Velcro straps. A laser indicator was used to position the x-ray arm prior to scanning.

8.5.2 High-Resolution Peripheral Quantitative Computed Tomography

HRpQCT was used to measure volumetric density, morphology, and microarchitecture at the ultradistal radius and tibia (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland).

The participant's limb was placed in an anatomically shaped carbon-fibre shell to immobilise it during the scan. HR-pQCT is sensitive to small movements so participants were asked to

remain completely still during the scanning procedure. An anteroposterior scout view was used to acquire and identify the region of interest. The metaphyseal site proximal to the tibial plafond was located using a manufacturer mask. The rationale for selecting this site was due to its ability to define the region of interest using an anatomic landmark. Data acquisition for each site took 3 minutes and exposed participants to less than 0.01 mSv.

Measurements were performed at the non-dominant wrist and leg unless there was a history of fracture at those sites, in which case the non-fractured side was measured. The standard evaluation procedure provided by the manufacturer (software version 6.0) was used to derive the following outcome variables: total, trabecular, and cortical vBMD (mg HA·cm^3); trabecular bone volume (%); trabecular area (mm^2); trabecular number (1•mm); trabecular thickness (mm); trabecular spacing (mm); cortical area (mm^2); cortical thickness (mm); cortical perimeter (mm); cortical pore diameter (mm) and cortical porosity (%). Finite element analysis was performed using manufacturer software, as described previously (Vilayphiou *et al.*, 2011) to calculate biomechanical properties stiffness (kN•mm) and failure load (kN) under uniaxial compression.

In addition to the standard evaluation protocol provided by the HR-pQCT manufacturer, a detailed cortical bone analysis by a semi-automated segmentation technique was performed as previously described (Ackerman *et al.*, 2012; Burghardt *et al.*, 2010a; Nishiyama *et al.*, 2010; Buie *et al.*, 2007). The 3D HR-pQCT images were used to perform linear µFEA and calculate apparent biomechanical properties under uniaxial compression, as previously described (Ackerman *et al.*, 2012; Boutroy *et al.*, 2008; Burghardt *et al.*, 2010b).

A single investigator performed all contouring to ensure consistency. The coefficient of variation (CV) for parameters in our laboratory ranges from 0.1% to 3.2% for geometry, density, and microarchitecture, and from 9.5% to 10.0 for cortical porosity (chapter 9).

8.6 Statistical Analysis

Statistical analysis varied for each study and the methodology is detailed in the relevant chapters.

9 Intra Operator Reliability of High-Resolution Peripheral Quantitative Computed Tomography and Dual Energy X-Ray Absorptiometry Scans

9.1 Summary

Dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT) are widely used imaging tools to assess the macro and microstructure of bone. These techniques are used in this thesis to estimate bone strength by using measures of bone macro and microstructure to predict likelihood of BSI (Chapter 9) and to monitor bone changes in response to teriparatide treatment (Chapter 13).

The aim of this prospective study is to investigate the utility of DXA and HR-pQCT when comparing bone material properties and the reliability of any conclusions drawn from data collected from them.

9.2 Introduction

Dual X-Ray Absorptiometry measures whole-body areal BMD which is used clinically in the diagnosis of osteoporosis, and commonly used in research to assess the effect of exercise and nutritional interventions on bone health (Nana *et al.*, 2015; O'Leary *et al.*, 2019a). It provides areal BMD measuring integral BMD of cortical and trabecular bone. Dual energy X-ray absorptiometry cannot distinguish between cortical and trabecular bone, quantify volumetric BMD (vBMD), geometry, microarchitecture and mechanical properties (Geusens *et al.*, 2014; Cheung *et al.*, 2013), which make it insensitive for detecting changes in bone strength (Turner and Robling, 2003). The addition of HRpQCT – a three dimensional technique – enables measurement of volumetric BMD and characterisation of bone geometry, microarchitecture and mechanical properties of bone in the peripheral skeleton (Boutroy *et al.*, 2005). These are key contributors to bone strength (Dalle Carbonare and Giannini, 2004; Brandi, 2009; Seeman and Delmas, 2006; Augat and Schorlemmer, 2006; Fonseca *et al.*, 2014) which allow estimation of bone strength using finite element analysis (Vilayphiou *et al.*, 2011). Both DXA (Hind, Oldroyd and Truscott, 2010) and HR-pQCT (Hughes *et al.*, 2018a; Burghardt *et al.*,

2010a) can be used to assess longitudinal changes in bone quality and is important in clinical research to allow evaluation of disease progression, treatment and exercise interventions. They demonstrate good reliability but are influenced by a number of factors including the operator (Nana *et al.*, 2015; Bonaretti *et al.*, 2017).

Before drawing conclusions from these changes, it is important to first characterise the variability of the measures locally. The first aim of this study is to assess the intra-operator reliability of DXA and HR-pQCT outcomes.

The aims of this study are to:

- Investigate the reliability of DXA-derived measures of whole-body and regional areal BMD, fat mass and fat-free mass;
- Investigate the reliability of HR-pQCT measures of trabecular and cortical vBMD, geometry, microarchitecture and mechanical strength of the tibia.
- geometry, microarchitecture and mechanical strength of the tibia.

9.3 Method

In this prospective study convenience sample of male and female, civilians, or military personnel, were recruited. Participants were aged between 18 and 36 years inclusive. Potential participants were excluded if they were pregnant, had a history of back problems (including previous spinal surgery, structural abnormalities of the spine or spinal cord) or had metal implants or plates to the tibia, radius, hips or spine.

The study was advertised by email (including attached patient information sheet) among colleagues and associates at Army Health and Performance Research, Army Recruiting and Initial Training Command, Infantry Training Centre Catterick and University Collaborators. Potential participants were asked to contact the investigators following which they were given a full verbal brief about the study procedures and risks by phone or in person with the opportunity to ask questions.

Consenting participants visited the Clinical Research Facility at the Royal Military Academy, Sandhurst, on two occasions, separated by one week (all participants attended during Aug 2019). During each visit, a whole-body DXA scan, and two tibial HR-pQCT scans were performed.

Female participants underwent a pregnancy test before commencing any study measures.

9.3.1 Participants

Participants were recreationally active males (n=4) and females (n=6) (mean ± SD, age 25.9 ± 1.5 years, height 171.2 ± 8.5 cm, mass 69.0 ± 2.8 kg) from Military sites around the south of England.

9.3.2 Dual Energy X-ray Absorptiometry

A total body scan using a DXA scanner (7 min) (Lunar iDXA™, GE Healthcare) equipped with Encore 13 software was performed with participants lying supine on the scanning table. Participants were asked to wear shorts and t-shirt, and to remove shoes and jewellery. Velcro straps were placed around participants knees and ankles. Scans were performed by a trained operator with IR(ME)R qualification.

Whole body and regional aBMD, BMC, fat mass and lean mass were analysed from the scan. Daily calibration with a standardised manufacturer supplied control phantom (GE Healthcare) was conducted to validate the device prior to scanning. BMD was expressed in terms of absolute BMD values (g/cm^3) and T scores.

9.3.3 High-Resolution Peripheral Quantitative Computed Tomography

High-resolution peripheral quantitative computed tomography scans (Xtreme CTII, Scanco Medical) were performed at the ultradistal (4% of length) and diaphyseal sites (30% of length) of the non-dominant tibia – limb dominance was defined as the primary stabilising side - using the manufacturers standard in-vivo protocol. The leg was immobilised in a carbon-fibre cast for scanning. The XtremeCT scanner was equipped with software version 6.0. Daily calibrations with a standardised manufacturer supplied control phantom (Scanco Medical)

were conducted to standardise measurements. The reference line was set manually, with the region of interest defined using the anteroposterior scout view. A total of 110 slices were carried out at each site. The effective dose equivalent for each scan was lower than 10µSv for each patient and the measurement time was 2.8 min. Motion grading (1-3) of scans was assessed using the Scanco SOP scale and scans graded 3 were excluded from the analysis. At the ultradistal site the following measures were taken; (1) total vBMD (mg HA·cm^3) (2) trabecular vBMD (mg HA·cm^3) (3) cortical vBMD (mg HA·cm^3) (4) trabecular bone volume (%) (5) trabecular area (mm^2) (6) trabecular number (1•mm) (7) trabecular thickness (mm) (8) trabecular spacing (mm) (9) cortical area (mm^2) (10) cortical thickness (mm) (11) cortical perimeter (mm) (12) cortical pore diameter (mm) (13) cortical porosity (%). Finite analysis included stiffness (kN•mm) and failure load (kN). Scans were performed by a trained operator with IR(ME)R qualification.

At the diaphysial site the following measures were taken; (1) total vBMD (mg HA·cm^3) (2) cortical vBMD (mg HA·cm^3) (3) trabecular area (mm^2) (4) cortical area (mm^2) (5) cortical thickness (mm) (6) cortical perimeter (mm) (7) cortical pore diameter (mm) (8) cortical porosity (%). Finite analysis included stiffness (kN•mm) and failure load (kN).

9.4 Statistical Analysis

Data was tested for normality. Bone imaging outcomes were then compared between visit one and visit two using paired sample t-tests, or Wilcoxon-signed Rank tests for non-parametric data, to examine systematic bias. Reliability and variability will be determined using intraclass correlations (ICC2,1) and coefficient of variation (CV).

9.5 Results

DXA Phantom aBMD measurements were maintained within a tolerance of ±1.5% for the duration of the study. HRpQCT phantom vBMD measurements were maintained within a tolerance of ± 1.5% for the duration of the study. The reliability and variability of all measures are described in table 9-1.

Table 9-1: Intra-operator Reliability and Variability of aBMD (DXA) and vBMD (HRpQCT) Measures

	ICC (95%)	**CV (%)**	***P***
DXA			
Total Fat Mass	0.997 (0.985-0.999)	0.68	0.072
Total Lean Mass	0.998 (0.992-1.000)	0.70	0.074_a
Total Bone Mass	0.999 (0.997-1.000)	0.38	0.247
Bone Mineral Content	0.999 (0.997-1.000)	0.38	0.247
Total aBMD	0.998 (0.992-1.000)	0.44	0.098
Arms aBMD	0.981 (0.931-0.995)	1.40	0.270
Legs aBMD	0.997 (0.989-0.999)	0.60	0.304
Trunk aBMD	0.996 (0.986-0.999)	0.77	0.646
Ribs aBMD	0.993 (0.974-0.998)	0.95	0.674
Spine aBMD	0.964 (0.870-0.991)	1.45	0.254
HRpQCT			
Tibial Metaphysis (4% Site)			
Total vBMD (mg HA·cm^3)	1.000 (0.998-1.000)	0.43	0.508
Trabecular vBMD (mg HA·cm^3)	0.999 (0.996-1.000)	0.67	0.503
Cortical vBMD (mg HA·cm^3)	0.962 (0.840-0.991)	0.59	0.981
Trabecular bone volume (%)	0.999 (0.996-1.000)	0.90	0.688
Trabecular area (mm^2)	1.000 (1.000-1.000)	0.07	1.000
Cortical area (mm^2)	0.996 (0.984-0.999)	0.78	0.934
Cortical thickness (mm)	0.994 (0.949-0.999)	1.16	0.036*
Cortical perimeter (mm)	0.914 (0.641-0.980)	1.61	0.097
Trabecular number (1·mm)	0.922 (0.713-0.982)	3.08	0.401
Trabecular thickness (mm)	0.976 (0.900-0.994)	1.23	0.606
Trabecular spacing (mm)	0.977 (0.909-0.995)	2.45	0.481
Cortical pore diameter (mm)	0.870 (0.555-0.968)	3.20	0.230
Cortical porosity (%)	0.806 (0.336-0.953)	9.99	0.916
Stiffness (kN·mm)	1.000 (0.998-1.000)	0.02	0.162
Failure load (kN)	0.999 (0.995-1.000)	0.01	0.299

	ICC (95%)	CV (%)	*P*

Tibial Diaphysis (30% Site)			
Total vBMD (mg HA·cm^3)	0.988 (0.947-0.997)	0.59	0.995
Cortical vBMD (mg HA·cm^3)	0.994 (0.974-0.999)	0.15	0.572
Trabecular area (mm^2)	0.996 (0.982-0.999)	1.19	0.857
Cortical area (mm^2)	0.996 (0.981-0.999)	0.87	0.485
Cortical thickness (mm)	0.988 (0.949-0.997)	1.32	0.280
Cortical perimeter (mm)	0.999 (0.994-1.000)	0.28	0.930
Cortical pore diameter (mm)	0.754 (0.253-0.938)	0.28	0.106
Cortical porosity (%)	0.939 (0.738-0.986)	9.48	0.108
Stiffness (kN·mm)	1.000 (0.998-1.000)	0.42	0.162
Failure load (kN)	0.999 (0.995-1.000)	0.74	0.299

DXA, dual x-ray absorptiometry; HRpQCT, high-resolution peripheral quantitative computed tomography; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; ICC, interclass correlation; CV, coefficient of variation; *P*, probability.

a. Total lean mass was non-normal data, this P value was calculated using the Wilcoxon test.

9.5.1 Dual X-Ray Absorptiometry

There were no reported significant differences for total fat mass, total lean mass, total bone mass, bone mineral content or any of the BMD measures (Table 9-1). All measures demonstrated good reliability (Interclass correlation (ICC) range 0.964 – 0.999) with small variability (CV 0.38% – 1.95%) between the repeat scans. An example body composition analysis is shown in figure 9-1.

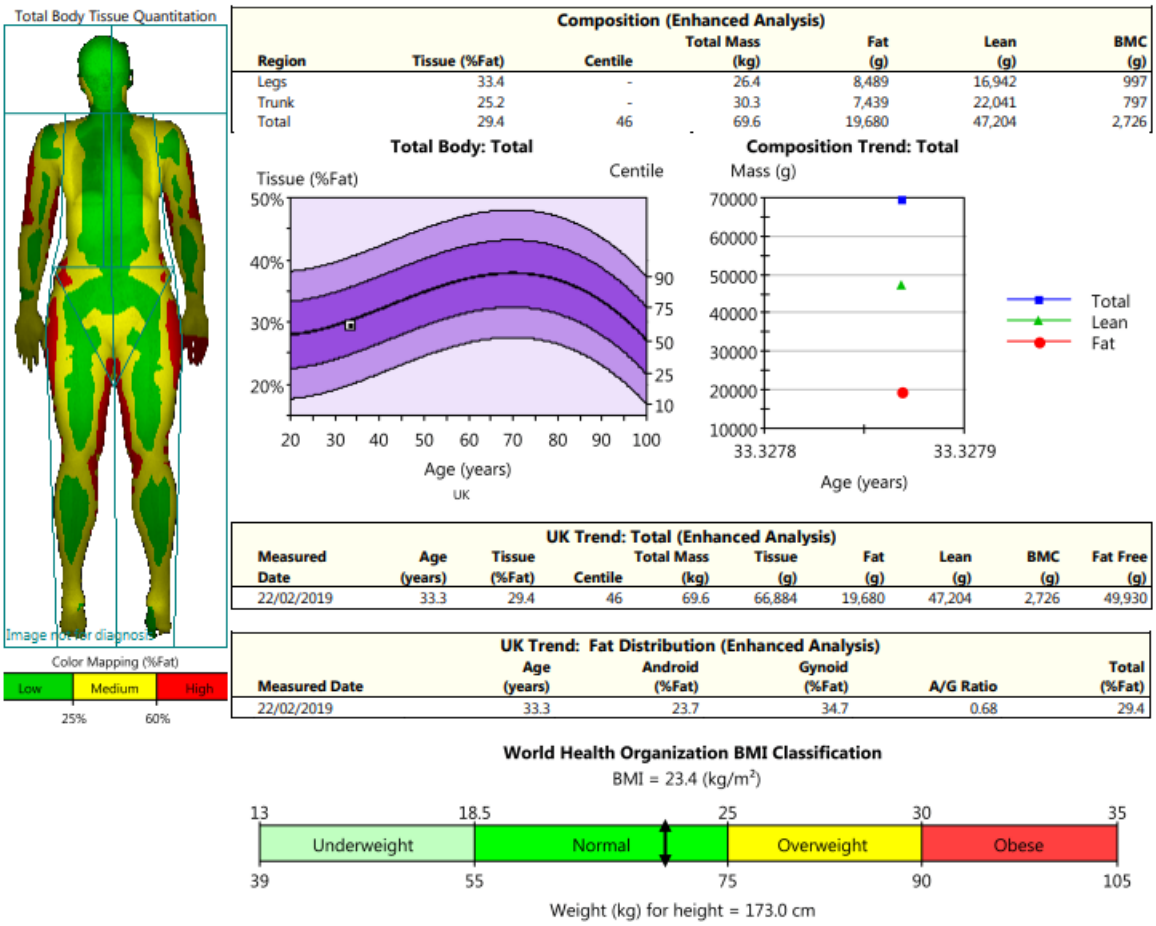


Figure 9-1: Example total body composition output from a dual x-ray absorptiometry scan.

Total Fat Mass. There were no reported differences between total fat mass between scans (t test, *P* = 0.072). The repeat scans demonstrated excellent reliability (ICC = 0.997) and small variability (CV=0.68%).

Total Lean Mass. There were no reported differences between total lean mass between scans (Wilcoxon test, *P* = 0.074). The repeat scans demonstrated excellent reliability (ICC = 0.998) and small variability (CV = 0.70%).

Total Bone Mass. There were no reported differences between total bone mass between scans (t test, *P* = 0.247). The repeat scans demonstrated excellent reliability (ICC = 0.999) and small variability (CV = 0.38%).

Bone Mineral Content. There were no reported differences in bone mineral content between scans (t test, *P* = 0.247). The repeat scans demonstrated excellent reliability (ICC = 0.999) and small variability (CV = 0.38%).

Total Areal BMD. There were no reported differences between total areal BMD between scans (t test, *P* = 0.098). The repeat scans demonstrated excellent reliability (ICC = 0.998) and small variability (CV = 0.44%).

Arms Areal BMD. There were no reported differences between arm areal BMD between scans (t test, *P* = 0.270). The repeat scans demonstrated excellent reliability (ICC = 0.981) and small variability (CV = 1.40%).

Legs Areal BMD. There were no reported differences between arm areal BMD between scans (t test, *P* = 0.304). The repeat scans demonstrated excellent reliability (ICC = 0.997) and small variability (CV = 0.60%).

Trunk Areal BMD. There were no reported differences between arm areal BMD between scans (t test, *P* = 0.646). The repeat scans demonstrated excellent reliability (ICC = 0.996) and small variability (CV = 0.77%).

Ribs Areal BMD. There were no reported differences between arm areal BMD between scans (t test, *P* = 0.674). The repeat scans demonstrated excellent reliability (ICC = 0.993) and small variability (CV = 0.95%).

Spine Areal BMD. There were no reported differences between arm areal BMD between scans (t test, *P* = 0.254). The repeat scans demonstrated excellent reliability (ICC = 0.964) and small variability (CV = 1.45%).

9.5.2 High Resolution Peripheral Quantitative Computed Tomography

Ultradistal Tibial (4%) Total vBMD. There were no reported differences in ultradistal tibial (4%) total vBMD between scans (t test, *P* = 0.508). The repeat scans demonstrated excellent reliability (ICC = 1.000) and small variability (CV = 0.43%).

Ultradistal Tibial (4%) Trabecular vBMD. There were no reported differences in ultradistal tibial (4%) trabecular vBMD between scans (t test, *P* = 0.503). The repeat scans demonstrated excellent reliability (ICC = 0.999) and small variability (CV = 0.67%).

Ultradistal Tibial (4%) Stiffness. There were no reported differences in the ultradistal tibial (4%) stiffness between scans (t test, *P* = 0.162). The repeat scans demonstrated excellent reliability (ICC = 1.000) and very small variability (CV = 0.02).

Ultradistal Tibial (4%) Failure Load. There were no reported differences in the ultradistal tibial (4%) failure load between scans (t test, *P* = 0.299). The repeat scans demonstrated excellent reliability (ICC = 0.999) and very small variability (CV = 0.01).

Diaphyseal Tibial (30%) Total vBMD. There were no reported differences in ultradistal tibial (4%) total vBMD between scans (t test, *P* = 0.995). The repeat scans demonstrated excellent reliability (ICC = 0.988) and small variability (CV = 0.59%).

9.6 Discussion

The findings from this technique study examine the intra-operator reliability of DXA and HRpQCT imaging techniques. The DXA parameters all had a CV value of less than 1.5% which is considered acceptable as per the International Society for Clinical Densitometry (Lewiecki *et al.*, 2016). Similar industry standards do not exist for HRpQCT but the CVs found in this study are similar or better than previously reported (Boutroy *et al.*, 2005) demonstrating that DXA and HRpQCT scans can be used reliably and repeatably by a single operator.

Statistical significance was accepted at 0.05, the only parameter that fell below this was cortical thickness (P = 0.036); this is thought to be a type one error (false positive) due to the high number of parameters analysed (n=35).

HRpQCT and DXA imaging tools are widely used to assess the macro and microstructure of the bone. HRpQCT is primarily used in research settings to measure changes to bone following interventions including drugs, exercise and nutrition (Nana *et al.*, 2015; O'Leary *et al.*, 2019a).

Whilst DXA is also used in research it is also commonly seen in clinical settings in the diagnosis of osteoporosis. DXA is the most widely used technique in clinical practice because of its ability to measure aBMD at the hip and spine, key sites for osteoporosis monitoring. Femoral Neck aBMD (g/cm^2) is used within the Fracture Risk Assessment (FRAX) tool which was developed to evaluate fracture risk in patients when combined with clinical risk factors. The FRAX algorithms give the 10-year probability of hip and major osteoporotic fracture (clinical spine, forearm, hip or shoulder) (Kanis *et al.*, 2009). Interpretation of DXA scans are affected by precision errors (investigated in this chapter), which are a measure of the reproducibility of BMD results in individual patients. Accuracy errors may also play a role: these are random errors caused by the inhomogeneous distribution of adipose tissue in the human body, that affects bone marrow and soft tissue, external to bone in the path of the X-ray beam.

DXA cannot, distinguish between cortical and trabecular bone, quantify vBMD, report geometric, microarchitectural or mechanical properties (Geusens *et al.*, 2014; Cheung *et al.*, 2013) and is insensitive to changes in bone strength.

HRpQCT can be used to assess cortical and trabecular vBMD, geometry and microstructure at peripheral sites. These measures are considered to be the key contributors to bone strength (Dalle Carbonare and Giannini, 2004; Seeman and Delmas, 2006; Brandi, 2009; Augat and Schorlemmer, 2006; Fonseca *et al.*, 2014) and can be used to predict bone strength using finite element analysis (Fonseca *et al.*, 2014). Both DXA (Vilayphiou *et al.*, 2011; Hind, Oldroyd and Truscott, 2010) and HRpQCT (Hughes *et al.*, 2018a) demonstrate good reliability but are influenced by a number of factors, in addition to the operator (Nana *et al.*, 2015; Burghardt *et al.*, 2010a; Bonaretti *et al.*, 2017). The findings of this study correspond to other studies which have reported similar low intra-operator variability (Bonaretti *et al.*, 2017; Burghardt *et al.*, 2010a).

9.7 Limitations

Although the aim of this study was to examine the reliability of intra-operator variability, limitations of this approach include transferability to the real-world setting where in longitudinal studies the operator conducting the baseline scan is often different to the one conducting the follow up.

9.8 Conclusions

In summary, these results suggest that repeat measures by HRpQCT and DXA imaging have good reliability and minor variability and are useful measures by which the effect of clinical interventions may be measured.

10 Bone Stress Injury Associations with Bone Geometry and Biochemical Markers of Bone Metabolism

10.1 Introduction

Bone stress injuries (BSIs) at weight-bearing sites are common in military training, particularly at the tibia (Sharma *et al.*, 2015; Milgrom *et al.*, 1985; Wentz *et al.*, 2011). Repetitive loading causes fatigue damage, whereby targeted bone remodelling temporarily increases cortical porosity (Hughes *et al.*, 2017). This temporary porosity weakens bone and, without sufficient recovery, can act as a site of concentrated stress leading to a BSI (Hughes *et al.*, 2017). Bone stress injuries range from i) periostitis; to ii) periosteal, endosteal and bone tissue oedema; to iii) partial or complete stress fracture (Beck and Drysdale, 2021). The resistance of the tibia to BSI is determined by bone strength and toughness, which, in turn is underpinned by structure (size, shape, and microarchitecture) and material properties (O'Leary, Rice and Greeves, 2021). The distal tibial microarchitecture distributes mechanical stress and joint forces to the cortex, and is an important determinant of bone strength, but little is known about how trabecular microarchitecture contributes to BSI risk.

Prospective and retrospective studies using peripheral quantitative computed tomography (pQCT) have reported lower total bone cross sectional area, cortical area, and estimated bone strength of the tibia in athletes and military recruits with stress fractures compared with uninjured controls (Popp *et al.*, 2020; Moran *et al.*, 2012; Moran, Evans and Hadad, 2008); some studies report no significant associations between pQCT outcomes and stress fracture (Moran, Evans and Hadad, 2008; Moran *et al.*, 2012). A wider bone increases resistance to bending forces by placing the cortex further away from the neutral axis (Warden *et al.*, 2005). This biomechanical advantage increases resistance to fatigue damage (Warden *et al.*, 2005). The low resolution of pQCT fails to detect the bone microstructure. Bone microstructure, including trabecular spacing, thickness and separation and cortical porosity, are important contributors to bone strength and may offer further insight into BSI risk (Wentz *et al.*, 2011; Izard *et al.*, 2016; O'Leary *et al.*, 2018b; Wilkinson, Rayson and Bilzon, 2008; Moreira and Bilezikian, 2017).

Post injury cross-sectional studies using high-resolution pQCT (HRpQCT) have demonstrated lower cortical vBMD, trabecular number, trabecular thickness, and a more *inhomogenous* trabecular network at the ultra-distal site of the tibia in soldiers with stress fractures compared with uninjured controls (Schanda *et al.*, 2019); lower trabecular vBMD and trabecular thickness at both ultra-distal and distal tibial sites (Ackerman *et al.*, 2015; Schnackenburg *et al.*, 2011); and, smaller cortical cross sectional area without an increase in cortical porosity (Zendeli *et al.*, 2021) at the tibia in civilian stress fracture cases compared with uninjured controls. No study has prospectively examined pre-injury bone microstructure and BSI risk.

This study used HRpQCT to investigate pre-injury ultra-distal tibial trabecular microarchitecture as a predictor of lower body BSI (including stress fractures to the pelvic girdle, sacrum, coccyx and lower limb) in men during military training. The military training environment is a unique opportunity to prospectively study skeletal risk factors during a controlled model of arduous training with a high incidence of BSI. Infantry training is more arduous than other basic military training courses, with an incidence of stress fracture at 64 per 1000 (Sharma *et al.*, 2015; HQ Army, 2016). The hypothesis of this study was that trabecular microarchitecture and estimated mechanical strength would be associated with lower body stress fracture injury sustained during basic military training.

10.2 Methods

10.2.1 Participants

Male British Army infantry recruits (n=1332) at the Infantry Training Centre, Catterick, UK, were assessed for eligibility for a study examining Army injury risk factors, with a convenience sample (those for whom timetabling enabled the option of participation), of 336 participants invited for HRpQCT (XtremeCT; Scanco Medical AG, Bassersdorf, Switzerland) measurements (Figure 10-1).

Participants were recruited during week 1 of basic military training between April 2014 and June 2016, passed a physician-screened medical assessment during week 1 of training, and were injury free at baseline. The nature and purpose of the study were fully explained both verbally and in writing to each participant. All participants provided written informed consent. Ethical approval was obtained from the UK Ministry of Defence Research Ethics Committee (MODREC/165). All study procedures were conducted in accordance with the Declaration of Helsinki (2013).

10.2.2 Experimental Protocol

Participants were undertaking either the Line Infantry 26-week or the Parachute Regiment 28-week training courses. Both courses are divided into two Phases: Phase One (14 weeks) teaches basic military skills and Phase Two (12 or 14 weeks) is infantry-specific combat training. The training courses include; endurance training, typically involving running in groups; load carriage; circuit training, consisting of high-repetition, low force exercises using all major muscle groups; agility-based gymnasium work using benches and ropes; assault courses; military drill, and; military field exercises (Richmond *et al.*, 2012). Energy expenditures can exceed 4500 kcal/day with body mass losses reaching 14.4kg; the greatest losses are observed in recruits of the Parachute Regiment who complete the most physically arduous training (Wilkinson, Rayson and Bilzon, 2008). At the time of data collection, higher physical standards were required by recruits to pass Parachute Regiment training who, as an example, were required to march 16.1 km in 110 mins (8.9 $km \cdot h^{-1}$) carrying a minimum of 23 kg compared with the Line Infantry who are required to march 12.9 km in 120 mins (6.4 $km \cdot h^{-1}$) carrying 25 kg.

All measurements were obtained in week one of the course, after the medical assessment and before starting physical training. Participants in this sub-analysis had a HRpQCT scan of the ultra-distal tibia for the measurement of bone density, geometry, microarchitecture, and estimated mechanical strength. Whole-body areal BMD (aBMD), lean mass, and fat mass were measured by dual energy x-ray absorptiometry (DXA) (Lunar iDXAä, GE Healthcare equipped with Encore 13 software). A venous blood sample was obtained for the assessment of biochemical markers of bone metabolism. Aerobic fitness, muscle strength, and muscle power were measured with field-based tests of physical fitness. Self-reported demographic and lifestyle characteristics were recorded by questionnaire.

10.2.3 High Resolution Peripheral Quantitative Computed Tomography

Three-dimensional HRpQCT scans (XtremeCT, Scanco Medical AG, Switzerland) were performed at the ultra-distal site of the non-dominant tibia. Limb dominance was defined as the leg primarily used to kick a ball (Hughes *et al.*, 2018b). Further detail regarding the methodology is described in chapter 8.

10.2.4 Dual X-Ray Absorptiometry

A whole-body DXA scan (Lunar iDXA™, GE Healthcare, UK.) was performed as described in chapter 8.

10.2.5 Blood Collection and Handling

Blood collection was as described in chapter 8.

10.2.6 Blood Analysis

Blood analyses were conducted as described in chapter 8.

10.2.7 Anthropometric Data

Height and weight were measured as described in chapter 8.

10.2.8 Physical Performance

Participants completed endurance, strength and power exercise performance assessments wearing Army shorts and T-shirt for all exercise performance assessments, and trainers for the 1.5-mile run.

10.2.8.1 Endurance Exercise Performance

Aerobic fitness was determined from a best effort 2.4 km run time, preceded by an 800 m warm-up led by a military physical training instructor on a standardised outdoor course during routine testing at the Infantry Training Centre, Catterick.

Time to complete the course was recorded to the nearest second. At the time of the study, the 2.4 mile run was used widely among military personnel, with performance indicative of an individual's maximal aerobic capacity (Friedl *et al.*, 2015) as results have a bearing on progression in their military careers.

10.2.8.2 Muscle Strength

Maximum dynamic lift strength was assessed using an incremental lift machine that simulates the power clean weight-lifting movement, as described previously (Fortes *et al.*, 2011). The device consisted of a vertically moving carriage with handgrips 0.3 m above the ground. Without shoes, participants started the incremental lifts with a mass of 20 kg, with the weight lifted to a point where the handgrips were 1.45 m from the ground: replicating the floor height of an Army four tonne truck. With each successful lift, the mass was increased by 5 kg with 1 minute rest between attempts. The test was terminated when participants failed to lift the weight on their second attempt, with the maximum weight successfully lifted recorded.

10.2.8.3 Peak Power Output

Peak power output was measured using a maximum vertical jump height determined using a digital jump meter (Takei Scientific Instruments, Tokyo, Japan) (Fortes *et al.*, 2011). Participants were instructed to jump as high as possible three times. Where an increase in jump height occurred across jumps 1–3, indicative of a learning effect, a final fourth jump was performed. Maximum vertical jump height was recorded as the highest score achieved from the four jumps.

10.2.8.4 Muscle Power Output

Peak power output was calculated from maximum jump height (from a maximum of four attempts) and body mass using a validated equation reflecting instantaneous power output (Sayers *et al.*, 1999):

Peak power output (W) = (51.9 x jump height (cm)) + (48.9 x body mass (kg)) – 2007

10.2.9 Bone Stress Injury Diagnosis

Lower body BSI diagnoses were retrieved from participants' individual medical record. BSI diagnoses were determined by magnetic resonance imaging (MRI) and graded according to the Fredericson scale (Fredericson *et al.*, 1995; Kijowski *et al.*, 2012).

10.3 Statistical Analysis

Data were analysed using SPSS (v. 25, SPSS Inc., IBM, USA). All data were initially checked for normality. All outcomes were compared between male recruits who sustained a BSI and those who did not, using chi-squared tests or independent sample t-tests (or Mann-Whitney U tests for non-parametric data) as appropriate. Forced entry binary logistic regression was used to determine risk factors for BSI. Model one included training regiment, age at start of basic military training, total body mass, lean body mass, height, leg aBMD (average of both legs), and circulating total 25(OH)D, PTH, adjusted calcium, iron, P1NP, and βCTX. Model two included the variables in model one *plus* physical performance (2.4 km run time, maximum dynamic lift strength, and peak power output). Model three included all the variables in model two *plus* distal tibia HRpQCT measures. All variables considered for inclusion were checked for collinearity using variance inflation factors; variables scores ≥ 10 were removed. Statistical significance was accepted at $P < 0.05$.

This was an exploratory analysis and a power calculation was not performed.

10.4 Results

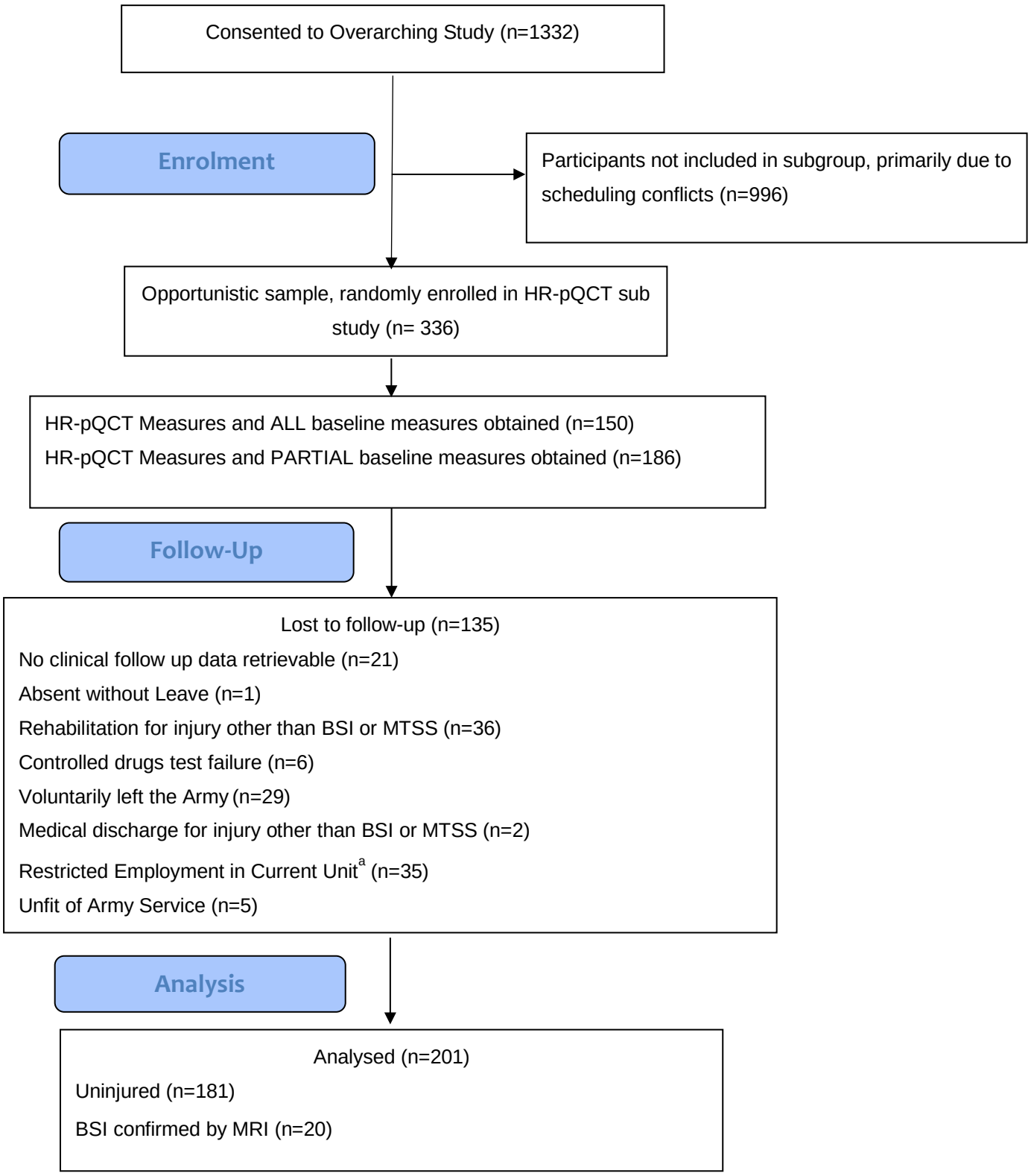
Participants (n=1332) provided consent and 336 participants were selected at random for HRpQCT scans. 135 participants were lost to follow-up because they did not complete training for a reason other than suffering a stress fracture. 201 participants were included in the analyses; 181 were uninjured (no musculoskeletal injury and completed the training course) and 20 sustained a BSI (Figure 10-1). The analysis included all available baseline data.

10.4.1 Bone Stress Injury Incidence

Twenty participants were diagnosed with a BSI by MRI representing 10.0 % of the population at risk, slightly higher than the previously reported rate of 6.3 % (HQ Army, 2016). BSIs were graded between 1 and 4b on the Fredericson scale (Fredericson *et al.*, 1995) and the median week of presentation with an injury was 6.5 [IQR 8.0] weeks after commencing training. The incidence of BSI was higher in the Parachute Regiment (n = 14, 21.9 % of the population) compared with Line Infantry (n = 6, 4.4 % of the population) ($P \leq 0.001$). Anatomical sites of BSI are listed in Table 10-1. No participant presented with multiple fractures.

10.4.2 Differences Between Stress Fracture Cases and Controls

Pre- injury demographics, body composition, physical performance, and biochemistry were not significantly different between non-BSI controls and BSI cases. Cortical area (P = 0.029), stiffness (P = 0.012), and estimated failure load (P = 0.011) were significantly lower in stress fracture cases compared with controls (Table 10-2).



[a] Recruit judged to not be suitable for Infantry training but may be re-deployed to another Corps.

Figure 10-1: A modified STROBE diagram detailing participation in the study.

Table 10-1: Full description of bone stress injuries recorded for participants in the ALLIP study.

Injury No.	Site of BSI	Grade[a]	Week of Presentation (Type of Training: Phase)
1	Metatarsal	3	9 (L:1)
2	Cuneiform	3	4 (L:1)
3	Cuneiform	4b	6 (L:1)
4	Calcaneus	4b	2 (P:1)
5	Calcaneus	4b	7 (P:1)
6	Metatarsal	4b	5 (P:1)
7	Calcaneus	4b	2 (P:1)
8	Calcaneus	4b	5 (P:1)
9	Metatarsal	Not Recorded	19 (L:2)
10	Tibia	1	6 (P:1)
11	Tibia	2	10 (P:1)
12	Tibia	2	9 (P:1)
13	Tibia	2	13 (P:1)
14	Tibia	2	2 (P:1)
15	Tibia	2	21 (P:2)
16	Tibia	4b	21 (P:2)
17	Pubic Rami	4a	4 (P:1)
18	Femur	4a	6 (L:1)
19	Neck of Femur	4	17 (P:2)
20	Site not recorded	3	10 (L:2)

[a] Grade a reported from MRI scan on Fredericson scale (Fredericson *et al.*, 1995; Kijowski *et al.*, 2012).
[b] (L)Lines infantry recruits undertake 12 weeks in phase one training, followed by 14 weeks in phase 2 for a total of 26 weeks in basic training. (P)Parachute regiment recruits undertake 16 weeks in phase one training followed by 12 weeks in phase 2 for a total of 28 weeks basic training.

Table 10-2: Participant characteristics by injury status in the ALLIP study. Categorical data are presented as total (percentage) with p-values calculated by Chi Squared. Continuous data are mean ± SD with P-values from independent t-tests or median [IQR] with P-values from Mann-Whitney U Tests.

	Overall (n=201)	**Non-BSI** (n=181)	**BSI** (n=20)	***P***
Demographics				
Regiment				
Line Infantry	137	131 (95.6%)	6 (4.4%)	< 0.001*
Parachute	64	50 (78.1.6%)	14 (21.9%)	
Alcohol Intake[a]				
Zero Intake	18	17 (94.4%)	1 (5.6%)	0.651
Light (1-90 units per month)	124	112 (90.3%)	12 (9.7%)	
Heavy (91-300 units per month)	47	40 (85.1%)	7 (14.9%)	
Very Heavy (>300 units per month)	1	1 (100.0%)	0	
Smoking[b]				
Never Smoked	76	69 (39.4%)	7 (35.0%)	0.412
Previous Smoker	54	60 (34.3%)	5 (25.0%)	
Current Smoker	65	46 (26.3%)	8 (40.0%)	
Ethnicity[c]				0.657
Asian	2	2 (100.0%)	0	
Black	3	3 (100.0%)	0	
Mixed	3	2 (66.7%)	1 (33.3%)	
White	187	168 (89.8%)	19 (10.2%)	
Other	1	1 (100.0%)	0	
Age at Start of Basic Military Training (years)	20.7 [4.3]	20.7 [4.1]	22.9 [5.0]	0.264
Body Composition and Physical Performance				
Height (cm)	177.7 ± 6.2	177.7 ± 6.2	177.7 ± 5.7	0.486
Body Mass Index (kg/m^2)	24.0 ± 2.7	24.1 ± 2.8	23.1 ± 2.0	0.112
Total Body Mass (kg)	76.0 ± 10.1	73.0 ± 8.6	76.3 ± 10.2	0.129
Lean Body Mass (kg)[e]	57.2 [6.8]	58.0 [7.0]	54.7 [4.1]	0.098
Leg aBMD ($g \cdot cm^2$)[e]	1.36 ± 0.13	1.36 ± 0.13	1.34 ± 0.14	0.441
2.4 km run time (s)[f]	597 [91]	598 [89]	568 [86]	0.130
Maximum Dynamic Lift (kg)[g]	73 [20]	75 [20]	70 [19]	0.414
Peak Power Output (W)[h]	3940 ± 540	3969 ± 536	3731 ± 532	0.083
Biochemistry				
Total 25(OH)D ($nmol \cdot L^{-1}$)	58.3 [43.2]	57.5 [42.9]	61.0 [49.3]	0.389
PTH ($pmol \cdot L^{-1}$)[d]	3.50 [1.51]	3.47 [1.62]	3.77 [1.39]	0.168
Adjusted Calcium ($mmol \cdot L^{-1}$)	2.39 [0.09]	2.39 [0.09]	2.40 [0.12]	0.390
Iron ($\mu mol \cdot L^{-1}$)	20.3 [8.5]	20.5 [8.5]	19.5 [11.7]	0.327
P1NP ($\mu g \cdot L$)[d]	92.8 [45.2]	92.8 [45.2]	97.0 [48.7]	0.650
CTX ($\mu g \cdot L$)[d]	0.47 [0.22]	0.47 [0.22]	0.43 [0.20]	0.175

	Overall (n=201)	Non-BSI (n=181)	BSI (n=20)	*P*
Bone Parameters				
Total vBMD (mg HA·cm^3)	350 ± 50	351 ± 50	338 ± 48.0	0.280
Trabecular vBMD (mg HA·cm^3)	231 ±33	232 ± 32	223 ± 37	0.206
Total Area (mm^2)	840 [188]	846 [194]	791 [165]	0.160
Trabecular Area (mm^2)	690 [205]	794 [207]	647 [172]	0.364
Cortical vBMD (mg HA·cm^3)	889 [47]	891 [45]	896 [60]	0.903
Trabecular Number (1·mm)	2.20 ± 0.29	2.21 ± 0.28	2.12 ± 0.35	0.192
Trabecular Thickness (mm)	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.946
Trabecular Spacing (mm)	0.364 [0.073]	0.361 [0.069]	0.369 [0.116]	0.237
Cortical Area (mm^2)	140 ± 21	141 ± 21	130 ± 17	0.029*
Cortical Perimeter (mm)	113.9 [12.7]	114.2 [12.7]	110.3 [11.0]	0.096
Cortical Porosity (%)	5 [2]	5 [2]	4 [2]	0.217
Cortical Thickness (mm)	1.32 ± 0.24	1.33 ± 0.25	1.26 ± 0.19	0.205
Cortical Pore Diameter (mm)	0.161 [0.022]	0.160 [0.021]	0.167 [0.026]	0.688
Stiffness (kN·mm)	284.0 ± 41.18	286.4±40.71	262.08 ± 39.9	0.012*
Estimated Failure Load (kN)	-14.2 ± 2.0	-14.3 ± 2.0	-13.1 ± 1.9	0.011*

[a]overall n=190, non-BSI n=170.
[b]overall n=195, non-BSI n=175.
[c]overall n=196, non-BSI n=176.
[d]overall n=200, non-BSI n=180.
[e]overall n=192, non-BSI n=173, BSI n=19.
[f]overall n=197, non-BSI n=178, BSI n=19.
[g]overall n=160, non-BSI n=143, BSI n=17.
[h]overall n=144, non-BSI n=128, BSI n=16.
[i]overall n=149, non-BSI n=131, BSI n=18.

10.4.3 Risk Factors for Bone Stress Injuries

In Model 1, training regiment was the only variable associated with BSI incidence ((OR 9.3 [95%CI, 2.6, 33.4]) Parachute *versus* Line Infantry, p ≤ 0.001) when training course, age at start of military training, total body mass, lean body mass, height, leg aBMD, and total 25(OH)D were included. Adding physical performance (2.4-km run time, maximum dynamic lift strength, and peak power output) (Model 2) identified both training course and 2.4-km run time as associated with BSI incidence; for every 1 second increase in run time, there was a 5.5% increase in BSI risk (1.06 [95%CI, 1.02, 1.10), *P* ≤ 0.04). Adding total area, cortical vBMD, trabecular thickness and cortical pore diameter (Model 3), did not change these findings; training course and 2.4-km run time remained the only associated factors (Table 10-3). Total vBMD, trabecular vBMD, trabecular area, trabecular spacing, cortical area, cortical vBMD, cortical perimeter, cortical porosity, cortical thickness, stiffness, and failure load were removed due to collinearity.

Table 10-3: Factors associated with BSI incidence in British Army infantry recruits.

Predictor Variable	Co-Efficient (95% CI)	*p*
Regiment †	748.90* (6.85 to 81866.10)	**0.006**
Age at Start of Military Training	0.94 (0.54 to 1.62)	0.813
Total Body Mass	0.83 (0.54 to 1.16)	0.282
Lean Body Mass	1.00 (1.00 to 1.00)	0.053
Height	1.05 (0.83 to 1.31)	0.698
Leg aBMD	0.98 (0.96 to 1.01)	0.288
Total 25(OH)D	1.03 (0.98 to 1.07)	0.223
2.4-km Run Time	1.05 (1.01 to 1.09)	**0.020**
Peak Power Output	1.00 (0.99 to 1.00)	0.196
Maximum Dynamic Lift Strength	1.00 (0.90 to 1.10)	0.721
Total Area	0.99 (0.97 to 1.01)	0.198
Cortical vBMD	1.01 (0.98 to 1.05)	0.487
Trabecular Thickness	2.08 (0.00 to 6.00×10^{65})	0.282
Cortical Pore Diameter	0.00 (0.00 to 3.35×10^{21})	0.368

†Line Infantry *or* Parachute Regiment
*Odds Ratio

10.4.4 Description of Parachute Regiment Participants

Most injuries were in Parachute Regiment trainees so exploratory analysis was conducted in this sub-group to better understand which, if any, factors contributed to the risk of BSI. Baseline demographics of the participants undergoing Parachute Regiment training overall, and by BSI status, are shown in table 10-4. Uninjured participants had significantly higher total lean mass ($P = 0.025$) and leg aBMD ($P = 0.012$) compared with BSI cases.

Table 10-4: Baseline demographics, alcohol intake, smoking status and ethnicity of participants undergoing Parachute Regiment training. Categorical data are presented as total (percentage), P-values calculated by Chi Squared. Continuous data are Mean±SD, P-value calculated by Independent T Test or Median [IQR], P-value calculated by Mann-Whitney U Test.

	Overall (n=64)	**Non-BSI** (n=50)	**BSI** (n=14)	***P***
Demographics				
Alcohol Intake[a]				
Zero Intake	4 (6.3%)	4 (8%)	0	0.089
Light (1-90 units per month)	42 (65.6%)	35 (70%)	7 (50%)	
Heavy (91-300 units per month)	18 (28.1%)	11 (22%)	7 (50%)	
Very Heavy (>300 units per month)	0	0	0	
Smoking[b]				
Never Smoked	31 (48.4%)	24 (48%)	7 (50%)	0.456
Previous Smoker	28 (43.8%)	21 (42%)	7 (50%)	
Current Smoker	5 (7.8%)	5 (10%)	0	
Ethnicity[c]				
Asian	2 (3.1%)	2 (4%)	0	0.644
Black	1 (1.6%)	1 (2%)	0	
White	61 (95.3%)	47 (94%)	14 (93.3%)	
Age at Start of Military Training (years)	21.61 [4.86]	21.33 [4.76]	22.63 [5.29]	0.697
Body Composition and Physical Performance				
Height (cm)	177.3 ± 6.2	177.4 ± 6.5	176.9 ± 5.2	0.400
Body Mass Index (kg/m^2)	24.0 ± 2.3	24.3 ± 2.4	23.0 ± 1.8	0.059
Total Body Mass (kg)	75.7 ± 9.5	72.2 ± 9.0	76.7 ± 9.5	0.123
Total Lean Mass (kg)[a]	59.1 ± 6.3	60.1 ± 6.6	55.6 ± 3.7	0.025*
Leg aBMD (g/cm^2)[a]	1.39 [0.21]	1.41 [0.22]	1.27 [0.17]	0.012*
2.4-km Run Time (secs)[b]	551 [35]	547 [47]	552 [35]	0.296
Maximum Lift (kg)[c]	75.0 [15]	75 [18]	74 [15]	0.338
Peak Power Output (W)[d]	4005 ± 559	4081 ± 549	3790 ± 555	0.122
Biochemistry				
Total 25(OH)D ($nmol \cdot L^{-1}$)	69.0 ± 25.9	68.6 ±25.9	70.5 ± 26.8	0.565
PTH ($pmol \cdot L^{-1}$)[e]	3.83 ± 1.22	3.84 ±1.32	3.82 ± 0.81	0.964
Adjusted Calcium ($mmol \cdot L^{-1}$)	2.37 ± 0.07	2.36 ±0.07	2.38 ± 0.07	0.264
Iron ($\mu mol \cdot L^{-1}$)	20.5 ± 7.4	20.7 ±7.3	20.1 ± 7.9	0.792
P1NP (µg/L)[f]	93.5 [51.8]	93.5 [51.2]	96.6 [55.6]	0.791
CTX (µg/L)[f]	0.45 [0.19]	0.45 [0.18]	0.46 [0.25]	0.710
Bone Parameters				
Total vBMD ($mg\ HA \cdot cm^3$)	353 ± 52	3547 ± 53	349 ± 50.0	0.744
Trabecular vBMD ($mg\ HA \bullet cm^3$)	232 ± 38	233 ± 38	229 ± 38	0.691
Total Area (mm^2)	850 ± 127	8638 ± 129	802 ± 107	0.114
Trabecular Area (mm^2)	697 ± 128	708 ± 131	660 ± 111	0.212
Cortical vBMD ($mg\ HA \cdot cm^3$)	888 [52]	888 [47]	893 [64]	0.826
Trabecular Number ($1 \cdot mm$)	2.13 ± 0.40	2.25 ± 0.30	2.13 ± 0.40	0.227
Trabecular Thickness (mm)	0.087 ± 0.013	0.087 ± 0.013	0.090 ± 0.010	0.351
Trabecular Spacing (mm)	0.36 [0.08]	0.353 [0.73]	0.37 [0.10]	0.363
Cortical Area (mm^2)	143 ± 20	145 ± 20	134 ± 17	0.075
Cortical Perimeter (mm)	114.1 [13.3]	115.9 [12.6]	109.7 [11.2]	0.077

	Overall (n=64)	**Non-BSI** (n=50)	**BSI** (n=14)	***P***
Cortical Porosity (%)	5 ± 2	5 ± 2	5 ± 2	0.645
Cortical Thickness (mm)	1.35 ±0.22	1.36 ±0.22	1.31 ±0.19	0.401
Cortical Pore Diameter (mm)	0.161 [0.019]	0.159 [0.019]	0.169 [0.019]	0.121
Stiffness (kN·mm)	283.72±47.76	288.58 ±48.86	266.37±40.55	0.125
Estimated Failure Load (kN)	-14.19 ±2.31	-14.44±2.38	-13.31±1.88	0.108

[a]overall *n*=58, non-BSI *n*=45, BSI *n*=13.
[b]overall *n*=61, non-BSI *n*=48, BSI *n*=13.
[c]overall *n*=52, non-BSI *n*=40, BSI *n*=13.
[d]overall *n*=43, non-BSI *n*=33, BSI *n*=10.
[e]overall *n*=46, non-BSI *n*=34, BSI *n*=12.
[f]overall *n*=53, non-BSI *n*=49.
[g]overall *n*=63, non-BSI *n*=49.

10.5 Discussion

BSIs are a financial and healthcare burden for defence, yet effective strategies to mitigate such injuries remain poorly understood. BSIs are caused by sudden changes in repetitive, high impact training loads. Basic military training is physically and mentally arduous (O'Leary *et al.*, 2018b; Wilkinson, Rayson and Bilzon, 2008; O'Leary *et al.*, 2018a), increases bone strength (tibial density, geometry, and microarchitecture) (Izard *et al.*, 2016; Hughes *et al.*, 2018a; O'Leary *et al.*, 2019b; O'Leary *et al.*, 2021; Gaffney-Stomberg *et al.*, 2014; Gaffney-Stomberg *et al.*, 2019), and results in a high incidence of lower limb BSIs (Sharma *et al.*, 2015). During this study 10.0 % of the population at risk were diagnosed with a BSI, slightly higher than the previously reported rate of 6.3 % (HQ Army, 2016).

Trabecular microarchitecture at the ultra-distal tibia was not associated with BSI incidence in this group of healthy male young adults undergoing arduous military training. Neither volumetric density, geometry and estimated bone strength of the ultra-distal tibia, nor biochemical markers of bone metabolism, were associated with lower body BSIs in the same population. We have demonstrated in both case-control comparisons and binary logistic regression that training Regiment is an important determinant of BSI; the incidence of injuries was higher in the Parachute Regiment compared with the Line Infantry during basic training. Physical fitness levels, including 2.4-km run time and maximum dynamic lift strength, were significantly higher in the Parachute Regiment, but controlled for in this study.

10.5.1 Trabecular Microarchitecture

Trabecular microarchitecture was measured at the ultra-distal tibia. The dense trabecular network has an anisotropic distribution aligned parallel to the mechanical stress axis and is important for absorbing and distributing mechanical stresses to the cortex (Turner, 2006). Therefore, trabecular microarchitecture acts to resist compressive forces and is an important contributor to bone strength (Dalle Carbonare and Giannini, 2004; Brandi, 2009; Chappard *et al.*, 2008; Seeman and Delmas, 2006). Our data show that trabecular microarchitecture was not associated with BSI incidence in this cohort of men during basic military training. A cross sectional study in a male military population reported lower cortical vBMD, trabecular

number, trabecular thickness, and greater *inhomogenous* trabecular network in the tibial bone stress injury group compared with uninjured controls (Schanda *et al.*, 2019). Bone microarchitecture did not play a role in BSI risk in our cohort of physically fit male infantry recruits, possibly due to mechanical adaptation experienced before entry to training; this supposition is supported by the observations that trabecular microarchitecture did not change in response to the same training course from a larger cohort in the same study (O'Leary *et al.*, 2019b). In females, increased trabecular thickness and number, decreased separation, and increased *estimated* strength, during basic military training have been observed in as little as 8 weeks (Hughes *et al.*, 2018a; O'Leary *et al.*, 2021); and, case-control studies reported lower trabecular vBMD and trabecular thickness (Ackerman *et al.*, 2015) at the ultra-distal site with no difference in vBMD, cortical or trabecular measures at the ultra-distal site (Schnackenburg *et al.*, 2011) in BSI cases compared with controls. The contribution of trabecular microarchitecture to BSI risk may depend on sex but further study would be required to elucidate this.

10.5.2 Bone Geometry

Cortical area was lower at baseline in recruits who sustained a BSI compared with uninjured controls in bivariate analysis, but no bone characteristics were associated with stress fracture risk in our binary logistic regressions. Retrospective HRpQCT studies in female athletes with a history of lower limb stress fractures reported lower cortical area at the distal tibia than those with no history of stress fracture injury (Schnackenburg *et al.*, 2011), possibly due to the unloading, or reduction in training load as a result of the BSI or its symptoms. A greater cortical area increases strength under axial loading as the tibial cortex is further from the neutral axis and the resistance to bending is increased (O'Leary, Rice and Greeves, 2021) ,which also likely explains the lower estimated failure load in stress fracture cases in our study. Another study in female athletes also reported lower stiffness and failure load in those with a history of multiple stress fracture (≥2) compared with those with <2 fractures (Ackerman *et al.*, 2015). Therefore, greater cortical area is likely protective against tibial stress fracture as most tibial stress fractures occur at the distal third of the tibia where the proportion of cortical bone and bending stresses are greatest (O'Leary, Rice and Greeves, 2021). Popp *et al.* reviewed skeletal properties at multiple tibial sites and reported differences in bone

properties at the proximal third (66%)—more slender tibias, lower stress strain indices, lower section moduli and smaller total cross sectional and cortical areas were observed in runners with bone stress injury compared with uninjured runners (Popp *et al.*, 2020). Izard *et al.* reported significant increases in cortical area, thickness and bone strength at the 38% site after undertaking 10 weeks of military training, which is the site of highest mechanical stresses during locomotion (Izard *et al.*, 2016) and these more proximal measures of geometry may be better predictors of fracture risk (Davey *et al.*, 2015).

10.5.3 Training Load

Training Regiment and 2.4-km run time were the only factors associated with BSI incidence in this study, with the Parachute Regiment having a 9-fold increased risk of BSI when controlling for other factors. The Parachute Regiment have faster 2.4-km run time entry standards than the Line Infantry, yet they suffered a higher BSI incidence. Accordingly, adding 2.4-km run time to the model markedly increased the odds ratio for the Parachute Regiment, but these coefficients should be interpreted with caution because the confidence intervals were wide and so the exact estimates are unclear. The small co-efficient associated with 2.4-km run time is likely a function of the combination of Parachute Regiment and Lines recruits. The higher entry fitness standards required for the Parachute Regiment are necessary to achieve their superior in-service role-related fitness standards; these occupational demands are reflected in a higher training intensity and volume of basic training that accentuates skeletal loading and, probably, increases propensity to BSI.

Slower 2.4-km run time has been consistently associated with increased risk of BSI injury in female recruits (Rauh *et al.*, 2006; Shaffer *et al.*, 2006). Slower run times suggest that recruits enter training with a lower level of aerobic fitness and are exposed to greater training demands when training alongside aerobically fitter peers. The higher training demands of the Parachute Regiment compared with other courses outweighs the risk of BSI from demographic, lifestyle behaviours and bone phenotype (Warden, Edwards and Willy, 2021). The physical demands of the Parachute Regiment training have been previously described in full (Richmond *et al.*, 2012; Wilkinson, Rayson and Bilzon, 2008). Both infantry populations in this study undertake a common military syllabus, but the training intensity and content is

variable, based on the specific role recruits are being trained for. The loaded march standards provide an example of the higher physical standards that must be attained by recruits to pass Parachute Regiment training. Parachute Regiment recruits carry marginally less weight than the line infantry (23 kg *vs* 25 kg), however, they march at a faster pace for a longer distance (8.9 km·h^{-1} [total of 16.1 km in 110 mins] *vs* 6.4 km·h^{-1} [total of 12.9 km in 120 mins]).

The difference in the rate and pattern of BSI risk between Line and Parachute Regiment, suggests differential magnitudes of bone loading. The skeleton is loaded externally from the ground reaction force (GRF) generated when the body impacts with the ground; faster running speeds increase GRF (Kohrt, Barry and Schwartz, 2009). Internal loading is elicited from muscle, tendon and ligament 'pull' on the bone. In Parachute Regiment trainees, their faster pace over longer distances might have increased cumulative GRF. In addition, all recruits carried the same weight for the loaded march, but those who suffered a BSI had a lower lean body mass than those who were uninjured, therefore, those with a BSI were carrying a greater proportion of their body mass and the GRF they experienced was likely disproportionately greater. Whilst a meta-analysis failed to identify an association between lower extremity stress fractures and GRFs (Zadpoor and Nikooyan, 2011) the studies populations and training status were unlike the demands and characteristics of military training. The majority of participants included were women, so different results may be seen in a cohort of solely male participants, although this is unlikely as the two studies considering males (Creaby and Dixon, 2008; Crossley *et al.*, 1999) did not report increased GRF in stress fracture sufferers. Zadpoor also considered vertical loading rate (VLR), (defined as the slope of the initial part of the vertical part of the vertical GRF-time curve, the time between foot strike and vertical impact peak (Munro, Miller and Fuglevand, 1987)) and found it to be significantly higher in the stress fracture group. Again, most participants were female, so the applicability of this finding to males is unknown.

In this study we found the training load required to carry load at faster speeds for longer distances is the most important determinant of BSI risk in men during physically arduous courses.

10.6 Strengths and Limitations

All bone stress injuries included in this study were diagnosed by MRI, which is considered the gold standard method for their diagnosis. We included all lower body BSIs in this study, but the contribution of bone size and shape to fracture resistance might be localised.
The ultra-distal tibia was selected as the focus of this study due to the dense trabecular bone, however Popp *et al* have previously identified that bone properties at the proximal (66% site) tibial site show the greatest difference between runners with bone stress injury compared with uninjured runners (Popp *et al.*, 2020).

Exercise levels prior to starting basic training were not formally recorded, but anecdotally recruits increase their training volume in preparation for basic training, consistent with the peak of BSIs occurring in the early weeks of training (Kardouni *et al.*, 2021). Low levels of physical activity and uniaxial loading in high school are associated with an increased risk of multiple stress fractures (Rudolph *et al.*, 2021). The 2.4-km best effort run time was used as a surrogate measure of aerobic fitness prior to joining the military as recollection of physical activity in formative years was deemed to be unreliable based on researchers previous experience in this participant cohort; but the 2.4-km best effort run time measurement may be influenced by physical preparation undertaken immediately prior to commencing initial training.

The sample size was small in this study with only 20 events (BSIs) recorded and so our ability to identify associated risk factors was limited when considered against the number of events per variable. The p values in this study must also be interpreted with caution as no correction was applied and a large number of factors were compared between our injured and non-injured groups; our results may, therefore, be subject to type I error. Women were not included in this study because they were not then eligible for infantry training; but, since the changes in employment policy to include women in ground close contact roles, further studies are required to establish the role of trabecular microarchitecture as a predictor of BSI risk in women.

10.7 Conclusions

Intrinsic risk factors, including ultradistal tibial density, geometry, and microarchitecture, were not associated with lower body BSI during arduous infantry training. The 9-fold increased risk of BSIs in the Parachute Regiment compared with Line Infantry suggests that injury propensity is primarily a function of training load and risk factors are population-specific.

11 Efficacy of Parathyroid Hormone in Fracture Healing: A Meta-Analysis

11.1 Introduction

Fractures are the most common large-organ trauma. All cause fracture rates in the UK were reported at 73.3 per 10,000 patients per year between 1988 and 2012 (Einhorn and Gerstenfeld, 2015; Curtis *et al.*, 2016). In England, fragility fractures alone cost £4.4 billion per year (Gormley, 2011), the largest proportions of this is hip fractures that account for £1.5 billion and 1.3 million hospital bed days per year (England, 2017). Fracture healing is a complex, but critical physiological process to recondition bone and restore its function (Fazzalari, 2011). Reducing fracture healing time is an important outcome because bone fractures, particularly those related to osteoporosis, are associated with high mortality, morbidity, disability and the need for long-term institutional care and are exacerbated by prolonged recovery times (Goldhahn *et al.*, 2012).

There are no licensed drug treatments for fracture healing. The current mainstay of treatment is surgical fixation were indicated and a programme of rehabilitation. There are several drug treatments licensed for the prevention of osteoporotic fracture. Bisphosphonates and denosumab are anti-resorptive agents that prevent the breakdown of bone. At the time of this review, Teriparatide (TPTD), a parathyroid hormone (PTH) analogue, was the only anabolic treatment currently on the market in Europe for the prevention of osteoporotic fracture; it increases bone mass and reduces bone loss leading to an increase in bone formation (Aslan *et al.*, 2012; Rubin and Bilezikian, 2003a; Rubin and Bilezikian, 2003b; Dempster *et al.*, 1993; Ito *et al.*, 2014; Varela *et al.*, 2017). Teriparatide, one of two PTH analogues commercially available, is the 1-34 N-terminal amino acid sequence of the endogenous human PTH. The second analogue is the full 1-84 amino acid PTH, which is was previously licensed for the treatment of osteoporosis and is currently licensed as an adjunctive treatment for chronic hypoparathyroidism. Since this review was conducted, romosozumab, a humanised monoclonal antibody (IgG2) with both anabolic and anti-catabolic activity has been licensed in Europe. Romosozumab increased bone formation while decreasing bone resorption. A PTHrP analogue is available in the US called abaloparatide.

Investigations are ongoing to understand the potential for PTH analogues to expedite bone healing. Animal studies have supported this hypothesis (Varela *et al.*, 2017; Komatsubara *et al.*, 2005; Komrakova *et al.*, 2011; Gardner *et al.*, 2007; Li *et al.*, 2012), but the evidence in humans is less clear. Some studies show that PTH analogue administration has a beneficial effect on fracture healing (Peichl *et al.*, 2011; Aspenberg *et al.*, 2010) while others show no effect on fracture healing rates or reduction in pain levels (Greenspan *et al.*, 2018; Tsuchie *et al.*, 2016). The six literature reviews published to date have focused on osteoporotic patients. The older reviews, that focus on case reports and series favour PTH treatment (Im and Lee, 2015; Zhang *et al.*, 2014), and conclude that whilst the evidence for TPTD is anecdotal it is sufficient to justify future prospective trials. Larger, prospective randomised trials have since been carried out. Reviews considering these trials concluded that the benefit of PTH analogue intervention in osteoporotic fracture healing was uncertain, however, the absence of adverse events justified further research (Kim *et al.*, 2017; Shi *et al.*, 2016). Lou *et al* reported PTH analogues to be effective in accelerating fracture healing and improving functional outcomes in osteoporotic women only (Lou *et al.*, 2016). Hong *et al* concluded the evidence supporting fracture healing indications was reasonably credible but more randomised controlled trials (RCTs) were required to verify differential effects in different populations (Hong *et al.*, 2019).

The purpose of this meta-analysis was to determine the efficacy of PTH analogues on fracture healing, updating and broadening previously published reviews (Shi *et al.*, 2016; Lou *et al.*, 2016; Hong *et al.*, 2019; Kim *et al.*, 2017; Im and Lee, 2015; Zhang *et al.*, 2014). Unlike the earlier reviews, this meta-analysis considers all fracture types and all controls (placebo, standard care and bisphosphonates) in addition to two recently published studies (Shigenobu *et al.*, 2019; Greenspan *et al.*, 2018). Consequently, more trials and a larger cohort of patients is considered than in previous reviews.

11.2 Methods

This meta-analysis protocol was reported according to the Preferred Reporting Items for Systematic Reviews and Meta- Analysis (PRISMA®) guidelines (Moher *et al.*, 2015). This study was not a human or animal experiment, so no ethical approval was required. This systematic review is registered on Prospero (registration no. CRD42019131967). The PICO (Population, Intervention, Comparison and Outcomes) model was used to define the inclusion criteria. The Risk of Bias 2 Tool was used to evaluate the risk of bias (Sterne *et al.*, 2019) and RevMan 5.3.5 (Nordic Cochrane Centre 2019) software was used to perform meta analyses.

11.2.1 Literature Search Strategy

An electronic database search for relevant articles was conducted in August 2019 using the following databases: MEDLINE, EMBASE and The Cochrane Library. No date restrictions were applied. The search was performed using a combination of key words and MESH terms. The detailed search strategy is provided in online resource 1. In general, the Cochrane search strategy was used to identify randomised clinical trials (RCTs) with the addition of: (1) Parathyroid Hormone; (2) Teriparatide; (3) Forsteo; (4) Forteo; (5) NatPar; (6) PREOS; (7) Preotact; (8) PTH; (9) Fracture.

Reference lists from trials, conference abstracts and reviews were examined to identify additional eligible trials. For completeness, www.clinicaltrials.gov was searched for RCTs that were registered as complete but not yet published; no relevant trials were identified by this route.

11.2.2 Trial Selection

A flow diagram illustrating the trial selection is shown in figure 11-1.

Results from the searches were combined and duplicates were removed. Two investigators (K. Eastman and Dr M. Gerlach) evaluated the title and abstract from each reference identified by the search. For inclusion, all trials were required to be prospective, randomised clinical trials in adult patients aged > 18 years old presenting with a fracture. Any type of fracture (delayed union, non-union or stress fracture) at any site (long bone, short bone, flat bone or irregular bone) were accepted. Trials where PTH analogues were used as an adjunctive

therapy to operative or conservative treatments were included. Treatments included TPTD, PTH 1-84, abaloparatide or other PTH analogues with any route of administration, dose or frequency. The outcomes included functional recovery, fracture union, pain, and adverse events.

All texts that were clearly irrelevant were excluded following abstract review, and full texts of the remaining articles were retrieved. Full texts that did not meet the inclusion criteria were excluded following further scrutiny. The reference lists of excluded articles were reviewed to identify any articles relevant to the subject area that met the inclusion criteria.

Any disagreement between authors regarding trial selection was resolved by means of consensus, involving a third investigator (Prof W.D. Fraser), according to *a priori* agreed criteria. Exclusion criteria were non-human studies, non-English language, and data sets with insufficient data to complete a review i.e., abstracts, review articles, editorials and letters.

11.2.3 Data Extraction

The results from each article were extracted using a standardised data collection form based on the pre-defined trial inclusion criteria. The main categories extracted from the articles were: author, year, title, trial overview, patient characteristics, type of fracture, duration of treatment, conclusions on primary outcomes and secondary outcomes where these related to fracture healing, pain, functional outcomes, adverse event or treatment discontinuations.

11.2.4 Quality Assessment of Included Articles

The quality of trial methods was independently rated by two investigators, who were not blinded to the article author, journal or institution, in accordance with Baker *et al* (Baker *et al.*, 2010). A Risk of bias evaluation was completed (Figure 11-2).

11.2.5 Data Analysis

The outcomes of the trials (bone healing rate, time to bone healing, functional recovery, pain and adverse events) were analysed. Forest plots were produced from meta-analyses performed by Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. Due to the variation within trial

designs and heterogeneity of results, not all data were suitable for meta-analysis. In these instances, analyses described in the eligible trials were extracted and reported in a systematic format as a narrative synthesis.

Odds Ratios (OR) were calculated for outcomes with dichotomous data including fracture healing rate (healing evaluated at set intervals), occurrence of adverse events and treatment discontinuations. The Mean Difference (MD) method was adopted for outcomes with continuous data including time to fracture healing, differences in pain (measured by visual analogue scale (VAS)) and functional outcomes (questionnaire scores or time as in the case of the 'timed up and go test').

Risk of bias was analysed using the *RoB 2 Tool* (Sterne *et al.*, 2019). This assessment reviews the risk of bias over 5 domains, bias arising from the randomisation process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in the measurement of the outcome and bias in the selection of the reported result.
Disease severity measurements such as BMD were excluded from analysis.

11.3 Results

11.3.1 Literature Search Strategies

The literature search identified 5781 publications; 626 were excluded due to duplication and 5144 did not meet the inclusion criteria. Following full-text scrutiny, 11 trials on efficacy of PTH analogues in fracture healing were included in the analysis. A flow diagram of the search strategy is at figure 11-1.

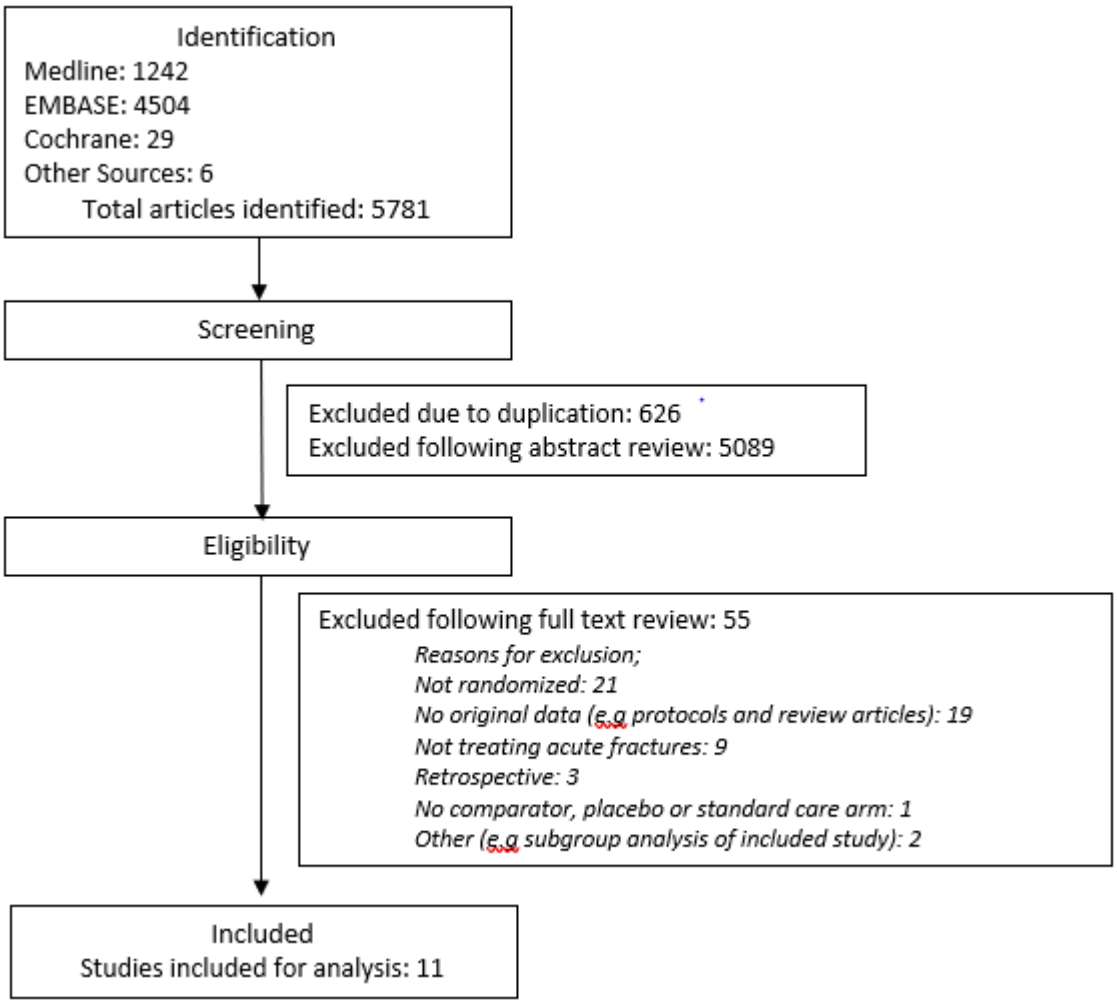


Figure 11-1: Flow diagram of the search strategy, Medline, EMBASE, Cochrane and other sources such as reference lists and clinical trials registers were reviewed. A total of 5781 articles were identified. After screening, 626 studies were excluded due to duplication and 5089 were excluded following abstract review. Sixty-six full text articles were scrutinised and 11 were selected for inclusion.

11.3.2 Patient Characteristics

The 11 articles selected for the systematic review included 1,452 patients, 91.8% (n=1,333) of whom were women. The mean age of patients was 72 years old. The anomaly was Almirol *et al.* who evaluated PTH analogues for lower extremity stress fractures in young female adults (n=13) with a mean age of 32 (± 5.8) and 31 (± 3.4) in treatment and control groups, respectively. Fracture sites in the 11 articles were vertebrae (n=789, 3 trials) (Shigenobu *et al.*, 2019; Zhao *et al.*, 2016b; Hadji *et al.*, 2012), femur (atypical) (n=13, 1 trial) (Greenspan *et al.*, 2018), hip (n=343, 4 trials) (Malouf-Sierra *et al.*, 2016; Chesser *et al.*, 2016; Bhandari *et al.*, 2016; Kanakaris, West and Giannoudis, 2015), tibia (n=13, 1 trial) (Almirol *et al.*, 2016), humerus (n=40, 1 trial) (Johansson, 2016) and radius (n=102, 1 trial) (Aspenberg *et al.*, 2010). Full details of patient characteristics are listed in table 11-1.

11.3.3 Trial Design

In all trials, patients were randomly assigned to treatment with a PTH analogue, standard care or a comparator drug. Randomisation approaches included sealed envelopes (Johansson, 2016; Chesser *et al.*, 2016), computer generated sequences (Malouf-Sierra *et al.*, 2016; Aspenberg *et al.*, 2010) and table based randomisation (Bhandari *et al.*, 2016).

Two parathyroid hormone analogue regimes were used 20µg TPTD/day (Greenspan *et al.*, 2018; Malouf-Sierra *et al.*, 2016; Almirol *et al.*, 2016; Chesser *et al.*, 2016; Zhao *et al.*, 2016b; Bhandari *et al.*, 2016; Johansson, 2016; Kanakaris, West and Giannoudis, 2015; Hadji *et al.*, 2012; Aspenberg *et al.*, 2010) and 56.5µg TPTD/week (Shigenobu *et al.*, 2019). Aspenberg *et al.* also included a 40µg TPTD/day dose but this was not used in analysis as it is not licensed in any territory (Aspenberg *et al.*, 2010). Comparator groups included: placebo, standard care (with the intention to initiate TPTD at 6-months), risedronate sodium (17.5mg or 35mg /week, 75mg / month) or alendronic acid (70mg or 35mg /week). The full trial schedules are shown in table 11-1. The duration of PTH treatment varied from 1 to 24 months. Calcium and vitamin D supplements were administered in 9 of the 11 trials. Characteristics of trials selected for inclusion in this review are listed in table 11-1.

11.3.4 Quality of Trials

The quality of trials was assessed by the risk of bias using the Sterne *et al* risk of bias tool (Sterne *et al.*, 2019). Overall 'low risk' and 'some concerns' were identified in seven trials. A high risk of bias was identified in four trials. The heterogeneous nature of the patient characteristics, treatments, comparators and outcomes of the trials was reflected in their risk of bias.

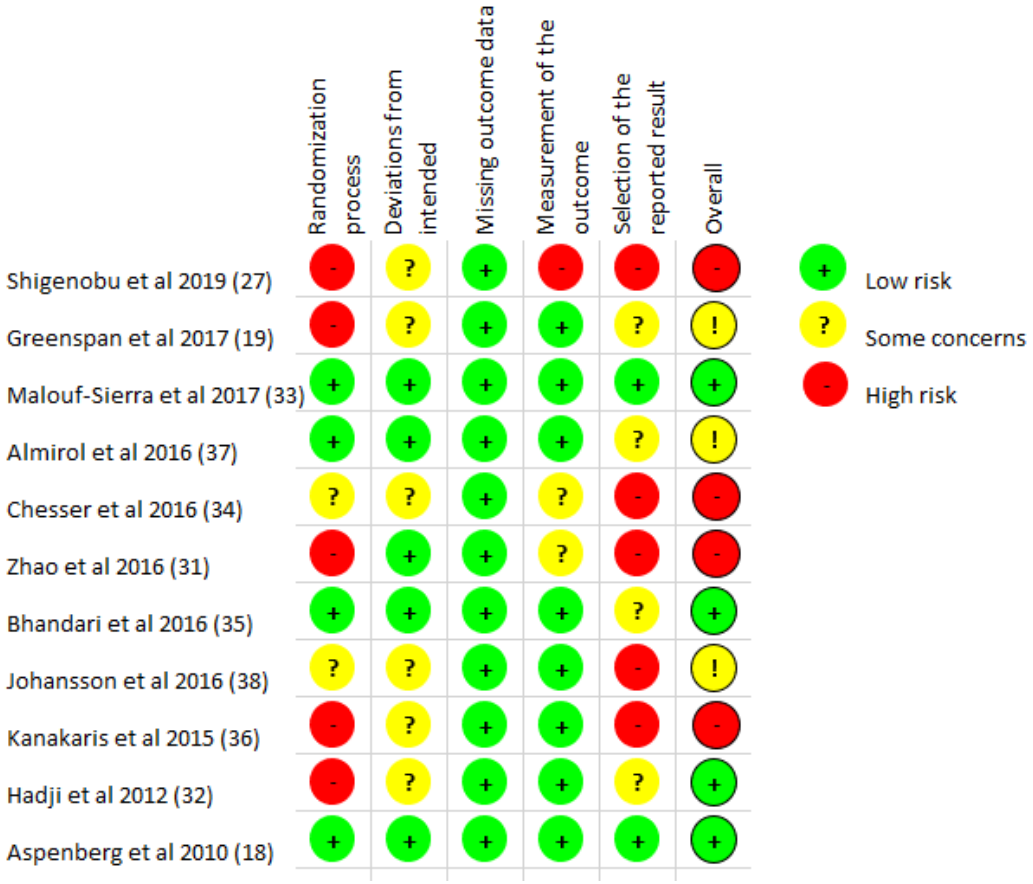


Figure 11-2: Assessment of the risk of bias for trials selected for the systematic review. Adapted from the Cochrane risk of Bias Tool 2. Bias is assessed based on five domains; the randomisation process, blinding (deviations from the intended), missing data, outcome measurement and selective or multiple outcome reporting. The results of the five domains are reported in the consolidated overall risk of bias.

11.3.5 Reporting of Outcomes

The primary outcomes were clearly identified in nine of the 11 trials. Six trials reported a single primary outcome related to fracture healing outcomes, including radiological healing (Greenspan *et al.*, 2018; Johansson, 2016; Aspenberg *et al.*, 2010), differences in pain (Hadji *et al.*, 2012), the requirement for surgical revision (Bhandari *et al.*, 2016) or changes in bone

biomarkers (Almirol *et al.*, 2016). Identification of, and distinction between secondary, exploratory or post-hoc analyses was poor in nine trials. Three trials (Greenspan *et al.*, 2018; Malouf-Sierra *et al.*, 2016; Almirol *et al.*, 2016) clearly stated secondary outcomes and one identified outcomes as 'exploratory' (Chesser *et al.*, 2016). An overview of the outcomes and findings of each trial is described in table 11-2.

Table 11-1: Characteristics of randomised controlled trials included for review including numbers of and mean age of participants. The experimental treatments included teriparatide 20ug/d and 56.6ug/wk. Controls included standard care, placebo, or a bisphosphonate (alendronic acid or risedronate sodium). Calcium, vitamin D supplementation, treatment duration, follow-up duration, fracture type and primary outcomes are also included.

	Trial	Number of Patients (women)	Mean Age Years ± SD	Treatment	Calcium and Vitamin D Supplementation	Treatment Duration	Follow-Up Durations Including Treatment	Fracture Type	Primary Outcome
1	Shigenobu *et al*. 2019 (Shigenobu *et al.*, 2019)	43 (38)	(n=24): 75.6 (n=19): 80.2 Overall: 78.1 (Range 61-93)	Teriparatide 56.6ug/wk Alendronic Acid 35mg/wk or Risedronate sodium 17.5mg/wk or 75mg/mo	No	12mo	12mo	Vertebral Compression Fracture	Not stated
2	Greenspan *et al*. 2018 (Greenspan *et al.*, 2018)	13 (13)	(n=7): 78.0 ± 3.3 (n=6): 69.8 ± 3.3	Teriparatide 20ug/d at fracture Teriparatide 20ug/d 6mo post fracture	Yes	6mo	12mo	Atypical femur	Radiological healing
3	Malouf-Sierra *et al.* 2016 (Malouf-Sierra *et al.*, 2016)	171 (132)	(n=86): 77.2 ± 8.0 (n=85): 76.4 ± 7.5 Overall: 76.8 ± 7.7	Teriparatide 20ug/d Risedronate sodium 35mg/wk	Yes	26wk blinded + 52wk unblinded	78wk	Post fixation low trauma pertrochanteric	Change from baseline in lumbar spine BMD at 78wk
4	Almirol *et al.* 2016 (Almirol *et al.*, 2016)	14 (14)	(n=6): 32 ± 5.8 (n=8): 31 ± 3.4	Teriparatide 20ug/d Placebo	Yes	8wk	12wk	Lower extremity stress fracture	Anabolic window in biomarkers for bone formation and resorption (P1NP and Yes OC Vs CTX and NTX)
5	Chesser *et al.* 2016 (Chesser *et al.*, 2016)	29 (29)	(n=15): 80.6 ± 8.8 (n=14): 78.6 ± 9.3	Teriparatide 20ug/d Standard Care	Yes	6wk	6mo	Trochanteric hip fracture	Pilot Study (Feasibility and acceptability of proposed methodology

6	Zhao *et al*. 2016 (Zhao *et al.*, 2016b)	49 (49)	(n=24): 68.9 ± 5.37 (n=25): 68.7 ± 5.74	Teriparatide 20ug/d Alendronic Acid 70mg/wk	Yes	16mo	12mo	Osteoporotic vertebral compression fracture	Not stated
7	Bhandari *et al*. 2016 (Bhandari *et al.*, 2016)	159 (117)	(n=78): 70 (Range 50-94) (n=81): 70 (Range 50-90)	Teriparatide 20ug/d Placebo	Yes	6mo	24mo	Femoral neck fracture followed be internal fixation surgery	Requirement for surgical revision at 12mo
8	Johansson *et al*. 2016 (Johansson, 2016)	40 (40)	(n=19)^: 67 (Range 54-82) (n=20): 69 (Range 54-94)	Teriparatide 20ug/d Standard Care	No	4wk	3mo	Proximal humorous	Radiological healing and callus formation at 7wk
9	Kanakaris *et al.* 2015 (Kanakaris, West and Giannoudis, 2015)	30 (24)	(n=9): 75 ± 8.98 (n=11): 75 ± 9.18 (n=10): 75 ± 8.89	Teriparatide 20ug/d Alendronic Acid 70mg/wk Standard Care	Yes	6mo	6mo	Hip fractures	Not stated
10	Hadji *et al.* 2012 (Hadji *et al.*, 2012)	710 (710)	(n=360): 70.5 ± 8.8 (n=350): 71.6 ± 8.1	Teriparatide 20ug/d Risedronate sodium 35mg/wk	Yes	18mo	18mo	Osteoporotic vertebrae	Greater than 30% reduction in worst back pain
11	Aspenberg *et al*. 2010 (Aspenberg *et al.*, 2010)	102 (102)	(n=34): 59.2 ± 9.6 (n=34): 62.8 ± 7.3 (n=34): 61.7 ± 8.6	Teriparatide 20ug/d Teriparatide 40ug/d Placebo	Yes	8wk	53wk	Distal Radius	Radiographic healing at 8wk

d: day(s); wk: weeks; mo: months; P1NP: N-terminal propeptide of type I collagen; OC: osteocalcin; CTX: Collagen-type I cross-linked C-telopeptide; NTX: collagen-type I cross-linked N-telopeptide. ^1 participant lost to follow -up, data not included in description of baseline characteristics.

Table 11-2: An overview of results of randomised controlled trials included in the review. Results are presented for primary outcomes and corresponding results, secondary and post-hoc outcomes, and corresponding results for each of the trials.

	Trial	Primary Outcome	Results	Additional Healing and Functional Outcomes Reported	Results
1	Shigenobu *et al*. 2019 (Shigenobu *et al.*, 2019)	Not stated	N/A	Radiological Healing (1,2,3, 6mo)	TPTD group improved healing at 12wk ($p<0.05$), no difference at 24wk
				Mean time to fracture healing	TPTD group had earlier fracture healing ($p<0.05$)
				Rowland-Morris Disability Questionnaire (RDQ) score	TPTD group RDQ score improved at all timepoints ($p<0.05$)
				EQ-5D score (2, 4, 8, 12,24wk)	No significant difference between arms
				Change in pain (by VAS) (2,4,8,12, 24wk)	No significant difference between arms
				Anabolic window in biomarkers for bone formation and resorption (P1NP Vs ALP and TRACP-5b)	P1NP significantly higher in the TPTD arm at 12 and 24wk
2	Greenspan *et al.* 2018 (Greenspan *et al.*, 2018)	Radiological healing	Cortical continuity on 2 of 4 cortexes at 6mo: immediate treatment = 3.1±0.1; delayed = 2.8±0.3 ($p=0.1032$)	SF-36 quality of life (6, 12mo)	No significant difference between arms
				Pain assessment (6, 12mo)	
3	Malouf-Sierra *et al.* 2016 (Malouf-Sierra *et al.*, 2016)	Change from baseline in lumbar spine BMD at 78wk	TPTD superior to RIS in the change of lumbar spine BMD at wk78 (mean difference 0.040 g/cm^2; 95%CI 0.025 to 0.55 g/cm^2; $p<0.0001$)	Timed Up and GoTUG test (6, 12, 18, 26wk	Shorter TUG in TPTD vs RIS at all timepoints ($p=0.021$)
				Radiological healing (6, 12, 26wk)	No significant difference in the rate of radiographic healing ($p=0.547$)
				Mechanical failure (26wk)	No significant difference in the rate of mechanical failure ($p=0.577$)
				Ability to walk (6, 12, 18, 26wk)	No significant difference in the ability to walk or use of aids ($p=0.8$)
				SF-36 Physical Function Component (6, 12, 18, 26wk)	No significant difference in SF-36 scores at any timepoint ($p=0.205$, 0.737, 0.435, 0.267 respectively)
4	Almirol *et al.* 2016 (Almirol *et al.*, 2016)	Anabolic window in biomarkers for bone formation and resorption (P1NP and OC vs CTX and NTX)	Significantly larger anabolic window in the teriparatide group (145.82±123.0) compared to placebo (5.99±48.4) ($p=0.05$)	MRI grade (8wk)	No difference between groups ($p>0.13$)
5	Chesser *et al.* 2016 (Chesser *et al.*, 2016)	Sample size calculation for full trial.	Detection of a one-point change is the SPPB at 12wk assuming 80% completion rate would require 405 patients.	EQ-5D score (12wk)	Significance not calculated
				SPPB (12wk)	Significance not calculated
6	Zhao *et al.* 2016 (Zhao *et al.*, 2016b)	Not stated	N/A	mJOA-BPEQ (6,12mo)	TPTD group improved mJOA-BPEQ scores at 6 & 12 vs ALEN ($p<0.05$)
				Change in pain (by VAS) (6, 12mo)	TPTD group improved pain scores at 6 & 12 vs ALEN ($p<0.05$)
				Biochemical makers of bone turnover (P1NP and TRACP-5b at 6, 12 mo)	Increased P1NP and CTX in TPTD group ($p<0.05$). No change in ALEN group

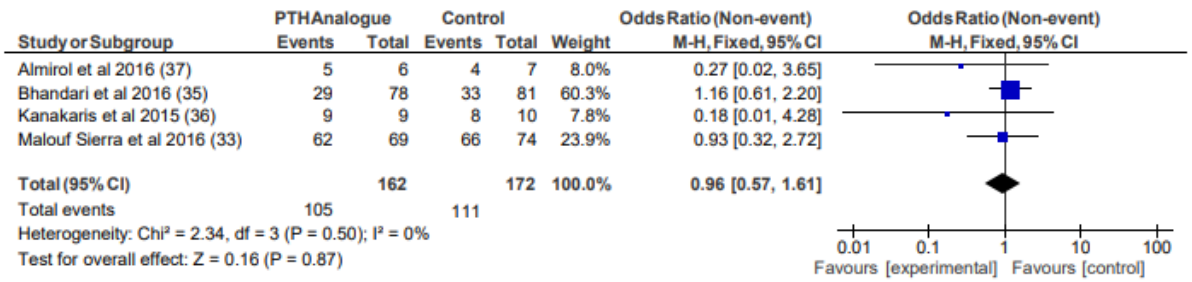
				Kyphotic angle and anterior border heights of fractured vertebrae	Significantly larger anterior border height in the TPTD group vs ALEN (p<0.05). Significantly smaller kyphotic angle in the TPTD group vs ALEN (p<0.05)
7	Bhandari *et al*. 2016 (Bhandari *et al.*, 2016)	Requirement for surgical revision at 12mo	There was no significant difference in the requirement for revision surgery at any time point	Radiographic assessment of fracture healing (10wk, 6,12mo)	No difference between groups at any timepoint
				Pain control (12mo)	No difference between groups
				Gait speed (12mo)	Improved gate speed in the TPTD arm
				Composite measure of fracture healing	No difference between groups at 12mo
8	Johansson *et al.* 2016 (Johansson , 2016)	Radiographic healing and callus formation at 7wk	No positive effect	Reduction in pain (by VAS) (8wk)	No difference between groups
				Change in DASH scores	
9	Kanakaris *et al*. 2015 (Kanakaris, West and Giannoudis, 2015)	Not stated	N/A	Johansson Hip Rating Questionnaire (6wk, 3, 6mo)	No meaningful statistical analysis due to small sample size
				Non unions (6mo)	
10	Hadji *et al*. 2012 (Hadji *et al.*, 2012)	Greater than 30% reduction in worst back pain	Greater than 30% reduction in worst back pain not seen at 6, 12 or 18 mo	Patients with worsening or worst or average back pain (12, 18mo)	More reports of worsening or 'worst' and 'average' back pain in the RIS arm vs TPTD arm (both p=0.04)
				New vertebral fractures	Fewer new vertebral fractures in the TPTD group (p=0.01). Subjects in the TPTD group had less height loss at 18mo (p=<0.05)
11	Aspenberg *et al.* 2010 (Aspenberg *et al.*, 2010)	Shorten time to cortical bridging	The time to healing was shorted in the teriparatide 20ug group compared to placebo (9.1 vs 7.2 wks) (p=<0.001)	Patient-Rated Wrist Evaluation Score (total)	No difference in TPTD score Vs Placebo
				Grip strength as a percentage of the uninjured hand	

d: day(s); wk: weeks; mo: months; RDQ: Rowland-Morris Disability Questionnaire; EQ-5D: EuroQol-5D, VAS: Visual Acuity Score; P1NP: N-terminal propeptide of type I collagen; ALP: Alkaline Phosphatase; TRACP-5b: Tartrate-resistant acid phosphatase 5b; TPTD: Teriparatide; SF-36: Short Form 36; JOA-BPEQ: Japanese Orthopedic Association Back Pain Evaluation Questionnaire; BMD: Bone mineral density; RIS: Risedronate sodium; TUG: Timed Up and Go; OC: osteocalcin; CTX: Collagen-type I cross-linked C-telopeptide; NTX: collagen-type I cross-linked N-telopeptide; ALEN: Alendronate; SF-12 PCS: Short Form-12 Physical Component Summary; SF-12 MCS: Short Form-12 Mental Health Component Summary; DASH: Disabilities of the arm, shoulder and hand;

11.3.6 Radiological Assessment of Fracture Healing

Radiological assessment of fracture healing was used in nine trials and was the primary outcome in three trials (Greenspan *et al.*, 2018; Johansson, 2016; Aspenberg *et al.*, 2010). Four trials used plain film radiographs (Greenspan *et al.*, 2018; Malouf-Sierra *et al.*, 2016; Bhandari *et al.*, 2016; Johansson, 2016) and one trial used magnetic resonance imaging (MRI) (Almirol *et al.*, 2016). Two trials used a combination of X-Ray and computed tomography (CT) (Aspenberg *et al.*, 2010; Kanakaris, West and Giannoudis, 2015). Kanakaris *et al* reported radiological non-union at 6 months as an outcome but did not describe the radiological method used (Kanakaris, West and Giannoudis, 2015).

The criteria used to grade the scans and the detail to which it was described differed greatly between the trials. Radiological healing was categorised as two outcomes — fracture healing rate (healing at set time points) and fracture healing time (days). Shigenobu *et al* used a non-standard method and did not provide adequate description of this method (Shigenobu *et al.*, 2019); consequently, this trial was excluded from the analysis of radiological fracture healing. The fracture healing rate between the PTH analogue and comparator or control groups was examined in four trials (334 patients) at the first reported timepoint. The forest plot of the Odds Ratios (95% CI) across trials is shown in figure 11-3. There was no difference in the fracture healing rate when PTH analogues were compared with comparator and control groups (OR 0.96, 95% CI 0.57 to 1.61, $P = 0.87$).



M-H: Mantel-Haenszel test.

Figure 11-3: Forest plot evaluating the change in fracture healing rate between experimental (PTH analogues) and control groups. Data are Odds Ratio (95% CI, Confidence Intervals). There was no difference in the fracture healing rate when PTH analogues were compared with comparator and control groups (OR 0.96, 95% CI 0.57 to 1.61, P = 0.87). Data are from the first reported timepoint: Almirol et al at 8 weeks (Almirol et al., 2016) ; Bhandari et al at 10 weeks (Bhandari et al., 2016); Malouf-Sierra et al at 12 weeks (Malouf-Sierra et al., 2016); Kanakaris et al at 6 months (Kanakaris, West and Giannoudis, 2015).

The fracture healing time between the PTH analogue and comparator / control groups was examined only by Greenspan *et al* and, therefore, not suitable for analysis by forest plot (Greenspan *et al.*, 2018). Using standard care as a comparator, the fracture healing time was confirmed by radiograph and defined as cortical continuity in 2 of 4 cortexes. Fracture healing time was shorter in the TPTD treated group (13.6 vs 12.3 weeks $P \leq 0.001$).

11.3.7 Pain

Pain was reported as an outcome in nine of the 11 trials, eight of which used an 11-point visual analogue scale (VAS) (0 = no pain, 10 = greatest pain imaginable) (Malouf-Sierra *et al.*, 2016; Chesser *et al.*, 2016; Zhao *et al.*, 2016b; Bhandari *et al.*, 2016; Johansson, 2016; Hadji *et al.*, 2012; Aspenberg *et al.*, 2010; Shigenobu *et al.*, 2019). Two trials (n=78) published sufficient data for meta-analysis (Chesser *et al.*, 2016; Zhao *et al.*, 2016b). The results are shown in figure 11-4. PTH analogue-treated groups reported less pain compared with comparator / controls in the trials that were suitable for meta-analysis (MD -4.55, 95% CI -7.47 to -1.63, *P* = 0.002). The Short Form 36 (SF-36) questionnaire (Greenspan *et al.*, 2018) was also used for pain reporting.

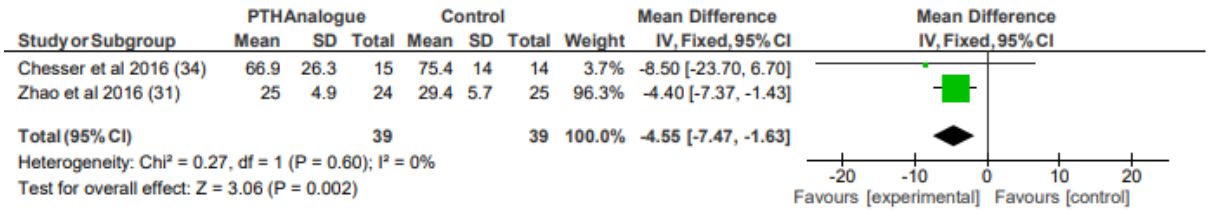


Figure 11-4: Forest plot evaluating the differences in pain between patients treated with PTH analogues and comparators or controls following fracture. Data are Mean Difference (95% CI, Confidence Intervals). PTH analogue-treated groups reported less pain compared with comparator / controls in the trials that were suitable for meta-analysis (MD -4.55, 95% CI -7.47 to -1.63, P = 0.002).

Seven trials reported insufficient data on pain for inclusion in the meta-analysis. Two trials reported no significant differences in pain but did not publish the data (Greenspan *et al.*, 2018; Shigenobu *et al.*, 2019). Three trials reported no significant differences in pain between treatment and control, but these data were not included in the forest plot because they were published as a percentage change rather than as raw data (Bhandari *et al.*, 2016; Hadji *et al.*, 2012; Aspenberg *et al.*, 2010). Another trial reported a significant reduction in pain during the 'timed up and go' test, but the results were reported as a time change (Malouf-Sierra *et al.*, 2016). One trial did not report standard deviations (Johansson, 2016) so was excluded from the meta-analysis.

11.3.8 Functional Outcomes

Nine trials reported functional outcomes (Malouf-Sierra *et al.*, 2016; Shigenobu *et al.*, 2019; Chesser *et al.*, 2016; Zhao *et al.*, 2016b; Bhandari *et al.*, 2016; Johansson, 2016; Kanakaris, West and Giannoudis, 2015; Hadji *et al.*, 2012; Aspenberg *et al.*, 2010), and three of these used multiple assessment methods (Shigenobu *et al.*, 2019; Chesser *et al.*, 2016; Hadji *et al.*, 2012). In total, 13 different methods of assessment were used, including a mixture of physical tests and questionnaires.

Validated functional multi-activity assessments were used in nine of these trials, and speed gait was used in one (Bhandari *et al* (Bhandari *et al.*, 2016)). Four trials used self-reported functional scores and published data detailed enough to be included for meta-analysis; the results are shown in figure 11-5. These analyses show a statistically significant improvement in functional outcome for participants treated with PTH analogues (MD -1.59, 95% CI -1.97 to -1.21, $P \leq 0.00001$). Of the remaining trials, two reported no significant difference in

functional outcomes but did not publish the data (Johansson, 2016; Hadji *et al.*, 2012), Bhandari *et al* and Shigenobu *et al* did find a significant improvement in functional outcomes in the PTH analogue group vs control (Bhandari *et al.*, 2016; Shigenobu *et al.*, 2019) and Aspenberg *et al* reported an improvement at the week 13 timepoint only (Aspenberg *et al.*, 2010) but none of these trials published sufficient data for a meta-analysis.

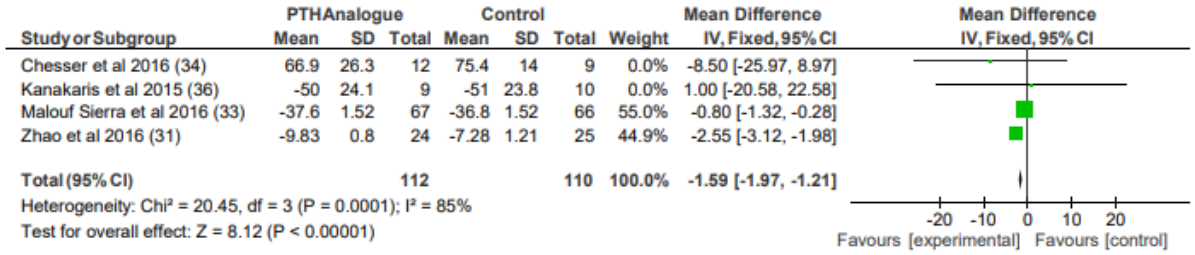


Figure 11-5: Forest plot evaluating the differences in functional outcomes between patients treated with PTH analogues and controls during fracture healing. Controls included standard care (Chesser et al., 2016; Kanakaris, West and Giannoudis, 2015) and bisphosphonates (Malouf-Sierra et al., 2016; Zhao et al., 2016b). Data are Mean Difference (95% CI, Confidence Intervals). PTH analogues improved functional outcomes (MD -1.59, 95% CI -1.97 to -1.21, P ≤ 0.00001).

11.3.9 Biochemical Markers of Bone Turnover

Three trials reported biochemical markers of bone formation and resorption (Almirol *et al.*, 2016; Shigenobu *et al.*, 2019; Zhao *et al.*, 2016b). All trials demonstrated significant increases in serum N-terminal propeptide of type I procollagen (P1NP) following PTH analogue treatment vs placebo (Almirol *et al.*, 2016) and vs bisphosphonates (Zhao *et al.*, 2016b; Shigenobu *et al.*, 2019), and no significant change in CTX, resulting in a greater anabolic window. Insufficient data were included for meta-analysis.

11.3.10 Adverse Events

Eight trials reported adverse events, and five (1182 patients) provided enough data for a meta-analysis (Figure 11-6). There was no statistical difference between PTH analogue treated groups and comparators / controls (OR 0.74, 95% CI 0.45 to 1.02, *P* = 0.07).

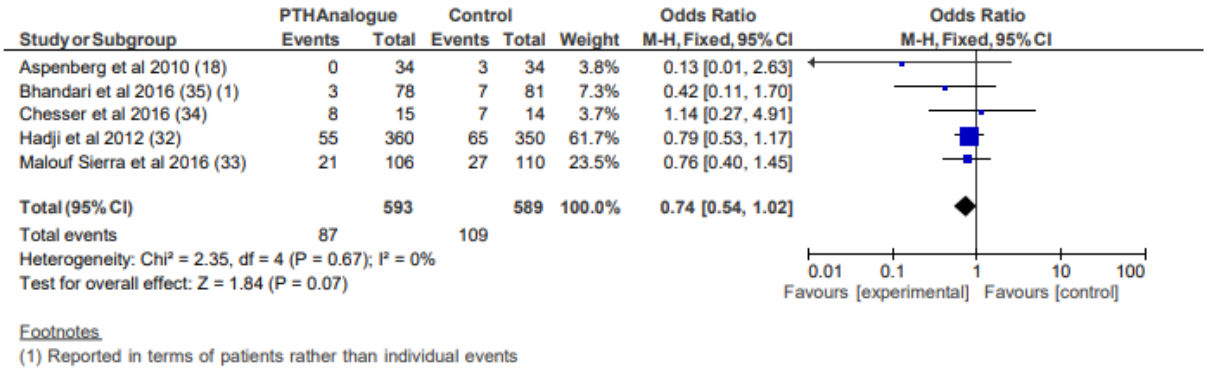
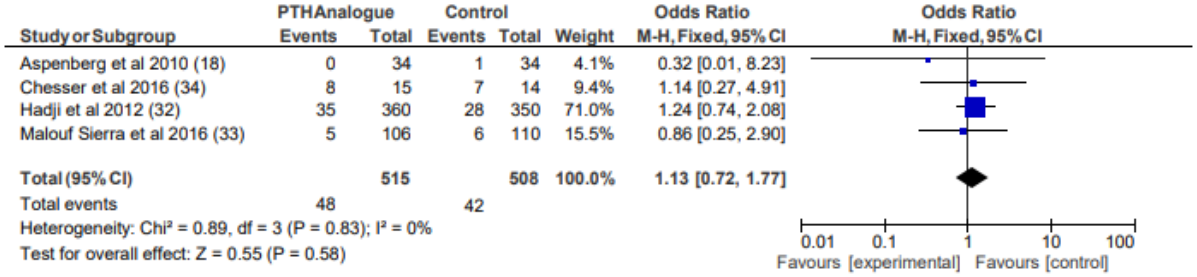


Figure 11-6: Forest plot evaluating the difference in adverse events between patients treated with PTH analogues and controls for fracture healing. Controls included standard care (Chesser et al., 2016), placebo (Aspenberg et al., 2010; Bhandari et al., 2016) and bisphosphonates (Hadji et al., 2012; Malouf-Sierra et al., 2016). Data are Odds Ratio (95% CI, Confidence Intervals). M-H: Mantel-Haenszel test. There was no difference between PTH analogue treated groups and comparators / controls (OR 0.74, 95% CI 0.45 to 1.02, P = 0.07).

Four trials (1023 patients) reported treatment discontinuation of PTH analogues as an outcome measure. The results are described in figure 11-7. There was no statistical difference between PTH analogue treated groups and comparators / controls (OR 1.13, 95% CI 0.72 to 1.77, *P* = 0.58).

Two trials did not report serious adverse events (SAE's) but did report no significant difference in mild or minor adverse events (Almirol *et al.*, 2016; Johansson, 2016).



M-H: Mantel-Haenszel test.

Figure 11-7: Forest plot evaluating the difference in treatment discontinuations between patients receiving PTH analogues and comparator / controls for fracture healing. Data are Odds Ratio (95% CI, Confidence Intervals). There was no statistical difference between PTH analogue treated groups and comparators / controls (OR 1.13, 95% CI 0.72 to 1.77, P = 0.58).

11.3.11 Choice of Comparator group

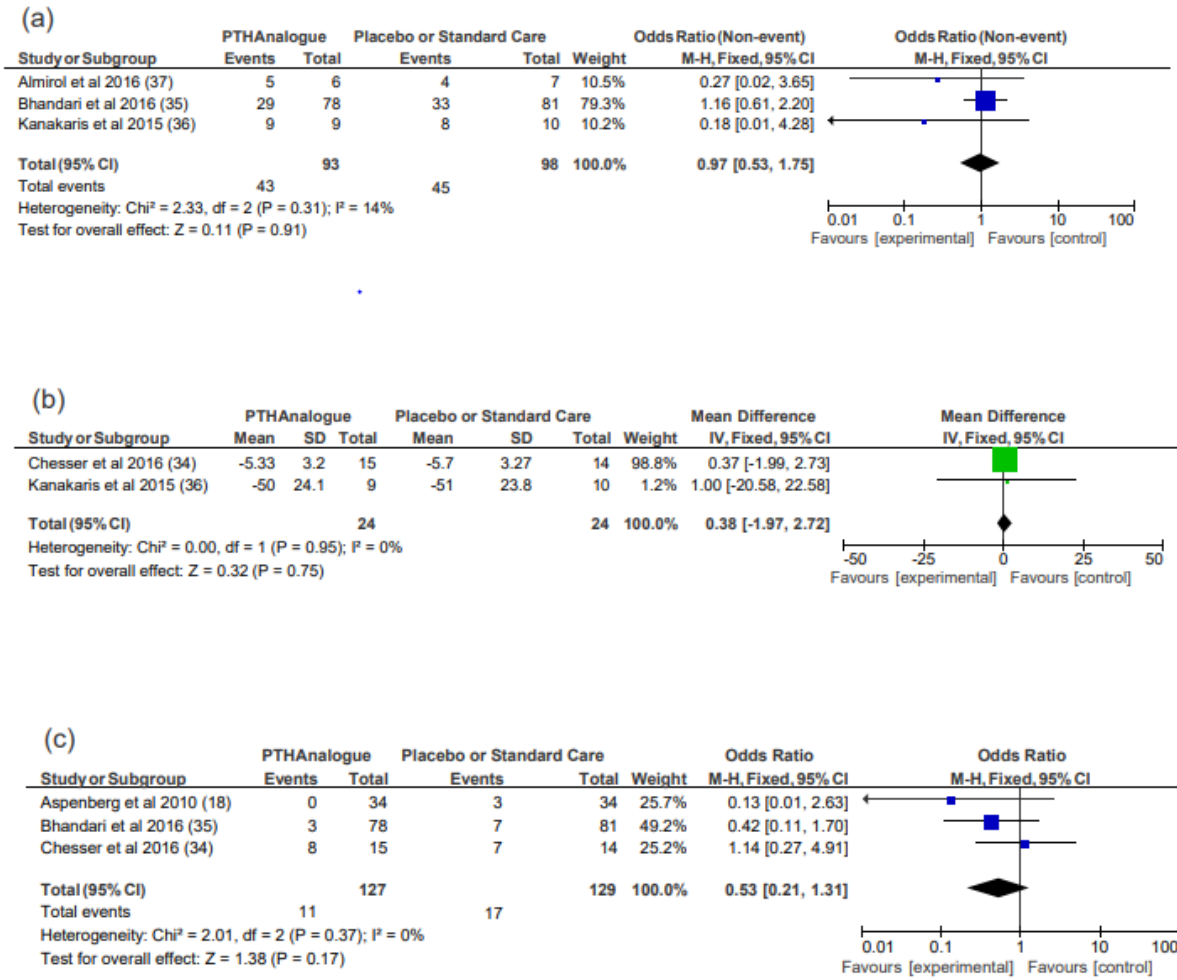
Sub-analysis was performed on the comparator groups as six trials used placebo (Almirol *et al.*, 2016; Bhandari *et al.*, 2016; Aspenberg *et al.*, 2010) or standard care (Greenspan *et al.*, 2018; Chesser *et al.*, 2016; Johansson, 2016) and four used a bisphosphonate (Shigenobu *et al.*, 2019; Malouf-Sierra *et al.*, 2016; Zhao *et al.*, 2016b; Hadji *et al.*, 2012). One trial had both a standard care arm and a bisphosphonate arm so was included in both analyses (Kanakaris, West and Giannoudis, 2015).

11.3.11.1 Sub-Analysis of Fracture Healing Rate, Pain, Adverse Events and Treatment Discontinuations Using Placebo or Standard Care as a Comparator

Consistent with the overall evaluations, there was no difference in the fracture healing rate, (OR 0.45, 95% CI 0.27 to 0.73, *P* = 0.001) or adverse events (OR 0.53, 95% CI 0.21, 1.31 *P* = 0.17) between the PTH analogue and placebo or standard care. Unlike the overall evaluations, functional outcomes were not improved with PTH analogue treatment in this sub-analysis (MD 0.38, 95% CI -1.97, 2.72, *P* = 0.75). These results are shown in figure 11-8.

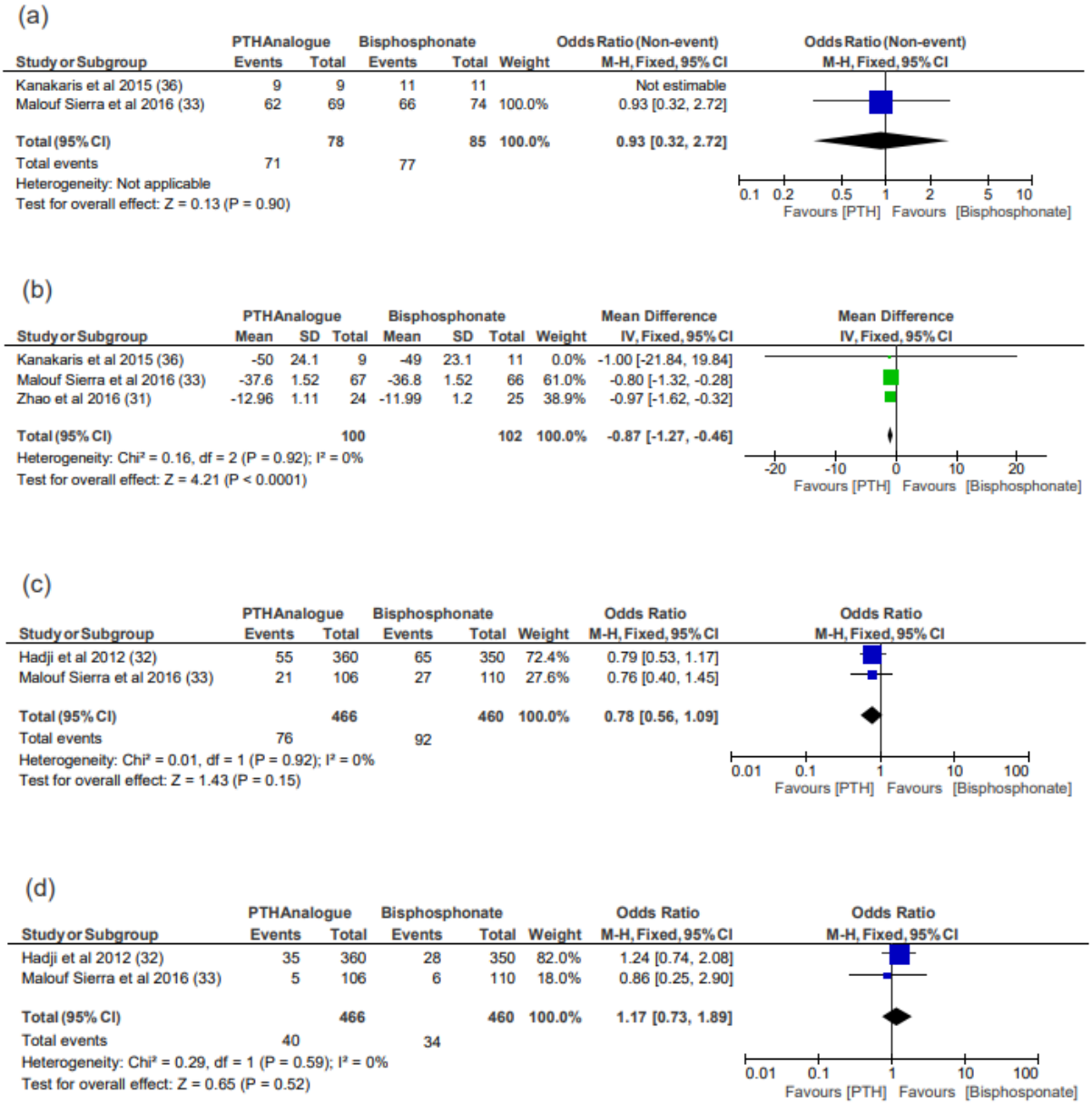
11.3.11.2 Sub-Analysis of Fracture Healing Rate, Pain, Adverse Events and Treatment Discontinuations Using a Bisphosphonate as a Comparator

Result of sub-analysis of trials comparing PTH analogues with bisphosphonates were consistent with the overall evaluations. Functional outcomes were improved (MD -0.87, 95% CI -1.27, -0.46, *P* ≤ 00001) and no difference was identified in fracture healing rate (OR 0.93, 95% CI 0.32 to 2.72, *P* = 0.90), the rate of adverse events (OR 0.78, 95% CI 0.56, 1.09, *P* = 0.15) or treatment discontinuations (OR 1.17, 95% CI 0.73, 1.89, *P* = 0.52). These results are shown in figure 11-9.



M-H: Mantel-Haenszel test.

Figure 11-8: Forest plots evaluating the difference between PTH analogue treatment and placebo or standard care. (a) Forest plot evaluating the difference in healing rate at first reported time point. Data are Odds Ratio (95% CI, Confidence Intervals). There was no difference between the healing rate between PTH analogue treated group and placebo or standard care (OR 0.97, 95% CI 0.53 to 1.75, P = 0.91), (b) Forest plot evaluating the difference in functional outcomes. Data are Mean Difference (95% CI, Confidence Intervals). There was no difference in functional outcomes between the PTH analogue treated group and placebo or standard care. (MD 0.38, 95% CI -1.97, 2.72, P = 0.75), (c) Forest plot evaluating the difference in adverse events. Data are Odds Ratio (95% CI, Confidence Intervals). M-H: Mantel-Haenszel test. There was no difference in adverse events between the groups (OR 0.53, 95% CI 0.21, 1.31, P = 0.17).



M-H: Mantel-Haenszel test.

Figure 11-9: Forest plots evaluating the difference between PTH analogue treatment and bisphosphonate treatment, (a)Forest plot evaluating the difference in healing rate at first reported time point. Data are Odds Ratio (95% CI, Confidence Intervals). There was no difference in healing rate between groups (OR 0.93, 95% CI 0.32 to 2.72, P = 0.90), (b) Forest plot evaluating the difference in functional outcomes. Data are Mean Difference (95% CI, Confidence Intervals). Functional outcomes were improved in the PTH treated group (MD -0.87, 95% CI -1.27, -0.46, P = 00001), (c) Forest plot evaluating the difference in adverse events. Data are Odds Ratio (95% CI, Confidence Intervals). There was no difference in adverse events between the groups (OR 0.78, 95% CI 0.56, 1.09, P = 0.15), (d) Forest plot evaluating the difference between treatment discontinuations. Data are Odds Ratio (95% CI, Confidence Intervals). There was no difference in treatment discontinuations between the groups (OR 1.17, 95% CI 0.73, 1.89, P = 0.52).

11.4 Discussion

This systematic review and meta-analysis reviewed the effect of PTH analogue treatment in fracture healing. The currently available PTH analogues – PTH (1-34) and PTH (1-84) – have been licensed in the UK since June 2003 and April 2017 for the treatment of hypoparathyroidism and the prevention of fractures in osteoporotic women. PTH (1-84) was licensed for osteoporosis treatment prior to 2017 by Nycomed and its sister company NPS Pharmaceuticals, but this formulation was withdrawn due to 'production difficulties'.

Several case reports and case series have suggested that PTH analogues are also efficacious in fracture healing (Stanciu and Popa, 2016; Kastirr *et al.*, 2016; Yang *et al.*, 2016; Coppola, Del Buono and Maffulli, 2015; Kim *et al.*, 2015; Mancilla *et al.*, 2015; Nozaka *et al.*, 2014; Bednar, 2013; Borges, 2015; Cortés Franco *et al.*, 2013; Tamai, Takamatsu and Kazuki, 2013) and a number of trials have begun to explore this indication, predominantly with TPTD in osteoporotic fractures (Kim *et al.*, 2017; Lou *et al.*, 2016). Previously published literature reviews of these treatments have reached conflicting conclusions; the efficacy of TPTD is reported as uncertain (11 trials, 1602 patients (Kim *et al.*, 2017)), effective in reducing fracture healing time (5 trials (inc. 1 using PTH (1-84), 251 patients (Lou *et al.*, 2016)), or not effective in reducing time to union, union rate, or reduction in pain (5 trials, 380 patients (Shi *et al.*, 2016)). The most recently published review, which included PTH(1-84) (Hong *et al.*, 2019), concluded that the evidence to support the use of PTH analogues to improve fracture healing was reasonably well established. Previous reviews (Lou *et al.*, 2018; Hong *et al.*, 2019) report that PTH analogues are not harmful to fracture healing and there is no evidence that they have a higher incidence of adverse events compared with control arms. This updated review considers eleven trials; the intervention arms were PTH analogues (TPTD 20 µg / day or TPTD 56.5 µg / week) given post fracture trialled against a variety of comparators.

In this review we found that PTH analogue treatment improves the functional outcomes of patients, but there was no evidence of improved fracture healing rate compared with comparators although one trial did show reduced time to fracture healing (Greenspan *et al.*, 2018). A reduction in pain is reported but while statistically significant, the clinical relevance

is questionable (Gallagher, Liebman and Bijur, 2001). There was no difference in SAEs or treatment discontinuations.

Treatment durations varied from one (Johansson, 2016) to 18 months (Hadji *et al.*, 2012), however, fracture healing endpoints were determined as 12 months or earlier. The optimum treatment duration for fracture healing is yet to be defined, but the three trials treating for 8 weeks or less did not show any difference in radiological healing, supporting a longer duration of treatment with PTH analogues.

The sensitivity of the radiological methods used in the trials may have affected outcomes. Most used plain-film x-ray, that might not have been sensitive enough to differentiate different phases of healing. One trial reported significant improvements in radiological healing using CT (Aspenberg *et al.*, 2010). The reproducibility of serial CT scans in the clinical setting would be difficult to justify given the radiation dose required to maintain image quality, despite dynamic protocols that keep the radiation dose to a minimum. Typically, CT scans are only used to assess cortical bridges in traumatic fractures that appear to be healing on plain film radiographs. MRI, the primary tool for diagnosing and following up insufficiency fractures, could be a safer alternative but this was only used in one trial (Almirol *et al.*, 2016). Metal artefact reduction sequences that allow assessment of marrow signal next to prothesis make this suitable for patients undergoing internal fixation, which is a large proportion of the patient population considered in this review.

The value of reporting time to radiological healing as an outcome is questioned as it is an estimate taken from radiological assessment at set intervals and assumes a normal distribution of healing. Earlier reviews have estimated the standard deviation of these values and incorporated them into meta-analysis. We did not analyse time to radiological healing in this review as the multiple estimations (particularly where the p value is reported as <0.05 rather than the precise value) required to calculate these limits their reliability. Greenspan *et al* (Greenspan *et al.*, 2018), Johansson *et al* (Johansson, 2016) and Aspenberg *et al* (Aspenberg *et al.*, 2010) described radiological outcomes as the primary outcome of the trial, and they all used plain film x-ray. The intervals of analysis varied greatly between all the trials and there

was no common analysis point between the trials using radiological outcomes as a primary endpoint. The assessment criteria were different for all trials; Almirol *et al* (Almirol *et al.*, 2016) used the validated Fredericson scale (Fredericson *et al.*, 1995). Other assessment criteria included healing graded on continuity of the cortices, but definitions were inconsistent with trials reporting this as either 'normal' or 'better' (Johansson, 2016), or as 'cortical continuity in 2 of 4 cortices' (Greenspan *et al.*, 2018) . Zhao *et al* investigated vertebral fractures and reported kyphotic angle changes as part of this assessment criteria, but this is not considered as a metric for fracture healing and was excluded from this analysis (Zhao *et al.*, 2016b).

Rarely was sufficient information reported to enable repetition of the analysis. One such example is Bhandari *et al* (Bhandari *et al.*, 2016); these authors set a statistical significance level at the one-side 10% level (a two-sided 20% level) rather than the conventional two-side 5% level, i.e. allowing p-values four times the size of convention to be declared sufficient evidence of a beneficial effect. Consistent with the 10% one-sided significance level, would be the use of an 80% confidence interval but this is reported at 90%. The methods section does not discuss the rationale for the non-standard statistical significance levels (Bhandari *et al.*, 2016).

A sub-analysis was performed comparing PTH analogues with either no intervention (placebo or standard care) and treatment with bisphosphonates, but the reduced number of trials and smaller sample of patients reduced statistical power. These results suggested no difference in fracture healing rate, adverse events, or treatment discontinuations between any of the groups, but PTH analogues did improve functional outcomes compared with bisphosphonates. We did not undertake sub-analysis by fracture type due to the heterogeneity in the pattern of fractures and the methods of evaluation used in trials.
Most of the trials included in this review included elderly patients. Only one trial examined a younger population (n=13) (Almirol *et al.*, 2016). There is evidence to suggest that age is a complicating factor in fracture healing and can lead to delayed union (Gruber *et al.*, 2006; Gaston and Simpson, 2007). This is an area that is still being explored in human subjects, but animal models (mice and rat) show age-related changes that compromise bone regeneration

(Gruber *et al.*, 2006). This limits the relevance of the findings of these trials in a younger population.

The variability in trial design compounds the complexities of comparison. The lack of a standard comparator arm is one such factor. The National Institute of Clinical Excellence (NICE)[2] does not recommend any standard treatment for fractures, yet there are placebo, standard care, oral bisphosphonate and TPTD weekly arms. Fracture locations, primary outcomes and time points at which these are measured vary considerably between the trials further limiting any comparisons.

The trials in this review mainly involve osteoporotic women, but there is no evidence that the results should not be relevant to men since no gender differences in fracture healing has been shown between the sexes (Gaston and Simpson, 2007).

This review selected all fracture types to maximise the inclusion of all RCTs of PTH analogues and fracture healing, but this approach increased the heterogeneity of the results. The quality of some of the selected trials was limited, and often contained insufficient data to enable meta-analyses; authors were contacted for further detail, but no responses were received.

This meta-analysis showed that teriparatide improved functional outcomes and reduced pain without an increase in adverse events in all fracture types. The single pilot study that investigated the use of teriparatide in stress fracture reported improved outcomes (Almirol *et al.*, 2016). The evidence reviewed in this meta-analysis supports the further investigation of teriparatide in a high quality well powered randomised controlled trial in young adults.

[2] https://www.nice.org.uk/

11.5 Conclusion

Meta-analysis of published data supports the use of PTH analogues to improve functional outcomes across a range of fracture types with no additional incidence of adverse events compared with bisphosphonates and standard care. The hypothesis that PTH improves fracture healing rate or reduction of pain was not proven, but the low-quality and heterogeneity of trial designs justifies further investigation as there is no evidence that PTH treatment caused harm or impeded fracture healing.

Only one pilot study has been performed in a young patient population (Almirol *et al.*, 2016) and a high quality well powered randomised controlled trial is required to confirm the benefit of PTH analogues in this patient cohort. Further work is required to establish the optimum duration of treatment, which probably exceeds 8 weeks.

These findings support the further investigation of PTH analogues, particularly teriparatide in the treatment of stress fractures in military personnel given the potential benefits of reducing the time spent in rehabilitation for all military personnel diagnosed with stress fractures. This efficacy of teriparatide for accelerating stress fracture healing in military personnel will now be reviewed. The hypothesis is that treatment with a teriparatide will accelerate: i) the healing of a stress fracture injury; ii) the cessation of pain associated with the injury, and iii) the return to training compared to standard care.

The quality of healing is also of importance to the MOD and so changes in bone density and micro architecture, assessed by DXA and HR-pQCT will be investigated. In the longer term the study will also assess the long term (5 year) refracture rate of the treatment group versus standard care, however this is outside the scope of this thesis.

12 Pharmacokinetic and Pharmacodynamic Analysis and Evaluation of Teriparatide (PTH 1-34) Formulations Between Sexes: The PHAB Study

12.1 Introduction

This chapter presents findings from an open label, parallel intention-to-treat Phase 3 clinical trial investigating the pharmacokinetics (PK) and pharmacodynamics (PD) of teriparatide in young healthy male and female adults. This study was a pre-cursor to the RETURN trial (Chapter 13) investigating the efficacy of teriparatide for accelerating stress fracture healing in Army infantry recruits (EudraCT: 2018-002130-20). Infantry recruits suffer a high risk of bone stress injuries (BSIs) during initial military training (HQ Army, 2016) and, until 2019, the infantry was only open to men. Government has since opened front line roles to women, and their risk of BSI is likely to be higher than their male counterparts (HQ Army, 2016). The number of women joining infantry training was expected to be low. The PHAB trial examined sex differences in the PK and PD of teriparatide in a population matched to Army recruits to understand if the results of RETURN could be extrapolated to women and underpin an inclusive policy for the treatment of stress fractures in service personnel.

Teriparatide, first licensed for the prevention of osteoporotic fractures in 2003, is a human recombinant preparation of parathyroid hormone (PTH) 1-34. The licensed dose is a 20 µg sub-cutaneous (SC) injection delivered once daily using a pre-filled pen (Lilly, 2017). Published PKPD data are based on adult pre-menopausal women (Takács *et al.*, 2019). Eli Lilly did not publish their original 'First in Human' data, therefore, there are a lack of available data in men. The patent on the Eli Lilly formulation expired in August 2019 and three teriparatide biosimilars have since been released onto the UK market. Gedeon Richter and Thornton and Ross presented data in osteoporotic patients to the MHRA to license their products as biosimilars. Teriparatide has also been investigated in the treatment of stress fractures (Almirol *et al.*, 2016), since publication of this study, terminology has changed the study included what would now be termed Bone Stress Injuries (BSI's). The study included BSI's

graded 1-4 (Kijowski *et al.*, 2012). There are no published data comparing the PKs and PDs of these drugs in a representative population of young military recruits.

Evidence to support the pharmacokinetic and pharmacodynamic equivalence of teriparatide between sexes is limited. Haden *et al*. used citrate and calcium infusions to characterise sex differences in PTH dynamics of twelve females with mean age ± SD of 26.4 ± 1.6 years and 12 men with mean age of 26.6 ± 1.3 years, and reported no sex differences in serum PTH concentrations (Haden *et al.*, 2000). Wang *et al*. reported that 2-weeks of intermittent daily PTH administration in mice was anabolic for cortical bone, with a greater effect observed in male compared with female rodents (Wang *et al.*, 2006). There are no published studies investigating differences in PK or PD or the efficacy of teriparatide injection between sexes in humans. The study presented in this chapter may help to improve the understanding of how teriparatide can be used in men and women.

12.2 Research Questions and Objectives

12.2.1 Research Questions

1. Is there a difference in the PKPD profile of a single dose of teriparatide subcutaneous injection between young adult males and females?
2. Are the PK and PD measurements reliable and reproducible?

12.2.1.1 Primary Objective and Outcome Measure

To test for a difference in the PKPD of a single dose of teriparatide PTH (1-34) between healthy male and female volunteers, matched by age, BMI and absence of medical conditions that are a bar to entry to the military recruits in training (see section 12.3.1 for further detail).

The primary outcome measure was the difference between sexes in plasma concentration of teriparatide (PTH (1-34)) over time, assessed by the Area Under the Curve (AUC), and maximum concentration (C_{max}) following a single 20 µg dose of teriparatide.

12.2.1.2 Secondary Objective and Outcome Measures

The secondary outcome measures were the difference between sexes in i) the time at C_{max} of circulating teriparatide (T_{max}) ii) AUC from T_0 to the last measurable concentration (AUC_{0-t}) Plasma Cyclic Adenosine Mono Phosphate (cAMP), iii) nephrogenous cAMP generation measured in urine, iv) changes in serum Adj Ca, PO_4 and ALP and v) changes in urinary Ca and PO_4. following a single 20 µg dose of teriparatide.

The reproducibility of the secondary outcome measures following a repeated dose of 20 µg of teriparatide was determined for: i) concentration over time (assessed by the AUC) and C_{max} of teriparatide PTH (1-34), ii) T_{max}, iii) AUC_{0-t} of cAMP, iv) nephrogenous cAMP generation, v) changes in serum Adj Ca, PO_4 and ALP and vi) changes in urinary Ca and PO_4.

12.3 Methods

12.3.1 Participants

The study enrolled 39 eligible participants comprising male and female British Military personnel or matched civilians aged 18–36 years, with a BMI between ≥18.5 and ≤28 kg/m^2, and laboratory results (full blood count, urea & electrolytes, bone profile, lipid profile, liver profile and 25 hydroxy vitamin D3) within the appropriate reference range performed within 30 days of the experimental testing visit; minor abnormalities (e.g. single result within 5 % of reference range with no other abnormalities or low serum creatinine) were evaluated by the Principal Investigator (PI) and participants were included if the PI deemed them to be of no clinical importance. Participants self-reported routinely performing a minimum of 3 sessions / week of 40 minutes or more of moderate or vigorous exercise, were free of any clinically significant illness (free from immune, cardiovascular or metabolic diseases and absence of medical conditions that would be a bar to joining the Army) and were vitamin D sufficient (25(OH) D3 >50 nmol/L). Participants who otherwise met the eligibility criteria but presented with 25(OH) D3 <50 nmol/L were offered supplementation and with their consent were re-screened once their course of treatment (Vitamin D3, 4000 IU / day for 30-days) was complete. The exclusion criteria included: hypersensitivity to the active PTH or any of the excipients listed in the Summary of Medicinal Product Characteristics (SmPC); pre-existing hypercalcemia; participants with skeletal malignancies or bone metastases; musculoskeletal injury or fracture within the last 6 months; digoxin and any other concurrent therapy that has a known interaction with teriparatide (as per the SmPC). Female participants were required to use a medically accepted method of contraception (defined as; reliable use of oral contraceptive, hormonal intrauterine device, non-hormonal intrauterine device with condom, diaphragm with condom, condom with spermicide), declaration of abstinence from sexual intercourse or be surgically sterile (having undergone bilateral tubal ligation, bilateral oophorectomy, or hysterectomy) throughout, the CONSORT diagram in Figure 12-1 gives further detail.

12.3.2 Ethical Approval

Sponsorship for this study was provided by the Norfolk and Norwich University Hospital NHS Foundation Trust and the trial site was the Norwich Clinical Trial Facility. The Army Scientific Advisory Committee approved the protocol in January 2021 and MODREC provided a favourable opinion in February 2021 (ref: 932MODREC18), followed by MHRA (EudraCT: 2018-002130-20) and HRA approval in May 2021. Trial procedures and data collection commenced on 24 May 2021 and completed on 8 July 2021.

12.3.3 Trial Design

This is an open-label, parallel, clinical trial in two separate groups, including both males and females.

12.3.4 Site Selection

Experimental testing was conducted at the Clinical Research Facility (CRF) (Quadram Institute, Norwich). This site was selected based on the sponsor approval and the facilities available on site, which included a laboratory for sample handing, easy access to Norfolk and Norwich University Hospital (NNUH) for sample delivery and medical support if required, catering, and ensuite treatment rooms. Screening visits were conducted at the CRF and the Royal Military Academy Sandhurst (RMAS).

12.3.5 Experimental Procedures

The participants were admitted to the CRF following a 12-hour overnight fast, and baseline blood and urine samples were obtained immediately. Full blood counts were analysed instantly, and results returned prior to teriparatide administration. Teriparatide was administered between 60 – 90 minutes after admittance to the CRF. Participants remained fasted for 4 hours after teriparatide administration but were allowed to eat and drink, avoiding tea, coffee, fizzy drinks and eating or drinking anything containing caffeine. Participants were not allowed to smoke or exercise from 48 hours prior to the day one teriparatide dose and for the duration of trial procedures.

Participants remained in the CRF for 12 hours post drug administration, and during this period serial blood samples were drawn following the schedule listed in table 12-1. To avoid repeated venepuncture, a peripheral venous catheter was placed in an antecubital vein and flushed with 1mL saline after each sample was taken. Prior to collection of the subsequent samples, 1.5mL of blood was discarded to ensure the sample was free of the flushing solution.

To determine plasma teriparatide and cAMP concentrations, blood samples were collected in (4 mL) EDTA-di-potassium-containing tubes at the following time points: baseline and at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360 and 420 minutes after administration. Samples were centrifuged immediately at ~ 3000 g for 10 min at 4° C. Plasma was separated, aliquoted, and stored at -80° C.

To determine the bone metabolic profiles (Calcium (Ca) Albumin, Alkaline Phosphatase (ALP), phosphate (PO_4), blood samples were collected in 3.5 mL plain tubes at the following time points: baseline, and at 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, 600, 660 and 720 minutes after administration. Samples were transferred to the NNUH for immediate analysis, where they were centrifuged at ~ 3000 g for 10 min at 4° C. Following analysis, serum was aliquoted and stored at -80° C for repeat if required.

The complete 24-hour urine output was collected from participants. This was split into 4 hourly collections throughout the day (from time zero to 12-hours post dose), and a 12-hour collection overnight. The volume of each urine passed was recorded to enable nephrogenous cAMP generation calculation. Experimental trial assessments are described in detail in table 12-1.

12.3.6 Intervention

A single dose of 20 ug teriparatide was administered subcutaneously on two occasions (the morning of experimental testing days 1 and 3), separated by 36 hours, by a research nurse. After the first administration of teriparatide, participants were closely monitored for 12 hours in clinical research ward setting and then sent home where they were requested to collect urine for a further 12 hours, followed by a 24-hour washout period prior to the second PTH

administration. The teriparatide (TERROSA, Gedeon Richter, Budapest, Hungary, as described in Chapter 8) was obtained from NHS commercial supply routes.

12.3.7 Adverse Event Reporting

Participants were monitored for adverse events throughout the study by direct questioning, spontaneous self-reports, and clinical parameters including blood pressure and heart rate.

12.3.8 Blood Analysis

All screening, baseline and biochemistry bone profiles (Ca, albumin, PO_4 and ALP), were analysed at the Norfolk and Norwich University Foundation Trust (Norwich, UK). The FBCs were measured by a combination of photometry, optical counting and fluorescence analysis on an Abbott diagnostics Alinity HQ (Illinois, U.S.A). Urate, albumin, bilirubin, alanine transaminase (ALT), ALP, high density lipoprotein (HDL), total cholesterol, sodium, potassium, urea, creatinine, Ca, PO_4 and magnesium were measured using standard automated methods on Abbott diagnostics Alinity C (Illinois, U.S.A). Low Density Lipoprotein was calculated by subtracting HDL from total cholesterol. Intact PTH was measured using an immunoassay on a Roche COBAS 6000 using a Roche (Burgess Hill, UK). 25(OH)D was measured using an immunoassay on an Abbott diagnostics Alinity I (Illinois, U.S.A).

The research biochemical analyses are described in chapter 8.

Table 12-1: PHAB Trial assessment schedule

Day	Time Schedule	**Action** (Parameter)
1	Baseline	Admit, confirm consent. Cannulation, **baseline assessments** (FBC[a], Ca, PO_4, Albumin, ALP, Chol, Triglycerides, HDL, ALT, total protein, total bilirubin, Na, K, urea, Cr, Mg, PTH (1-84), PTH (1-34), 25-OH Vit D, cAMP, urinary measures; nephrogenous cAMP, PYP, DYP, Ca, PO_4.)
	Time 0	Injection of first teriparatide dose Post dose urine collection start (nephrogenous cAMP, PYP, DYP, Ca, PO_4)
	5 mins	PTH (1-34), cAMP
	10 mins	PTH (1-34), cAMP
	15 mins	PTH (1-34), cAMP, Bone Profile[b]
	20 mins	PTH (1-34), cAMP
	25 mins	PTH (1-34), cAMP
	30 mins	PTH (1-34), cAMP, Bone Profile
	45 mins	PTH (1-34), cAMP, Bone Profile

	60 mins	PTH (1-34), cAMP, Bone Profile
	75 mins	PTH (1-34), cAMP, Bone Profile
	90 mins	PTH (1-34), cAMP, Bone Profile
	120 mins	PTH (1-34), cAMP, Bone Profile
	150 mins	PTH (1-34), cAMP, Bone Profile
	180 mins	PTH (1-34), cAMP, Bone Profile
	240 mins	PTH (1-34), cAMP, Bone Profile **Urine Collection Changeover** (nephrogenous cAMP, PYP, DYP, Ca, PO_4)
	300 mins	PTH (1-34), cAMP, Bone Profile
	360 mins	PTH (1-34), cAMP, Bone Profile
	420 mins	PTH (1-34), cAMP, Bone Profile
	480 mins	Bone Profile **Urine Collection Changeover** (nephrogenous cAMP, PYP, DYP, Ca, PO_4)
	540 mins	Bone Profile
	600 mins	Bone Profile
	660 mins	Bone Profile
	720 mins	Bone Profile **Urine Collection Changeover** (nephrogenous cAMP, PYP, DYP, Ca, PO_4)
2	1440 mins	**Final dose one urine collection ends** (nephrogenous cAMP, PYP, DYP, Ca, PO_4)
3	Baseline	Cannulation, **baseline assessments** (FBC, Ca, PO_4, Albumin, ALP, Chol, Triglycerides, HDL, ALT, total protein, total bilirubin, Na, K, urea, Cr, Mg, PTH (1-84), PTH (1-34), 25-OH Vit D, cAMP, urinary measures; nephrogenous cAMP, PYP, DYP, Ca, PO_4.)
	Time 0	Injection of second teriparatide dose Post dose urine collection start
	5 – 720 mins	Blood Sampling and urine collection changeovers as per day 1
4	1440 mins	Final dose two urine collection ends

[a] Full blood count (FBC) includes white blood cells, red blood cells, platelets, reticulocytes and haemoglobin.
[b] The bone profile includes assays for Calcium (Ca), albumin, phosphate (PO_4) and alkaline phosphatase (ALP).
Cholesterol (Chol), High density lipoproteins (HDL), Alanine transferase (ALT), Sodium (Na), Potassium (K), Creatinine (Cr), Magnesium (Mg), Parathyroid Hormone (PTH), 25 hydroxy vitamin D3 (25-OH Vit D), Cyclic adenosine mono phosphate (cAMP), Pyridinoline (PYD), deoxypyridinoline (DPD).

12.3.9 Urine Collection and Handling

Single point urine samples were collected in 50 mL sterile containers using no preservatives. 24-hour urine collections were collected in 3 x 4-hour (0-4, 4-8, 8-12 hours post dose) and 1 x 12-hour (12-24 hours post dose) collections each in a 5L sterile container using no preservatives.

12.3.10 Urine Analysis

Urinary Ca and PO_4 were measured at the Norfolk and Norwich University Hospital Foundation Trust (Norwich, UK) using the Abbott Diagnostics immunoassay on the Alinity I (Illinois, U.S.A). Ca and PO_4 excretion were calculated by multiplying the concentration in

mmol/L by the total urine volume (L). Where the concentration was below the lower limit of quantification (LLQ), the LLQ was used in the calculation (LLQ Ca = 0.5 mmol/L, LLQ PO_4 = 1.6 mmol/L).

Urinary pyridinoline, deoxypyridinoline and cyclic AMP were measured at the BioAnalytical Facility (Norwich, UK) using in-house LCMS methods (Al Riyami, 2017; Tang *et al.*, 2016)

12.3.11 Statistical Methods

12.3.11.1 Power Calculation

To address the primary objective, thirty individuals were recruited, 15 men and 15 women. This sample provided 80% statistical power to detect a between group difference of approximately 1 SD between the two groups in any PK or PD variable.

To address the secondary objective, an intra-class correlation coefficient (ICC) was used to calculate the reproducibility of each measure. The standard error of each ICC was dependent upon the value of the estimate. Thirty individuals, measured twice, resulted in a standard error of less than 0.035 for an ICC of around 0.90, and a standard error of less than 0.018 for an ICC of around 0.95.

12.3.11.2 Statistical Analysis

Data were analysed using SPSS (v. 25, SPSS Inc., USA). All data were initially checked for normality. Descriptive statistics were calculated for the sample. Statistical significance was accepted at $p \leq 0.05$.

A linear mixed effects model was used to model within subject repeated measures for UcAMP, cAMP, Adj Ca, ALP, PO_4, urine Ca and urine PO_4 to determine differences between the male and female groups after adjusting for their age. Different co-variance structures (compound symmetry, unstructured and auto regressive) were modelled, and the co-variance structure resulting in the best fitting model adopted. The analysis was repeated for dose one (day one) and two (day three). Differences in PTH-1-34 between males and females was assessed using AUC, C_{max} and T_{max}.

The remaining blood and urine measurements that were only recorded at 0 min were analysed using an analysis of covariance adjusting for age to compare results between males and females. Graphical representation was created by GraphPad PRISM and results are shown as median [95% Confidence Intervals].

12.4 Results

12.4.1 CONSORT Data

Ninety participants were assessed for eligibility, 34 were excluded at pre-screening, 26 because they did not meet the entry criteria (no exercise n = 6, excluded health condition or medication n = 14, BMI out of range n = 5, no contraception n = 1), five were unavailable for testing dates and two declined to participate after a study brief. Fifty-six participants provided consent and underwent a screening visit, 13 were excluded because they did not fit the entry criteria (anomalies in blood tests n =10, excluded health conditions or medications n =1, BMI out of range n =1, no contraception n =1). One declined to participate any further and one had an adverse event at screening (vaso-vagal following venepuncture) and it was decided this person would be unsuitable for cannulation. Eligibility was confirmed in 41 participants, 39 of which were invited to the experimental testing visit, the remaining two (last recruited) were kept as reserves in case of withdrawals. Thirty-three participants completed the study, a further three (all females) completed the first day only (failure to cannulate on Day 2 [n =2], adverse event on Day 1, [n =1]). Three participants were withdrawn on Day 1 having had no data collected (unable to cannulate [n =1], adverse event [n =1], cannula tissued in first 30 mins, could not re-cannulate [n =1]). Figure 12-1 shows a modified CONSORT diagram describing enrolment, intervention, follow-up and analysis.

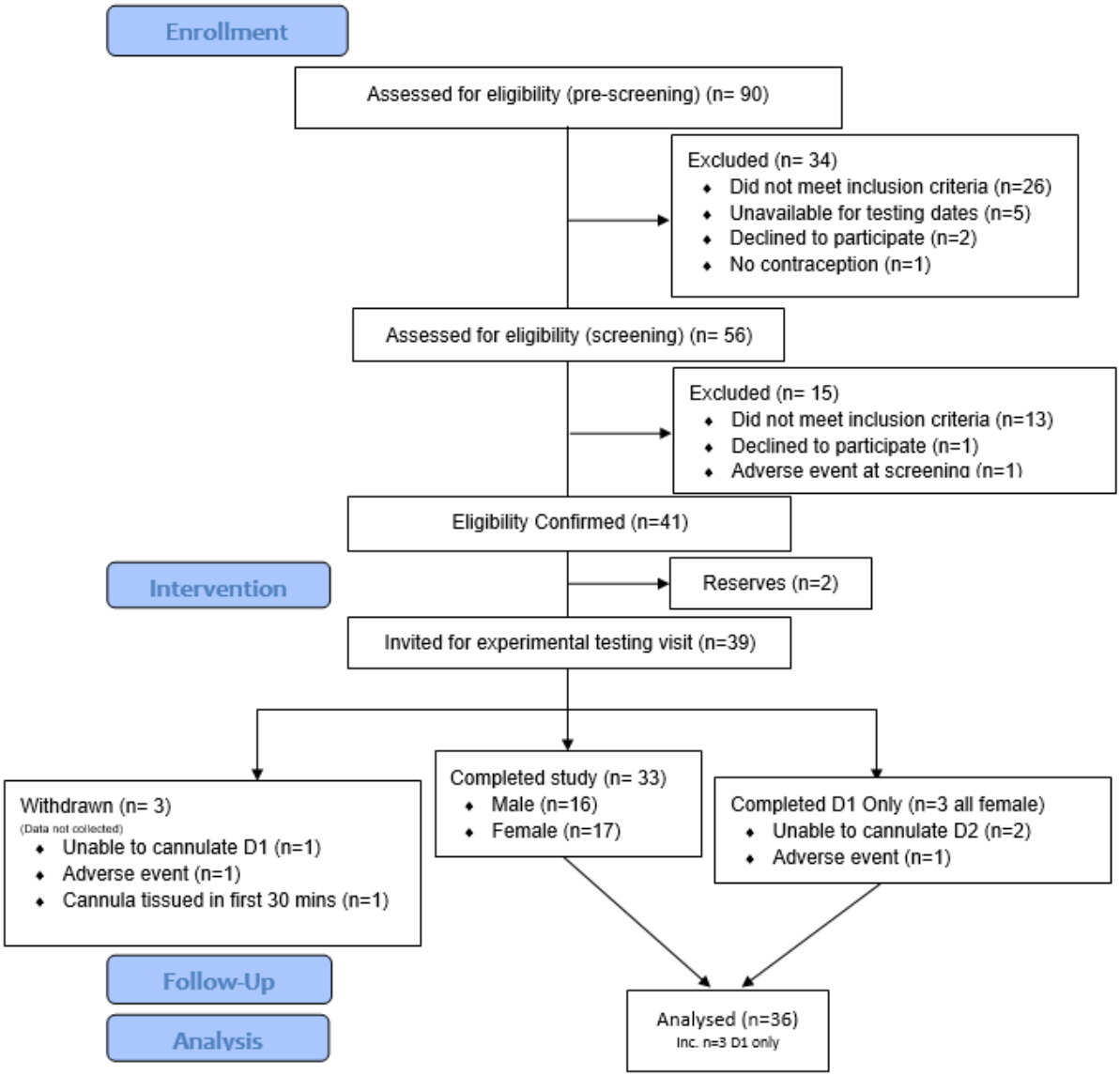


Figure 12-1: A modified CONSORT diagram describing enrolment, intervention, follow-up, and analysis for participants.

12.4.2 Description of Participants

Thirty-three participants completed the study with a further three provided complete data on Day one. Participant characteristics are shown in table 12-2. Males were older (P = 0.026) and had a higher BMI (P = 0.022) than females. There was no significant difference in alcohol intake, smoking or ethnicity between groups.

Table 12-2: PHAB participant characteristics by sex

Categorical data are presented as total (percentage) with p-values calculated by Chi Squared. Continuous data are mean ± SD with p-values from independent t-tests, or median [IQR] with p-values from Mann-Whitney U Tests.

	Overall (n=36)	**Male** (n=16)	**Female** (n=20)	***p***
Demographics				
Age at Consent (years)	22.2 [7.7]	26.1 [9.3]	20.6 [3.9]	0.026*
Alcohol Intake[a]				
Zero Intake	3 (8.3%)	1 (6.2%)	2 (10.0%)	
Light (1-20 units per week)	29 (80.6%)	12 (75.0%)	17 (85.0%)	0.412
Heavy (21-67 units per month)	4 (11.1%)	3 (18.8%)	1 (5.0%)	
Smoking[b]				
Never Smoked	31 (86.1%)	13 (81.2%)	18 (90.0%)	
Previous Smoker	4 (11.1%)	3 (18.8%)	1 (5.0%)	0.302
Current Smoker	1 (2.8%)	0 (0.0%)	1 (5.0%)	
Ethnicity[c]				
White	35 (97.2%)	15 (93.7%)	20 (100.0%)	
Multiple ethnic group	1 (2.8%)	1 (6.3%)	0 (0.0%)	0.257
Body Composition				
Body Mass Index (kg/m^2)	23.7 [4.5]	25.6 [4.7]	23.1 [4.0]	0.022*

* Denotes significant result.

12.4.3 Area Under the Curve Following a Single Dose of Teriparatide

Teriparatide (PTH 1- 34) area under the curve (AUC) following a single 20 µg dose was higher in males than females (22385.0 pmol*h/mL [17554.0 to 27216.0] *versus* 14078.0 pmol*h/mL [9817.3 to 18339.0], *P* = 0.016) (Figure 12-2).

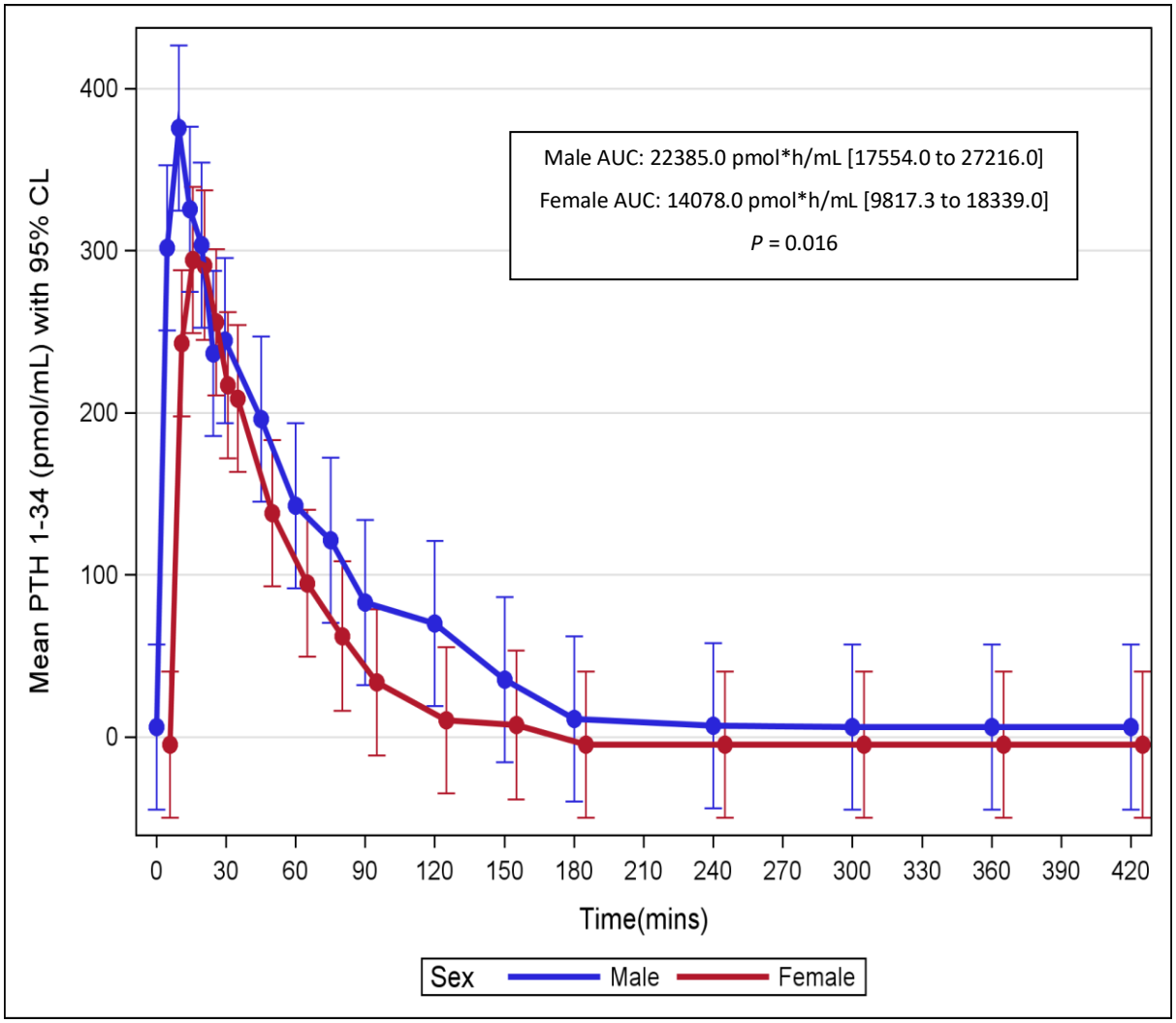


Figure 12-2: The mean serum concentrations (with 95% Confidence Levels (CL)) of teriparatide following a single 20 µg dose in males (blue) and females (red). Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

12.4.4 Secondary Outcome Measures

12.4.4.1 Males versus Females Following a Single 20 µg Dose of Teriparatide.

There was no difference in the maximum concentration (C_{max}) or time of maximum concentration (T_{max}) between males and females following a single 20 µg dose of teriparatide. The full results are shown in table 12.3.

Table 12-3: Description of maximum teriparatide concentration (C_{max}) and time at maximum (T_{max}) teriparatide concentration following a single 20µg dose of teriparatide. Data are mean ± 95% CL.

	C_{Max} Estimate (nmol/L)	P Value	T_{Max} Estimate (mins)	P Value
Male	442.1 [319.9 to 564.3]		13.9 [6.4 to 21.4]	
Female	322.8 [215.0 to 430.6]		6.1 [-0.3 to 12.5]	
Difference Male Vs Female	119.3 [-48.6 to 287.3]	0.157	7.9 [-1.9 to 17.6]	0.110

The area under the curve from time zero to last measurable concentration (AUC_{0-t}) of plasma cyclic Adenosine Mono Phosphate (cAMP) was significantly higher in males ($P = 0.007$). A plot comparing the mean plasma cAMP following a single 20 µg dose of teriparatide between males and females is shown in figure 12-3.

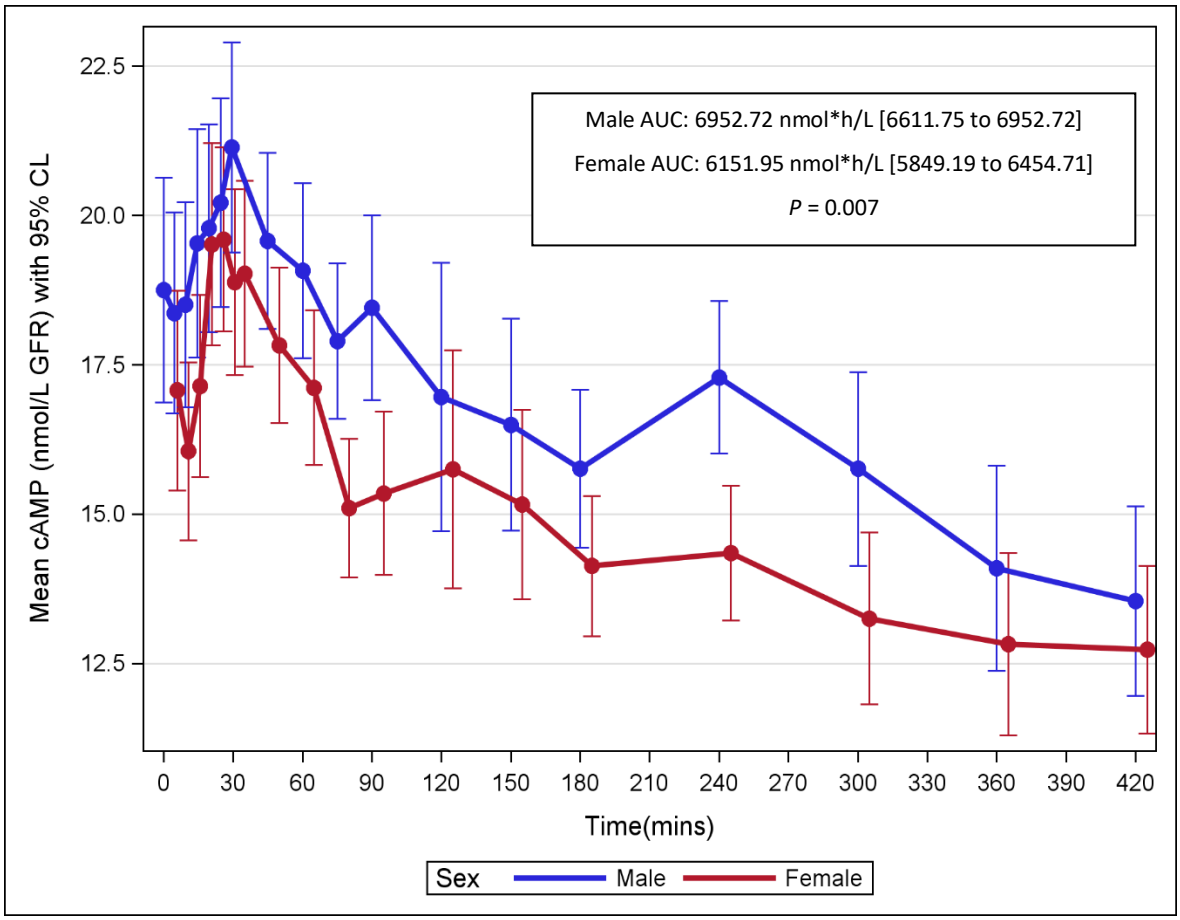
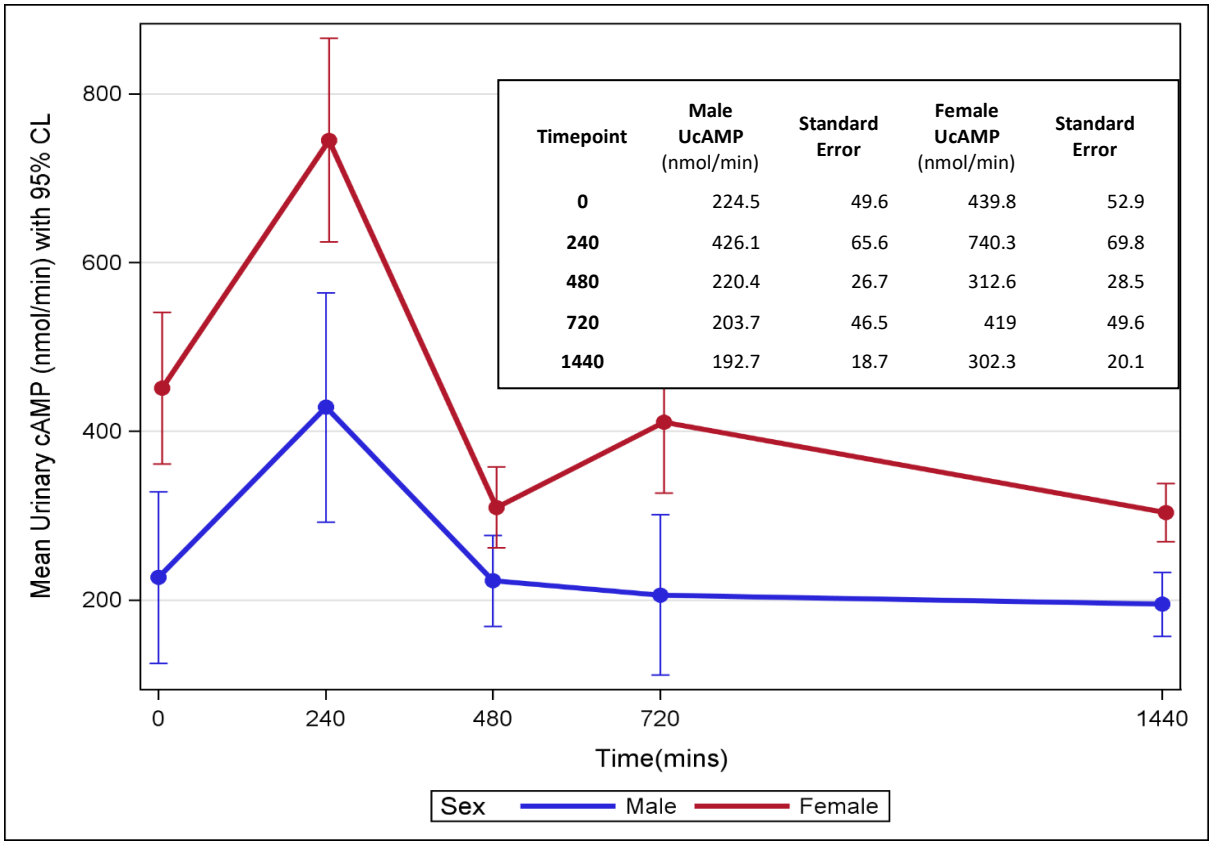


Figure 12-3: The mean plasma concentrations (with 95% Confidence Levels (CL)) of cAMP following a single 20 µg dose of teriparatide in males and females. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

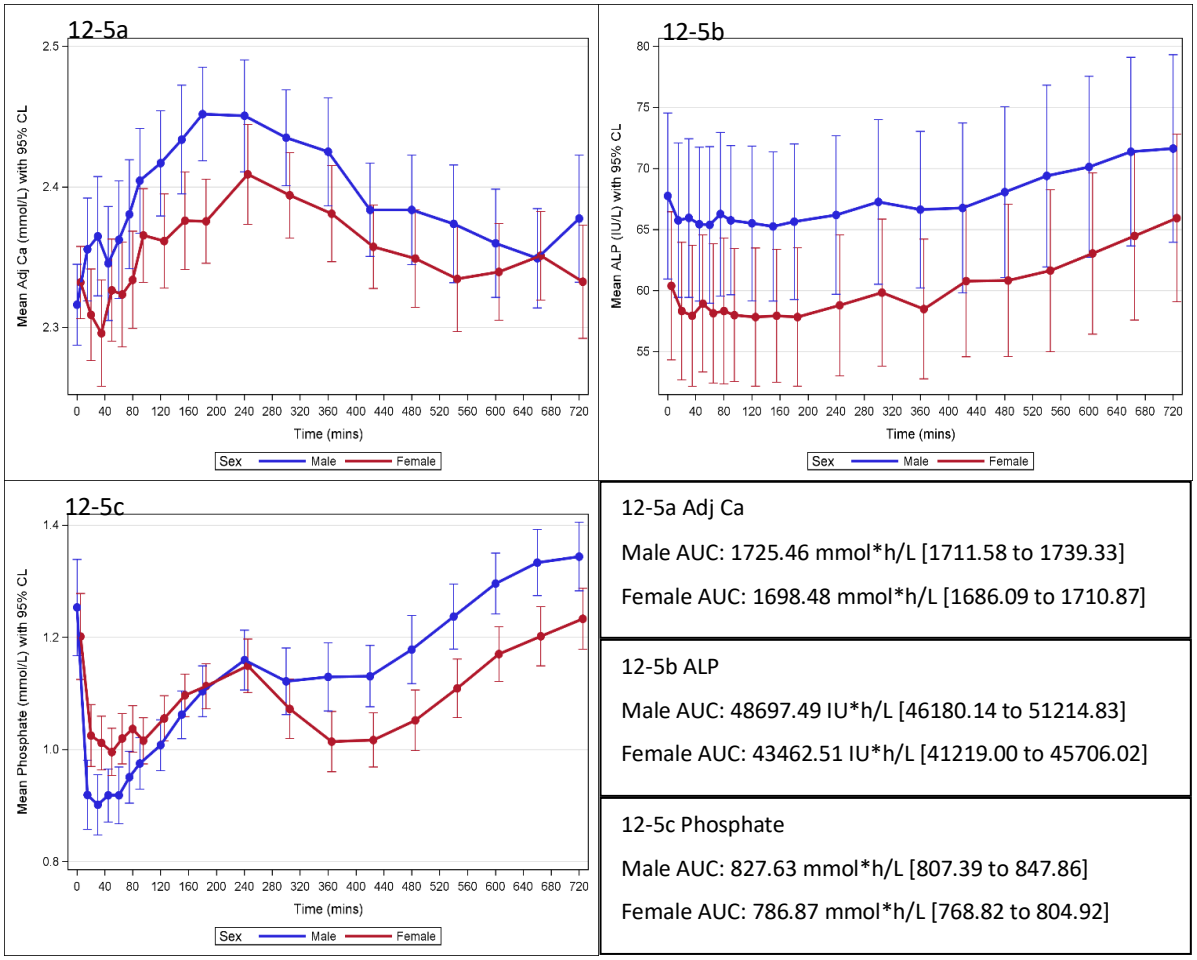
The cAMP in urine (UcAMP), which is a combination of the filtered load and nephrogenous cAMP, was significantly higher in females compared to males ($P \leq 0.001$). A comparison of the mean UcAMP following a single 20 µg dose of teriparatide in males and females is shown in figure 12-4.



Timepoint	Male UcAMP (nmol/min)	Standard Error	Female UcAMP (nmol/min)	Standard Error
0	224.5	49.6	439.8	52.9
240	426.1	65.6	740.3	69.8
480	220.4	26.7	312.6	28.5
720	203.7	46.5	419	49.6
1440	192.7	18.7	302.3	20.1

Figure 12-4: The mean urine cAMP (with 95% Confidence Levels (CL)) following a single 20 µg dose of teriparatide in males and females. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

Following a single 20 µg dose of teriparatide, females have a significantly lower Adjusted Ca measurements over time ($P = 0.0079$) (Figure 12-5a), but there was no statistical difference in ALP ($P = 0.1046$) (Figure 12-5b) and PO_4 ($P = 0.4552$) (Figure 12-5c) between males and females.



12-5a Adj Ca

Male AUC: 1725.46 mmol*h/L [1711.58 to 1739.33]

Female AUC: 1698.48 mmol*h/L [1686.09 to 1710.87]

12-5b ALP

Male AUC: 48697.49 IU*h/L [46180.14 to 51214.83]

Female AUC: 43462.51 IU*h/L [41219.00 to 45706.02]

12-5c Phosphate

Male AUC: 827.63 mmol*h/L [807.39 to 847.86]

Female AUC: 786.87 mmol*h/L [768.82 to 804.92]

Figure 12-5: The mean adjusted Ca (12-5a), ALP (12-5b) and phosphate (12-5c) (with 95% Confidence Levels (CL)) following a single 20µg dose of teriparatide in males and females. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

Females have significantly lower urine Ca (P = 0.009) (Figure 12-6) and significantly lower creatinine adjusted urine PO_4 concentrations ($P \leq 0.001$) (Figure 12-6) following a single 20 µg dose of teriparatide.

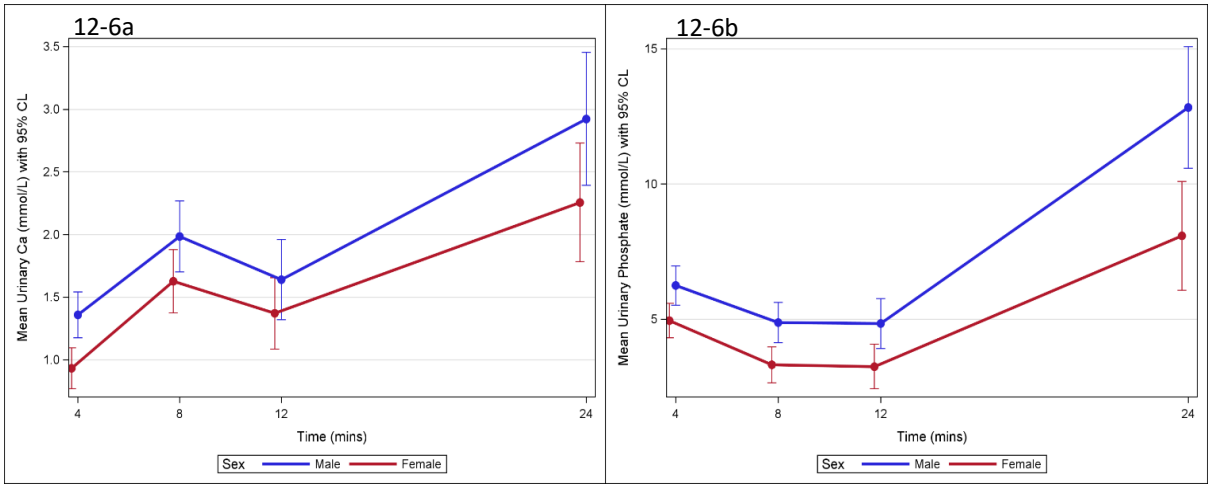


Figure 12-6: The mean urinary Ca (12-6a) and urinary phosphate (12-6b) (with 95% Confidence Levels (CL)) following a single 20µg dose of teriparatide in males and females. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

Compared to males, females had significantly lower cumulative urine Ca excretion (*P* = 0.0006) and significantly lower cumulative urine PO_4 excretion (*P* = <0.0001) following both a single 20 µg dose of teriparatide and a repeated dose 48 hours later (day 3) (Figure 12-7).

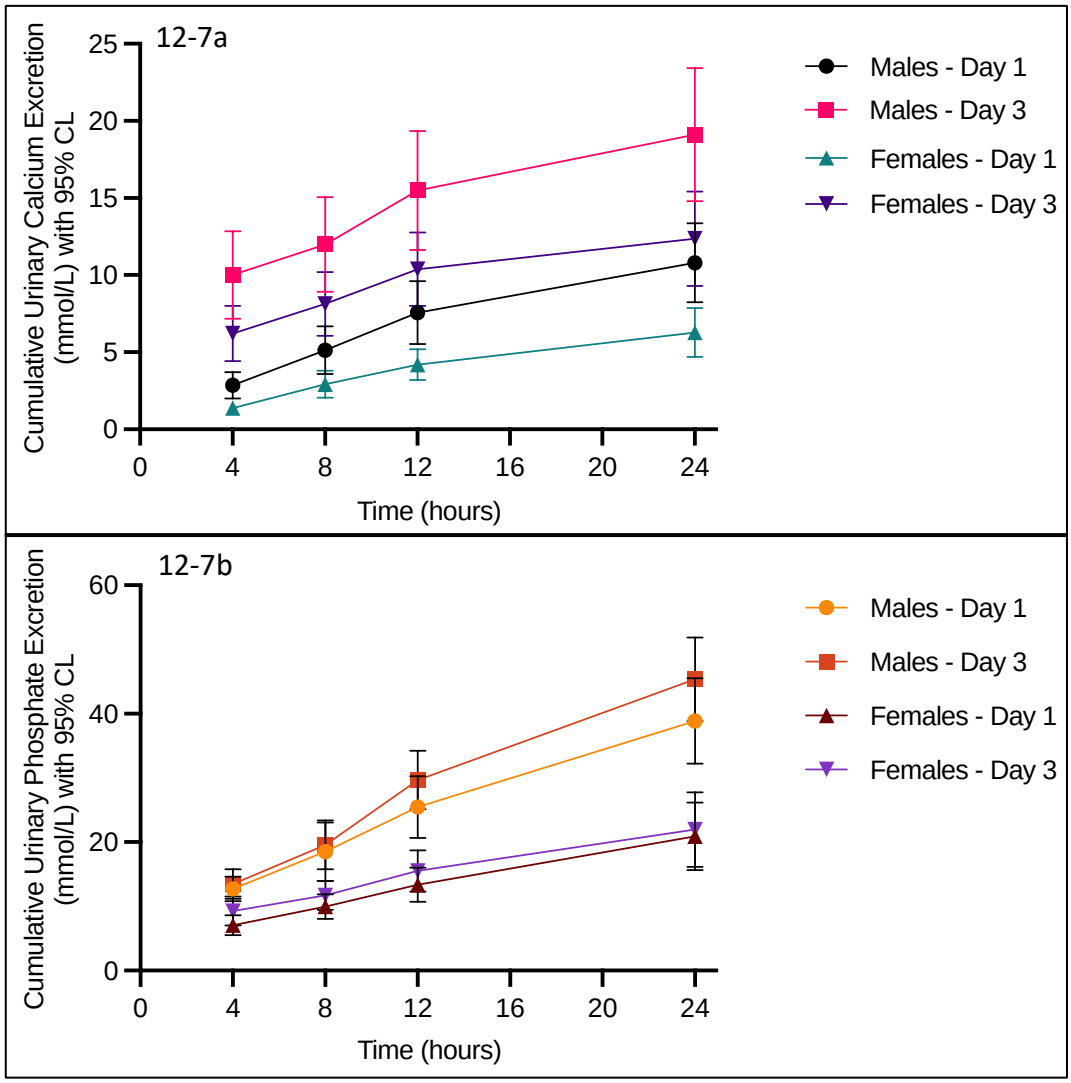


Figure 12-7: Comparison of cumulative urinary calcium excretion between males and females following a single 20µg dose of teriparatide and a second dose administered 48 hours later (12-7a) (with 95% Confidence Levels (CL)). Comparison of cumulative urinary phosphate excretion between males and females following a single 20µg dose of teriparatide and a second dose administered 48 hours later (12-7b) (with 95% CL).

12.4.4.2 Male Vs Female following a Repeated Dose of 20 µg dose of Teriparatide.

The concentration over time (AUC) of teriparatide following a repeated 20 µg dose (48-hours after the first dose) was the same in males compared to females (13475.0 [10060.0 to 16889.0] vs 15352.0 [12063.0 to 18640.0], *P* = 0.441. There was also no difference between C_{max} (mean difference 80.3 pmol/mL [-227.1 to 387.8] *P* = 0.601) and T_{max} (mean difference -1.4 mins [-7.2 to 4.4] *P* = 0.64) following a repeated 20 µg dose of teriparatide.

A comparison of the mean serum concentrations of PTH (1-34) following a repeated dose of teriparatide is shown in figure 12-8.

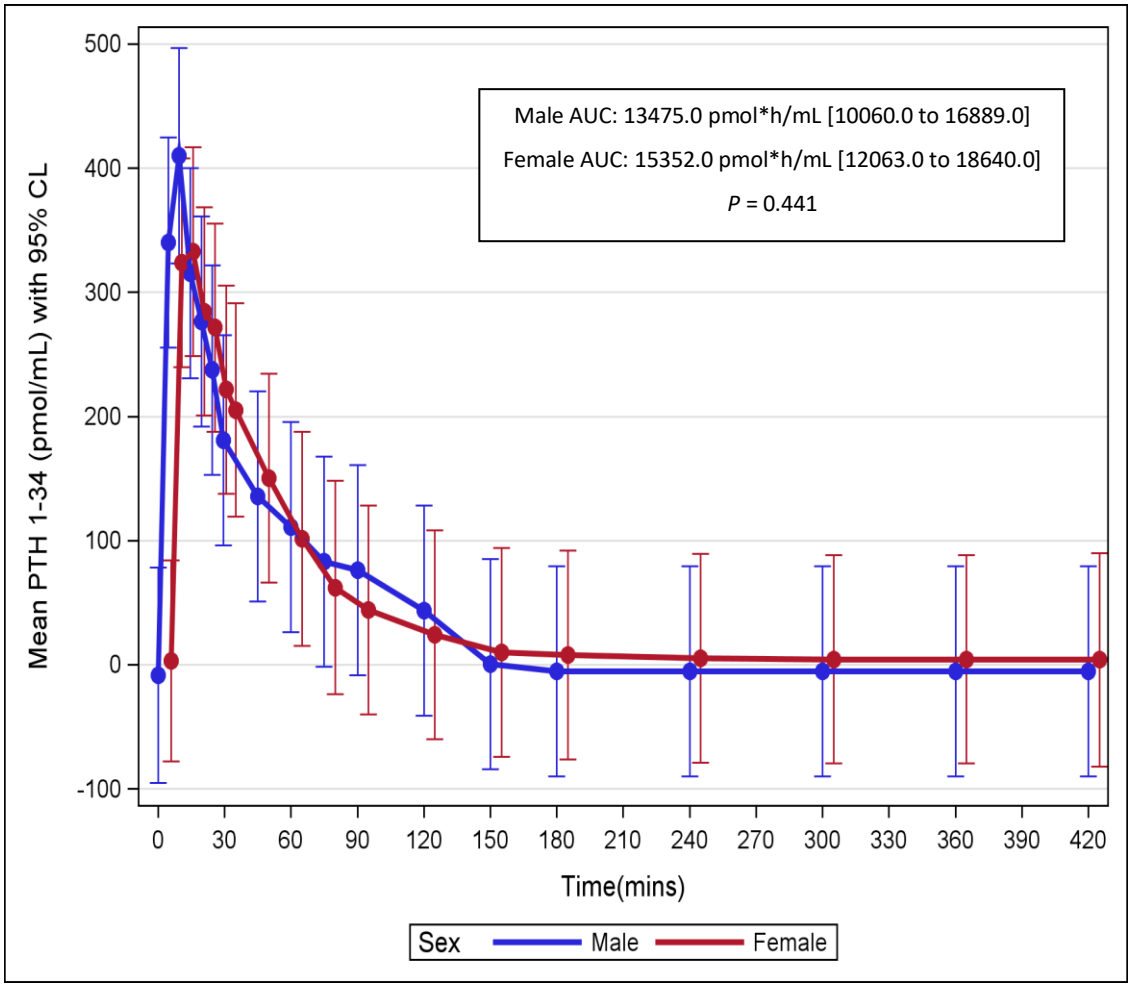
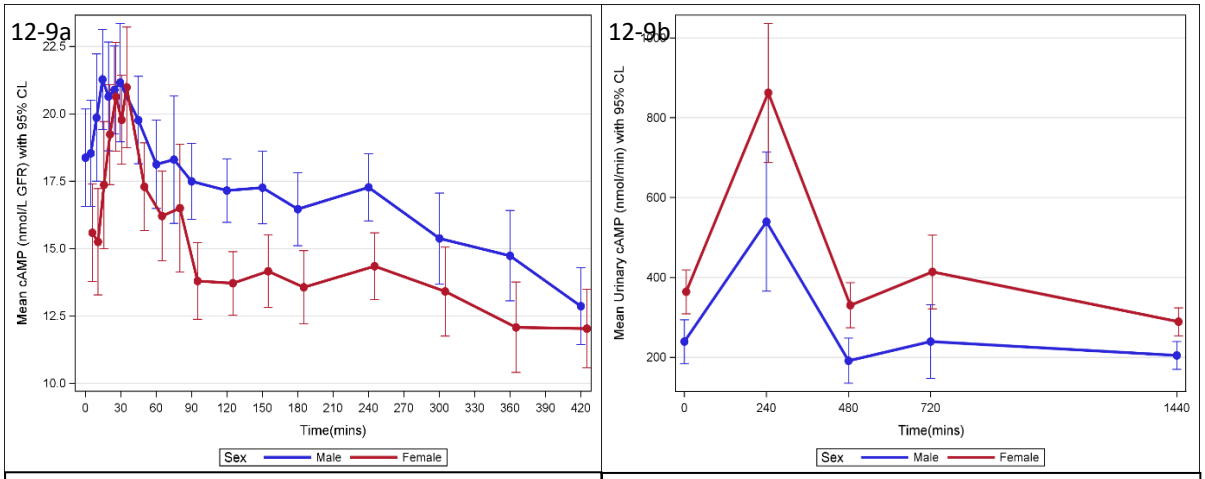


Figure 12-8: The mean serum concentrations of teriparatide (with 95% Confidence Levels (CL)) following a second 20 µg dose of teriparatide (48-hours after the first dose) in males and females. Dose administered at 0 mins. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

Following a repeated 20 µg dose of teriparatide (48-hours after the first dose) plasma cAMP and urinary cAMP were higher in males compared to females (P = 0.0004 and P = 0.0003 respectively). A comparison of the mean plasma cAMP and urinary cAMP following a repeated dose of teriparatide are shown in figure 12-9.



Plasma cAMP

Male AUC: 7012.08 nmol*h/L [6680.83 to 7012.08]

Female AUC: 5949.71 nmol*h/L [5618.43 to 6280.98]

Time-point	Male UcAMP (nmol/min)	SE	Female UcAMP (nmol/min)	SE
0	212.1	22.5	363.4	22.5
240	546.5	98.8	856.6	98.8
480	181.8	29.5	320.1	29.5
720	224.2	47.7	409.8	47.7
1440	202.6	12.1	306.3	12.1

Figure 12-9: Mean plasma cAMP (12-9a) and urinary cAMP (12-9b) following a second 20 µg dose of teriparatide (with 95% Confidence Levels (CL)) (48-hours after the first dose) in males and females. Dose administered at 0 mins. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

Following a repeated 20 µg dose of teriparatide (48-hours after the first dose) serum PO_4 was significantly different in males compared to females (P = 0.0008), concentrations initially decreased more so in males before increasing at a greater rate than in females. There was no difference between males cand females in serum adj Ca or ALP (P = 0.40 and P = 0.35 respectively). A comparison of the mean serum PO_4, adj Ca and ALP following a repeated dose of teriparatide are shown in figure 12-10.

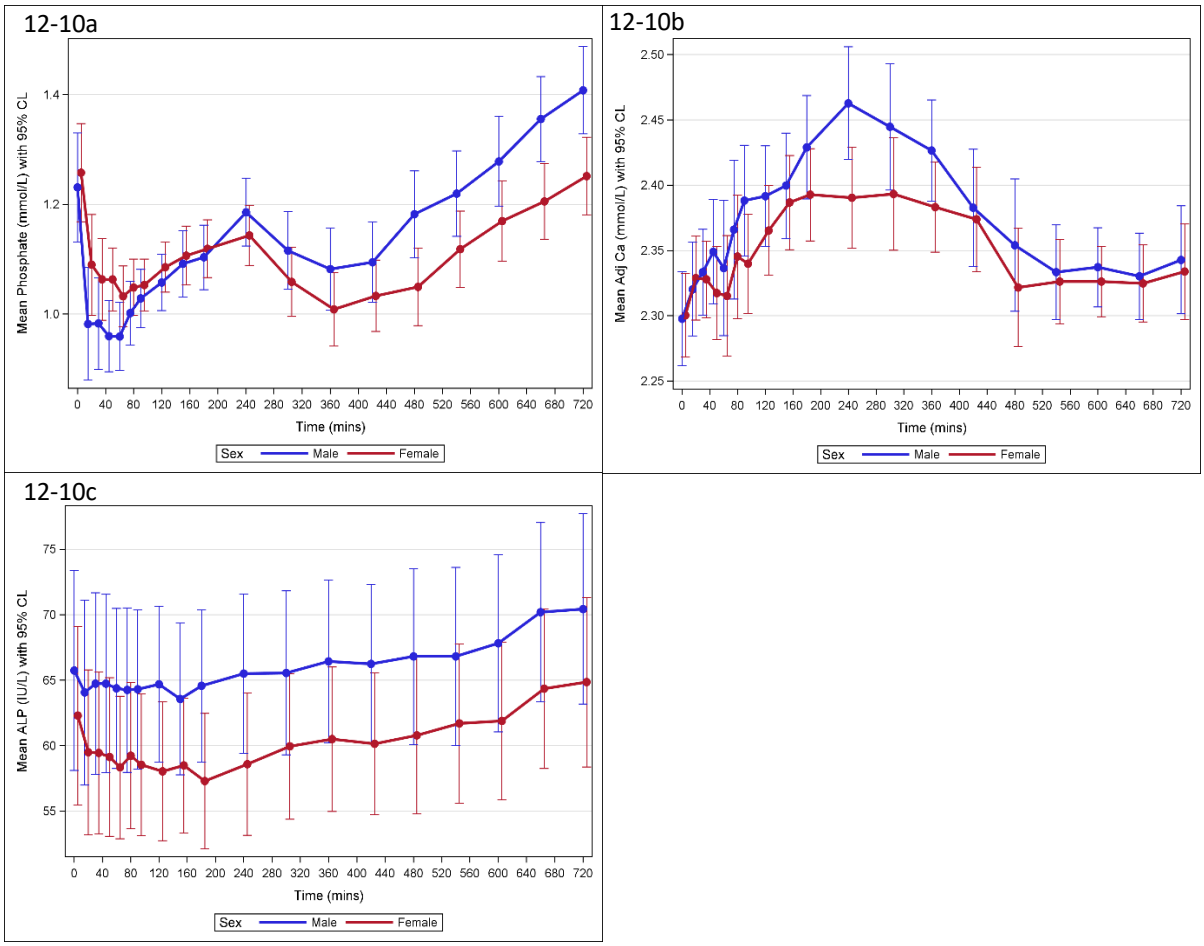


Figure 12-10: Mean serum phosphate (12-10a), adj Ca (12-10b) and ALP (12-10c) following a second 20 µg dose of teriparatide (48-hours after the first dose) (with 95% Confidence Levels (CL)) in males and females. Dose administered at 0 mins. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

Following a repeated 20 µg dose of teriparatide (48-hours after the first dose) there was no difference in mean urinary Ca and PO_4 between males and females (P = 0.408 and P = 0.234 respectively). A comparison of the mean urinary Ca and PO_4 following a repeated dose of teriparatide are shown in figure 12-11.

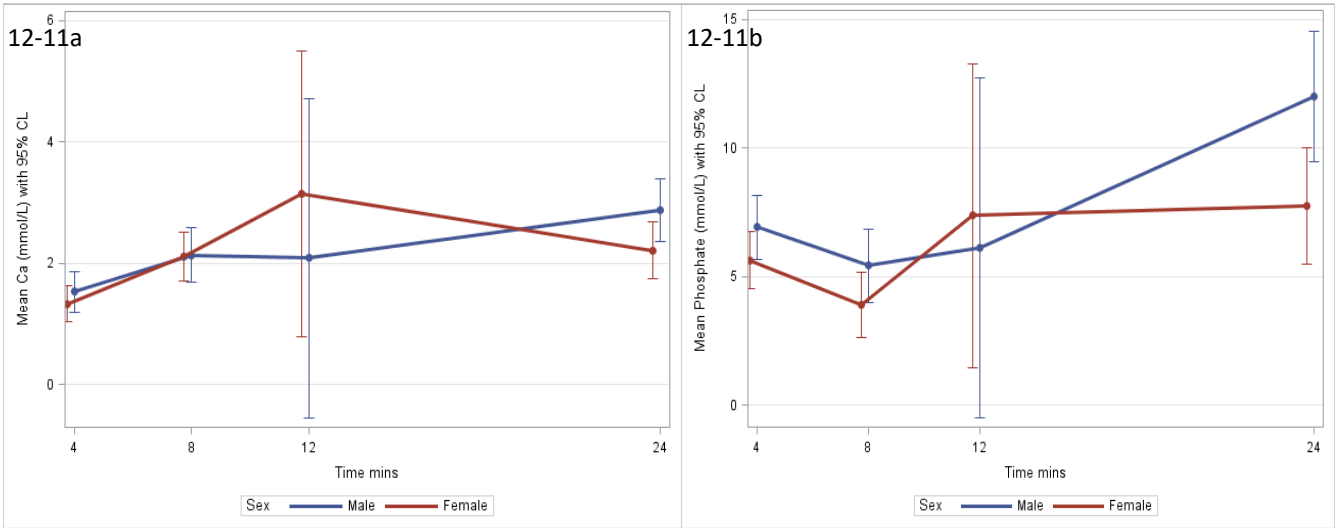


Figure 12-11: Mean urine calcium (12-11a) and phosphate (12-11b) following a second 20 µg dose of teriparatide (48-hours after the first dose) (with 95% Confidence Levels (CL)) in males and females. Dose administered at 0 mins. Male and female measurements were taken at the same timepoints, they are offset in the graph for clarity.

12.4.4.3 Dose 1 (day 1) Vs Dose 2 (day 3) Comparisons (Males and Females Combined)

No statistically significant differences were observed following dose one and dose two any of the analytes, examined when male and female data was combined.

12.4.4.4 Dose 1 (day 1) Vs Dose 2 (day 3) Comparisons (Males and Females Separate)

No statistically significant differences were observed in males between dose one and dose two in any of the analytes measured in serum or plasma (figure 12-11a-d). Similarly, no statistically significant differences were observed in females between dose one and dose two in any of the analytes measured in serum or plasma (figure 12-11e-h).

Males had a significantly higher cumulative urinary calcium excretion compared to females on both day 1 (P = 0.0006) and day 3 (P = 0.0028). On day 3, males and females tended to have a greater cumulative urinary calcium excretion compared with day 1. Figure 12-7a describes these results.

Males also had a significantly higher cumulative urinary phosphate excretion compared to females on both day 1 (P < 0.0001) and day 3 (P < 0.0001). There was no difference in cumulative urinary phosphate excretion for males or females between day 1 and day 3 (figure 12-7b)

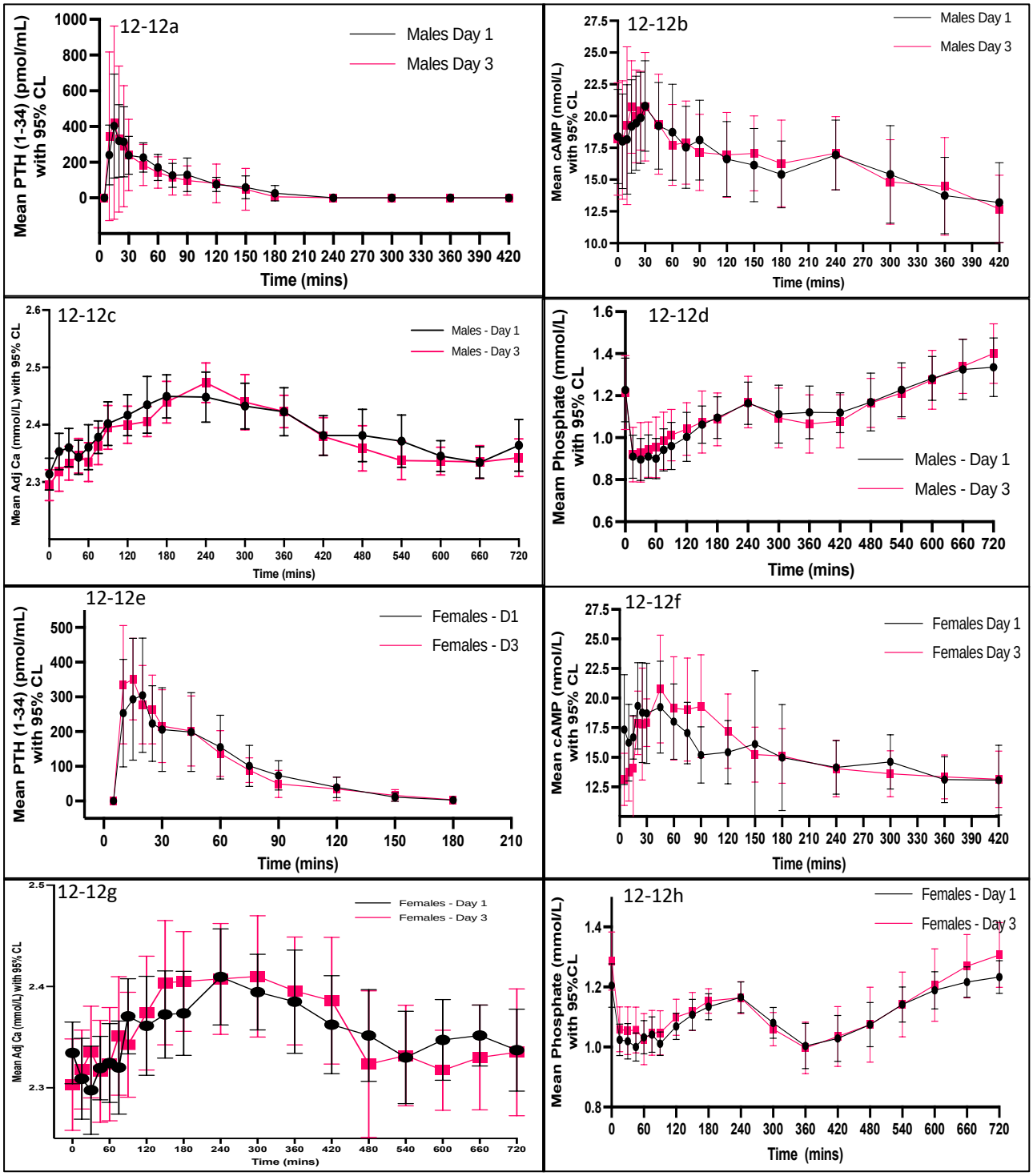


Figure 12-12: Pharmacokinetic and pharmacodynamic response of males and females to a second 20µg dose of teriparatide (with 95% Confidence Levels (CL)) (given on experimental testing day 3, after a 24 hour wash-out period following the first dose). Dose administered at 0 mins. (Male serum PTH (1-34) (12-12a), male plasma cAMP (12-12b), male serum albumin adjusted calcium (12-12c), male serum phosphate (12-11d), female serum PTH (1-34) (12-12e), male plasma cAMP (12-12f), male serum albumin adjusted calcium (12-12g), male serum phosphate (12-12h).

12.4.5 Baseline biochemistry

12.4.5.1 Haematology

Haemoglobin, haematocrit, and red blood cells were all lower prior to dose two than dose one (141 ± 12 vs 136 ± 14, *P* = <0.001), (0.42 [0.06] vs 0.41 [0.06], <0.001) and (4.63 ± 0.50 vs 4.50 ± 0.52, *P* = <0.001) respectively. These three parameters were also lower in females than males prior to dose one and two (all *P* = <0.001, see table 12-3 for full results).

12.4.5.2 Liver function

Baseline albumin, ALT and total protein concentrations were lower in females compared to males prior to both doses one and two (all *P* = ≤ 0.009 see table 12-3 for full results) but were not different overall (males and females combined) or for single-sex samples (males only, or females only). Bilirubin was lower in females than males prior to dose one and two (*P* = 0.006 and *P* = 0.016 respectively), and lower overall prior to dose two than dose one (*P* = 0.023), but there were no differences in single-sex groups prior to dose one and two.

12.4.5.3 Lipid Profile

There were several differences in lipid profile parameters between males and females and doses one and two. Prior to dose one, total cholesterol (3.4 [1.2] vs 2.7 [0.6], *P* = 0.009), LDL (2.8 [1.2] vs 1.9 [0.8], *P* = 0.032) and non HDL cholesterol (3.35 ± 1.30, 2.52 ± 0.70, *P* = 0.028) were all lower in females compared to males, whilst HDL (1.43 [0.41] vs 1.25 [0.30], *P* = 0.032) was higher in females compared to males. Males had lower total cholesterol (4.36 ± 0.75 vs 4.16 ± 0.66, *P* = 0.018) and non-HDL cholesterol (3.35 ± 1.30 vs 2.89 ± 0.65 *P* = 0.027) prior to dose two, compared to prior to dose one. There were no differences in the lipid profile between males and females prior to dose two and there was no difference pre- dose one and two in females.

12.4.5.4 Renal Function

Creatinine was lower in females than males prior to both doses one and two and was lower overall prior to dose one compared to immediately prior to dose two. Sodium was lower in females than males prior to dose one, but there was no difference prior to dose two. Calcium and adjusted calcium were lower overall in females and prior to dose two than dose one.

Magnesium was lower in females than males prior to dose one, it was also lower prior to day one than prior to dose two in overall and males vs males analysis.

A full description of the biochemistry at baseline is detailed in table 12-4.

12.4.6 Adverse Events

No severe adverse events were reported during the study. Three adverse events were reported following teriparatide dosing, one injection site reaction, one report of dizziness and one vasovagal episode. All adverse events were self-limiting and resolved before the participant left the clinical research facility. The participant experiencing the vasovagal episode was withdrawn from the study.

Table 12-4: Baseline biochemistry (Prior to day 1 (dose 1) and prior to day 3 (dose 2)) of participants in the PHAB trial.

		Day 1 Baseline Biochemistry				Day 3 Baseline Biochemistry				Overall D1 Vs Overall D3 Baseline	Male D1 Vs Male D3 Baseline	Female D1 Vs Female D3 Baseline
		Overall	**Male**	**Female**	***P***	**Overall**	**Male**	**Female**	***P***	***P***	***P***	***P***
Full Blood Count	**Hb** (g/L)	141 ± 12	149 ± 9	134 ± 12	<0.001*	136 ± 14	145 ± 10	129 ± 12	<0.001*	<0.001*	0.025*	<0.001*
	Basophils (10^9/L)	0.05 [0.05]	0.05 [0.04]	0.45 [0.06]	0.36	0.05 [0.04]	0.05 [0.05]	0.05 [0.04]	0.224	0.729	0.634	0.949
	Eosinophils (10^9/L)	0.17 [0.18]	0.20 [0.22]	0.15 [0.16]	0.962	0.14 [0.16]	0.20 [0.26]	0.12 [0.09]	0.725	0.530	0.528	0.149
	Hct	0.42 [0.06]	0.45 [0.36]	0.40 [0.05]	<0.001*	0.41 [0.06]	0.44 [0.04]	0.38 [0.05]	<0.001*	<0.001*	0.078	<0.001*
	Lymphocytes (10^9/L)	2.24 ± 0.56	2.29 ± 0.63	2.21 ± 0.51	0.673	2.21 ± 0.59	2.18 ± 0.64	2.25 ± 0.56	0.736	0.759	0.35	0.373
	MCH (pg)	30.58 ± 1.42	30.63 ± 1.46	30.55 ± 1.42	0.878	30.50 ± 1.28	30.45 ± 1.31	30.54 ± 1.29	0.842	0.186	0.223	0.583
	MCV (fL)	91 [7]	90 [6]	91 [7]	0.86	91 [5]	91 [6]	91 [6]	0.743	0.479	0.557	0.660
	Monocytes (10^9/L)	0.52 ± 0.12	0.54 ± 0.11	0.49 ± 0.13	0.227	0.50 ± 0.11	0.52 ± 0.13	0.47 ± 0.09	0.213	0.732	0.426	0.735
	Neutrophils (10^9/L)	2.91 [1.06]	2.52 [0.89]	3.04 [0.98]	0.115	2.96 ± 0.79	2.69 [0.78]	3.04 [1.48]	0.13	0.598	0.379	0.868
	Platelets (10^9/L)	240.8 ± 39.8	246.0 ± 37.6	234.0 ± 41.3	0.494	245 ± 41.1	246.9 ± 40.2	243.3 ±43.2	0.807	0.972	0.848	0.900
	Rbc (10^12/L)	4.63 ± 0.50	4.88 ± 0.39	4.42 ± 0.51	0.004*	4.50 ± 0.52	4.80 ± 0.43	4.22 ± 0.44	0.001*	<0.001*	0.046*	<0.001*
	Wbc (10^9/L)	6.08 ± 1.07	5.89 ± 1.02	6.17 ± 1.10	0.35	5.94 ± 1.11	5.66 ± 1.12	6.20 ± 1.08	0.17	0.535	0.208	0.614
Liver Function Test	**Albumin** (g/L)	42.4 ± 2.8	43.6 ± 2.6	41.4 ± 2.7	0.009*	41.9 ± 3.1	43.5 ± 2.6	40.4 ± 2.8	0.003*	0.311	0.795	0.283
	ALP (U/L)	62.2 ± 14.1	66.0 ± 13.5	59.0 ± 14.1	0.167	63.06 ± 13.8	65.4 ± 13.4	60.8 ± 14.1	0.343	0.946	0.613	0.776
	ALT (U/L)	15 [12]	20 [17]	12 [7]	0.004*	15 [14]	22 [14]	11 [7]	0.001*	0.403	0.533	0.805
	Globulins (g/L)	26.83 ± 3.12	27.19 ± 3.37	26.53 ± 2.95	0.545	26.76 ± 2.98	26.50 ± 2.73	27.00 ± 3.26	0.636	0.284	0.180	0.900
	Bilirubin (umol/L)	10 [6]	15 [8]	9 [5]	0.002*	8 [7]	11.5 [4]	7 [4]	0.016*	0.023*	0.119	0.098
	Total protein (g/L)	70 [4]	70 [5]	67 [4]	0.006*	70 [6]	70 [3]	67 [6]	0.042*	0.168	0.243	0.362
Lipid Profile	**Cholesterol** (mmol/L)	4.18 ± 1.0	4.36 ± 0.75	4.04 ± 0.51	0.237	4.10 ± 0.62	4.16 ± 0.66	4.04 ± 0.60	0.584	0.012*	0.018*	0.204
	Total Cholesterol/HDL Ratio	2.8 [0.98]	3.4 [1.2]	2.7 [0.6]	0.009*	2.9 [0.8]	3.3 [1.2]	2.7 [0.7]	0.004*	0.121	0.210	0.375
	HDL-C (mmol/L)	1.35 [0.39]	1.25 [0.30]	1.43 [0.41]	0.032*	1.28 [0.38]	1.22 [0.37]	1.51 [0.44]	0.014*	0.210	0.105	0.585
	LDL (mmol/L)	2.20 [0.9]	2.75 [1.2]	1.9 [0.8]	0.032*	2.1 [0.8]	2.6 [1.3]	2.1 [0.4]	0.053	0.046*	0.094	0.251
	Non HDL cholesterol (mmol/L)	2.77 ± 0.63	3.35 ± 1.30	2.52 ± 0.70	0.028*	2.68 ± 0.54	2.89 ± 0.65	2.48 ± 0.31	0.033*	0.008*	0.027*	0.124
	Triglycerides (mmol/L)	1.0 [0.5]	1.1 [0.4]	0.8 [0.6]	0.298	0.9 [0.6]	0.95 [0.3]	0.90 [0.7]	0.456	0.191	0.206	0.549
Urea and Electrolytes	**Creatinine** (umol/L)	74.42 ± 14.52	84.63 ± 13.92	66.5 ± 8.79	<0.001*	73.1 ± 12.56	81.44 ± 10.63	65.29 ± 8.66	<0.001*	0.040*	0.115	0.205
	Potassium (mmol/L)	3.99 ± 0.26	4.01 ± 0.30	3.97 ± 0.23	0.67	4.02 ± 0.21	4.01 ± 0.20	4.01 ± 0.24	0.738	0.231	0.800	0.158
	Sodium (mmol/L)	138 ± 1.6	139 ± 1.3	137 ± 1.6	0.005*	139 ± 1.4	139 ± 1.5	139 ± 1.4	0.121	0.235	0.907	0.090
	Urea (mmol/L)	4.74 ± 1.08	4.85 ± 1.00	4.65 ± 1.15	0.521	4.34 ± 0.85	4.61 ± 0.82	4.08 ± 0.82	0.077	0.070	0.237	0.172

		Day 1 Baseline Biochemistry				Day 3 Baseline Biochemistry				Overall D1 Vs Overall D3 Baseline	Male D1 Vs Male D3 Baseline	Female D1 Vs Female D3 Baseline
		Overall	**Male**	**Female**	***P***	**Overall**	**Male**	**Female**	***P***	***P***	***P***	***P***
Bone Group	**Calcium** (mmol/L)	2.36 [0.07]	2.37 [0.08]	2.36 [0.07]	0.103	2.32 [0.08]	2.33 [0.05]	2.30 [0.14]	0.129	0.010*	0.458	0.016*
	Adjusted Calcium (mmol/L)	2.32 ± 0.06	2.31 ± 0.05	2.33 ± 0.06	0.278	2.30 ± 0.08	2.29 ± 0.05	2.30 ± 0.08	0.815	0.006*	0.166	0.015*
	Phosphate (mmol/L)	1.22 ± 0.14	1.23 ± 0.15	1.22 ± 0.14	0.711	1.25 ± 0.18	1.21 ± 0.18	1.27 ± 0.18	0.31	0.435	0.214	0.050
	CTX (µg/mL)	0.61 ± 0.26	0.69 ± 0.31	0.54 ± 0.17	0.091	0.63 ± 0.25	0.68 ± 0.29	0.59 ± 0.20	0.281	0.222	0.029	0.044*
	P1NP (µg/mL)	81.30 ± 35.61	91.2 ± 42.7	72.5 ± 26.1	0.141	87.51 ± 37.60	94.4 ± 42.0	81.4 ± 33.3	0.322	0.008	0.382	0.003*
Other	**25 OH Vitamin D** (nmol/L)	77 ± 28	75 ± 35	79 ± 22	0.725	76 ± 15	75 ± 28	78 ± 23	0.741	0.655	0.690	0.840
	Magnesium (mmol/L)	0.79 ± 0.05	0.81 ± 0.04	0.77 ± 0.04	0.001*	0.77 ± 0.05	0.79 ± 0.03	0.76 ± 0.05	0.057	0.014*	0.005*	0.399
	Parathyroid Hormone (pmol/L)	3.31 ± 1.15	3.14 ± 0.98	3.31 ± 1.16	0.444	3.25 ± 1.11	3.14 ± 1.01	3.34 ± 1.21	0.614	0.696	1.000	0.597

* Denotes significant result.

A summary of the results of the PHAB study is presented in table 12-5.

Table 12-5: PHAB Study Summary of Results.

		Day One		Day Two	
		Pre – Dose One	**Post - Dose One**	**Pre – Dose Two**	**Post - Dose Two**
Demographics	**Age**	M > F (*P* = 0.026)	-	-	-
	Alcohol Intake	M = F	-	-	-
	Smoking	M = F	-	-	-
	Ethnicity	M = F	-	-	-
Body Composition	**Body Mass Index**	M > F (*P* = 0.022)	-	-	-
Primary Outcome	**Teriparatide Area Under the Curve**	-	M > F (*P* = 0.016)		M = F
Secondary Outcomes	**Teriparatide C_{max}**	-	M = F		M = F
	Teriparatide T_{max}	-	M = F		M = F
	cAMP (plasma)	-	M > F (*P* = 0.007)		M = F
	cAMP (urine)	-	F > M (*P* ≤ 0.001)		M = F
	Adj Ca (serum)	M = F	M > F (*P* = 0.008)	M = F	M = F
	PO_4 (serum)	M = F	M = F	M = F	Initially M < F then M > F (*P* ≤ 0.001). Crossover approx. 180 mins post dose
	Ca (urine)	-	M > F (*P* = 0.009)	-	M = F
	PO_4 (urine)	-	M > F (*P* ≤ 0.001)	-	M = F
	Cumulative Ca (urine)	-	M > F (*P* ≤ 0.001)	-	M > F (*P* ≤ 0.001)

		Day One		Day Two	
		Pre – Dose One	**Post - Dose One**	**Pre – Dose Two**	**Post - Dose Two**
	Cumulative PO_4 (urine)	-	M > F ($P \leq 0.001$)	-	M > F ($P \leq 0.001$)
Selected Biochemistry	**Hb**	M > F ($P \leq 0.001$)	-	M > F ($P \leq 0.001$)	-
	Rbc	M > F ($P = 0.004$)	-	M > F ($P \leq 0.001$)	-
Liver Function Test	**Albumin**	M > F ($P = 0.009$)	-	M > F ($P = 0.003$)	-
	ALT	M > F ($P = 0.004$)	-	M > F ($P \leq 0.001$)	-
	Total protein	M > F ($P = 0.006$)	-	M > F ($P = 0.042$)	-
Lipid Profile	**Total Cholesterol**	M > F ($P = 0.028$)	-	M > F ($P = 0.033$)	-
Urea and Electrolytes	**Creatinine**	M > F ($P \leq 0.001$)	-	M > F ($P \leq 0.001$)	-
	Magnesium	M > F ($P \leq 0.001$)	-	M > F ($P = 0.057$)	-

12.5 Discussion

This study examined sex differences in the PKPD in response to a single and then repeated dose (administered 48 hours later) of teriparatide (20 µg) and informed the use of teriparatide in the treatment of stress fractures in the British Army in Chapter 13.

Parathyroid hormone (1-34) was detected in serum immediately following a single 20 µg dose of teriparatide and persisted in circulation for up to 240 min analogous to findings of similar studies in post-menopausal women (Satterwhite *et al.*, 2010). In contrast to previous studies which reported no gender differences (Farías *et al.*, 2016) the PHAB study found a significantly higher PTH (1-34) AUC following a single subcutaneous dose of PTH in males compared to females (P = 0.016) suggesting the PTHr1 receptor response in these females is different to the males, this finding endured when results were adjusted for age and BMI. There was also a trend towards a lower C_{max} in females and faster metabolism (lower T_{max}), despite females having smaller blood volume than males. Individual variability in a small sample size is believed to be at least in part the reason why these findings did not reach significance.

The most likely cause of this difference in distribution between males and females is their different hormonal milieu which affects receptor sensitivity, response and subsequent PTH metabolism. Serum PTH has been shown to increase with age in pre-menopausal women and oestrogen-deficient postmenopausal women. This age-related increase in serum PTH was wiped out in the postmenopausal women receiving hormone replacement therapy (HRT) containing oestrogen long term (Khosla *et al.*, 1997). It has also been shown that the response to PTH can be modified by growth hormone levels in circadian rhythm studies (Fraser, Ahmad and Vora, 2004; Ahmad *et al.*, 2003; White *et al.*, 2007).

In response to a PTH (1-34) stimulus, there are three physiological effects, the primary action is PTH (1-34) acting on a G-coupled protein receptor complex that results in cAMP production. The secondary effects are inositol phosphate production (if the G-coupled protein receptors are bypassed) and calcium changes intracellularly.

In this study, I found that when PTH (1-34) acted on the G-coupled protein receptors there were differences in the cAMP response between males and females. Males had a greater concentration of cAMP in plasma (P = 0.007) but lower UcAMP ($P \leq 0.001$) compared to females, which goes some way to explain the effect on the excretion of Ca and PO_4. Urinary cAMP is a combination of the filtered cAMP from plasma plus the cAMP generated by the kidneys. These differing concentrations of cAMP indicate the response to PTH (1-34) in the kidneys is greater in females than males despite less circulating PTH, suggesting a greater level of pharmacological activity. Urinary cAMP following PTH stimulus is a more reliable reflection of PTH activity, as plasma cAMP is affected by several G-protein related hormones, including cortisol (Fraser *et al.,* submitted) which may have been increased by the testing environment and potentially stressful interventions such as siting cannulas. Urinary cAMP is predominantly nephrogenous cAMP, reflecting the effect of PTH on the kidney. Females excreted the cAMP and retained the Ca and PO_4. Males retained the cAMP and excreted the Ca and PO_4 indicating they have a better intracellular response to cAMP..

Circulating PTH acts on the kidney, bone cells (osteoblasts) via secondary messengers which promote Ca reabsorption by the kidney and PO_4 loss in urine. This phosphaturic effect following PTH administration is a second messenger response beyond the cAMP response. Stimulation of osteoblasts results in feedback to the osteoclasts, bone resorption and both Ca and PO_4 release from the bone (Evenepoel, Bover and Ureña Torres, 2016).

Mean total urinary Ca and PO_4 excretion is lower in females than males (Urine Ca, P = 0.009. Urine PO_4, $P \leq 0.001$ respectively) although the profile of urine Ca and PO_4 loss in response to PTH is almost identical in males and females. This suggests that females are retaining more Ca and PO_4 to support the formation of hydroxyapatite and the anabolic effects of PTH (1-34) treatment.

The plasma PO_4 profile following administration of PTH (1-34) differs between males and females, a smaller decrease in females is seen initially compared to males, this is consistent with the greater phosphaturic response seen in males indicating PTH has a greater effect on PO_4 secretion in males compared to females. This smaller initial decrease in plasma phosphate following PTH has not previously been reported. Plasma PO_4 then reverses with greater increase in males than in females 240 minutes after administration of PTH. The greater

phosphaturic effect in males, following PTH stimulus suggests females are conserving their phosphate to maintain plasma phosphate concentration, resulting in a far greater PO_4 response to PTH in males compared to females.

There were some differences in the biochemistry at baseline prior to dose 1 between males and females. The Hb, HCT and RBC were all lower in women than men prior to dose 1 and dose 2 (*P* = <0.001, <0.001, 0.004 respectively), this is probably due to differences in sex hormones, with androgens having a stimulatory effect on erythropoiesis (Murphy, 2014) and may be compounded by phase of the menstrual cycle.

Magnesium concentrations for both males and females were well within the reference range, at all points within the study, but they did differ between males and females prior to dose one (*P* = 0.001) and magnesium concentrations in men decreased following dose one (*P* = 0.005), but the clinical significance is unclear. Severe hypomagnesemia (< 0.4 mmol/L) impairs the release of endogenous PTH and causes skeletal resistance to the actions of PTH. Hypomagnesemia (serum magnesium <0.7 mmol/L) has been reported as a side effect during teriparatide treatment, particularly in older patients and those with lower baseline magnesium (Bégin et al., 2018). The decreases in magnesium observed in this study are much smaller than those seen in other studies albeit over a much shorter period. Slovik *et al*. 1981 reported decreases of -0.066 mmol/L following 3-4 weeks of treatment with 20µg of teriparatide daily (Slovik, Neer and Potts, 1981). Given the small changes observed in this study, it is unlikely this reduction in magnesium is affecting responses to PTH (1-34) but magnesium should be monitored if using teriparatide for long term treatment.

Every effort was made to remove variability from this study, injection site was limited to the abdomen as a previous study found a 21% slower rate of absorption if injected to the thigh compared to abdominal wall injections, which resulted in 18% reductions in T_{max} concentrations and a 1.5 minute increase in the time to C_{max} (Hodsman *et al.*, 2005). All administrations were made by a qualified nurse to remove the variability which may have been introduced by individual patients injecting for the first time.

The urinary response to PTH of healthy females when compared to males is similar to pseudohypoparathyroid type two patients, who have a greater cAMP response but a poorer phosphate response. The biochemical diagnosis of pseudohypoparathyroid disease is made by monitoring the total phosphate output in response to PTH, in type one pseudohypoparathyroidism there is no cAMP or phosphaturic response compared to type two pseudohypoparathyroidism where a cAMP response is seen but no phosphaturic response (Mantovani *et al.*, 2018; Linglart, Levine and Jüppner, 2018).

Overall, these findings imply the overall responses to a single dose of 20 µg of teriparatide by males and females seen in Ca and PO_4 are beyond the second messenger responses. This study focused on the cAMP, Ca and PO_4 related responses to PTH. Measurement of other PTH responses including the response of G-protein coupled receptors, conformational changes that take place on these receptors and intracellular calcium changes and their contribution to the differences in response were out of the scope of this study. The findings of this study suggest that the differences in response to PTH are in the second messengers, future work to elucidate the exact mechanism, should look at intracellular calcium and kinase responses. This could potentially include taking the kidney cells from male and female rats and comparing the second messengers intracellularly.

Long-term elevation of PTH (several hours) promotes intestinal absorption of calcium by stimulating the conversion of 25 hydroxy vitamin D3 (25 (OH) D3) to 1,25 dihydroxy vitamin D3 (1,25 $(OH)_2$ D3). The impact of these differences on the rate or quality of bone repair remains to be studied, and analysis of vitamin D metabolites, fibroblast growth factor (FGF23) and bone turnover markers may elucidate this further.

The findings of this study, although carried out in healthy participants, may have implications for the future research and treatment of hypoparathyroid patients, the majority of whom are female (Powers *et al.*, 2013). Female hypoparathyroid patients are potentially getting a poorer response from PTH (1-34) treatment than their male counterparts, suggesting that future studies should examine males and females independently, where at present standard practice is to combine them into one cohort. Further analysis of urinary Ca and PO_4 are

required to make a definitive hypothesis. No differences in tolerability between males and females was identified which supports further study.

This study was based on two doses separated by a washout period of 24 hours, the response seen to the second dose (administered on day 3) was the same as that seen to the first dose despite individuals previously having been exposed to one dose of teriparatide. Previous studies have shown an increased sensitivity at first exposure to teriparatide with the effect being greater the longer the patient has been hypoparathyroid. Once teriparatide (or another PTH analogue) is introduced the receptor sensitivity decreases (White *et al.*, 2007). The persistence of this effect long term is unknown, there is the potential for individuals to develop resistance to PTH, but this is unknown.

12.6 Strengths and Limitations

Initially, a gender comparison comprised a sub-arm of the RETURN trial (Chapter 13), however as the protocol matured it became clear that the trial in this format was not achievable within the allocated time and budget, and an independent PKPD gender comparison of teriparatide was a more efficient approach, hence the study was based on civilians rather than just military recruits, and despite the inclusion and exclusion criteria being written to limit differences as far as practicable, the lifestyles and diets of civilians are inevitably going to be different.

The age range specified in the inclusion criteria for the study was narrow (18-36 years), but even within this, there was a significant different in the ages between males and females (males 26.1 [9.3] years vs females 20.6 [3.9] years (P = 0.026)), whilst the implications of this are unclear in this participant group, in post-menopausal osteoporotic patients teriparatide response is independent of age (Marcus et al., 2003). In practice recruits vary in age, with soldier recruits being younger than officer recruits. In the 12 months ending 31 Mar 2021, 47.8% of soldier recruits joining were under the age of 20 years compared to 5.2% of officer recruits (Statistics, 2021). BMI also differed between the two groups (males 25.6 [4.7] vs females 23.1 [4.0] (P = 0.022)), but this variation is not unusual in British Army recruits. Previous studies of British Army recruits have reported similar mean ages and BMIs (Carswell et al., 2018; Coombs et al., 2021; O'Leary et al., 2018a; O'Leary et al., 2018b). The impact of these differences on the findings of this study must be considered in the context for which it was designed – to inform the use of teriparatide in the treatment of stress fractures in the British Army.

The eligibility criteria was almost identical to that of RETURN (Chapter 13) with the intention that the participants were matched however this did not prove to be the case as the majority of recruits enlisting into the Army were at the younger end of the age limit, the mean age of RETURN participants was lower than the male PHAB participants.

12.7 Conclusion

This clinical trial was the first investigating the pharmacokinetic and pharmacodynamic of generic teriparatide in healthy young males and females. This study was designed to inform the use of teriparatide in the treatment of BSIs in the British army and hypothesised that teriparatide had the same pharmacokinetic and pharmacodynamic profiles in males and females. The trial found, following a single dose of teriparatide 20 micrograms of teriparatide by S/C injection, the AUC of PTH (1-34) was higher in males than females. There were also differences in the secondary messenger cAMP, phosphate and calcium responses from which we conclude that teriparatide sensitivity is different in males compared to females in this age group.

No serious adverse events were recorded during this study. These findings should inform the way future trials of teriparatide (be that standard release sub-cutaneous, long-action sub-cutaneous or oral) are recruited and results analysed as it may be that males and females require different dosing schedules for the same therapeutic result.

13 Efficacy of Teriparatide Use in the Return of Recruits to Normal duty (RETURN): A Randomised Controlled Trial on the Treatment for Bone Stress Injuries

This chapter describes a randomised controlled trial investigating teriparatide for the treatment of bone stress injuries (BSIs) at a basic military training unit. RETURN is the first known randomised clinical trial using teriparatide for treatment of BSI in military personnel.

13.1 Introduction

One fifth of trainees with a musculoskeletal injury (MSKI) do not return to training and are discharged from the military. Medical discharge, which accounts for 10% of those discharged, is the worst-case outcome for trainees with an MSKI as this suggests the injury has life changing consequences for the affected individual. Other reasons for discharge following MSKI include; i) discharge as of right (personal choice to leave service) (52%), ii) defect in enlistment (e.g. health related reason or previously undeclared criminal activity that has come to light after enlistment) (24%), iii) unfit for Army service (e.g. failure to engage with the rehabilitation process) (14%), iv) services no longer required (e.g. following positive drugs test). Before the COVID-19 pandemic (2016 to 2020), 9.3% of male recruits and 6.6% of female recruits had an MSKI-related medical discharge, and after the COVID-19 pandemic, this decreased to 4.1% for males, but female recruit discharges remained similar at 6% (Chapman *et al.*, 2023).

Lower limb BSIs require the longest rehabilitation times of all overuse MSKI sustained during Infantry training, and a recruit spends an average of 92 ± 17 days recovering from a BSI (Sharma *et al*., 2015). Quality of healing, time to BSI healing, and return to training can have significant impact on recruits' career progression and risk of medical discharge; and, to the UK Ministry of Defence (MOD) for the cost of training days lost and economic cost of medical care.

Standard of care for BSI patients in the military is stratified by risk, based on the site and severity of the injury. High risk BSI sites (BSIs in zones of high tension or have poor blood supply) include, femoral neck at the superior cortex, tibial shaft at the anterior cortex, fifth metatarsal at the diaphyseal-metaphyseal junction, navicular, proximal to the second metatarsal, talus, medial malleolus and sesamoid) are referred for specialist orthopaedic opinion and, potentially, surgical intervention and, thereafter, discharged to Defence Primary Healthcare (DPHC); meanwhile, low risk BSIs are treated within DPHC. All recruits with a BSI stop training and undergo rehabilitation; they re-enter training if injury is healed or are medical discharged from service if the injury fails to heal. Healing is achieved when a recruit completes their rehabilitation programme and build-up training, passes a physical assessment at a level commensurate with the week of training they were in when they stopped due to BSI, and is deemed fit to return to training by their GP.

Teriparatide is an anabolic drug licensed for the treatment of osteoporosis; it is not licensed for the treatment of BSIs but has been used for this indication in case reports (Malhotra, Meena and Digge, 2013; Raghavan and Christofides, 2012).

Eleven randomised controlled trials have investigated the efficacy of teriparatide for healing osteoporotic fractures in older men and women. These studies have reported outcomes including fracture healing time, and pain and mobility, as reported in a number of literature reviews and meta-analyses (Kim *et al.*, 2017; Lou *et al.*, 2016; Shi *et al.*, 2016; Eastman *et al.*, 2021) with equivocal results Eastman *et al*., forms the basis of Chapter 11 of this thesis.

Most of the evidence for use of teriparatide is based on osteoporotic fracture risk in post-menopausal women, in line with the licensed indication, with only one study reporting its use on BSI healing in young women. An 8-week pilot study compared 20µg / day sub-cutaneous (SC) teriparatide to placebo in premenopausal women with BSIs. The study reported increased biochemical markers of bone formation in the teriparatide treatment group — Procollagen Type 1 N-terminal Propeptide (P1NP) and osteocalcin (OC) (both $P \leq 0.01$), demonstrating an anabolic window of bone formation ($p \leq 0.05$). Peripheral quantitative computed tomography (pQCT) measurements of bone structure in teriparatide-treated

women have demonstrated a larger cortical area and thickness compared to placebo at the 38% tibial site, while placebo-treated women had a greater total area and cortical density at the 38% tibial site. No changes at the radial sites were observed between groups. pQCT is more widely available than High Resolution pQCT (HRpQCT), which is used throughout this thesis. pQCT benefits from greater portability and unlimited gantry length enabling it to scan sites as proximal as the thigh, however, HRpQCT uses smaller voxel sizes (component of image quality) and a larger image stack (reduces noise and sharpens images) compared to pQCT so the output is clearer and more reliable.

Using MRI, 83.3% of the teriparatide- and 57.1% of the placebo-treated groups had improved or had healed BSIs by 8 weeks (P = 0.18); 66% of the teriparatide-treated group improved by two Magnetic Resonance Imaging (MRI) grades or more *versus* 29% in the control group (Almirol *et al.*, 2016)

13.2 Research Questions and Objectives

13.2.1 Research Questions

This trial investigated the efficacy of teriparatide for accelerating BSI healing in military personnel. The hypotheses for this study were that treatment with a teriparatide SC injection accelerates: i) the time to healing of a BSI injury; ii) the cessation of pain associated with the injury, and; iii) the return to training compared to standard care. The quality of healing is also important as on return to training the healed injury will be expected to withstand the training activities that caused the original injury, hence changes in bone density and microarchitecture, were assessed by Dual energy X-ray absorptiometry (DXA) and HR-pQCT.

13.2.2 Primary Objective

The primary objective was to investigate the effect of teriparatide plus the Army's standard care on lower body BSI healing in Army Infantry recruits, compared with Army standard care alone. The primary outcome was the improvement in radiological healing, assessed using Fredericson grading of MRI, by two grades or more, or reduction to grade zero, 8 weeks after randomisation and treatment initiation (Fredericson *et al.*, 1995; Kijowski *et al.*, 2012).

13.2.3 Secondary Objectives

Secondary objectives were to examine the effect of teriparatide on:

1) Time to radiological healing, assessed by MRI at week 8, 10, 12, 14, 16, 20, and 24, until healed.
2) Time to discharge from physical rehabilitation.
3) Health-related quality of life, using the Short Form (36) Health Survey version 2 (SF-36v2).
4) Pain, assessed by visual analogue scale.
5) Time to clinical healing and proportion clinically healed, assessed using a clinical severity score of injury signs and symptoms.
6) Adverse events

13.2.4 Exploratory Outcomes

The effect of teriparatide on the following exploratory outcomes was also examined:

1) Blood biochemistry (SRANKL (pmol/L), DKK1 (pmol/L), OPG (pmol/L), $1,25(OH)_2D$ (pmol/L), CTX (µg/mL), PINP (µg/mL), SOST (pmol/L)) at weeks 8 and 16.

2) Areal bone density of lumbar spine and hip, and tibial volumetric density and morphology assessed using dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT).

3) Physical activity levels measured using wrist-mounted accelerometers.

4) Long-term future BSI risk, by review of medical records for 5 years following last patient last visit of the initial study (beyond the scope of this thesis).

13.3 Methods

This study was a randomised prospective open label study of teriparatide plus standard care v standard care. The protocol for this trial has been published (Carswell *et al.*, 2021).

13.3.1 Participants

Thirty-four Army Infantry recruits (33 males, 1 female), described in chapter 8, with one or more lower body BSIs voluntarily participated in the study. All participants had a lower body (lower body is defined as pelvic girdle, sacrum, coccyx and lower limb) BSI confirmed by MRI. All participants underwent standard treatment for BSI at the Infantry Training Centre (Catterick) (ITC(C)) in the form of a personalised rehabilitation plan, according to usual military training care. The trial open-label and the rehabilitation team was advised of a patient's participation in the study, so they were permitted to attend study appointments and wear a wrist mounted accelerometer (trainees are not normally allowed to wear watches during training sessions - see section 13.3.6 for more detail) but this did not alter their standard treatment as it followed a standard procedure. A summary of British Army BSI Management Procedures for Infantry Recruits is at appendix 3.

13.3.2 Ethical Approval

The study protocol was approved by the Ministry of Defence Research Ethics Committee (MODREC), reference 932/MODREC/18, the Medicines and Health Regulatory Agency (MHRA) and the Health Research Authority (HRA), reference 19/HRA/6011.

The trial was registered with clinicaltrials.gov, reference NCT 04196955 and European Union Drug Regulating Authorities Clinical Trials Database (EudraCT), reference 2018-002130-20.

13.3.3 Trial Overview

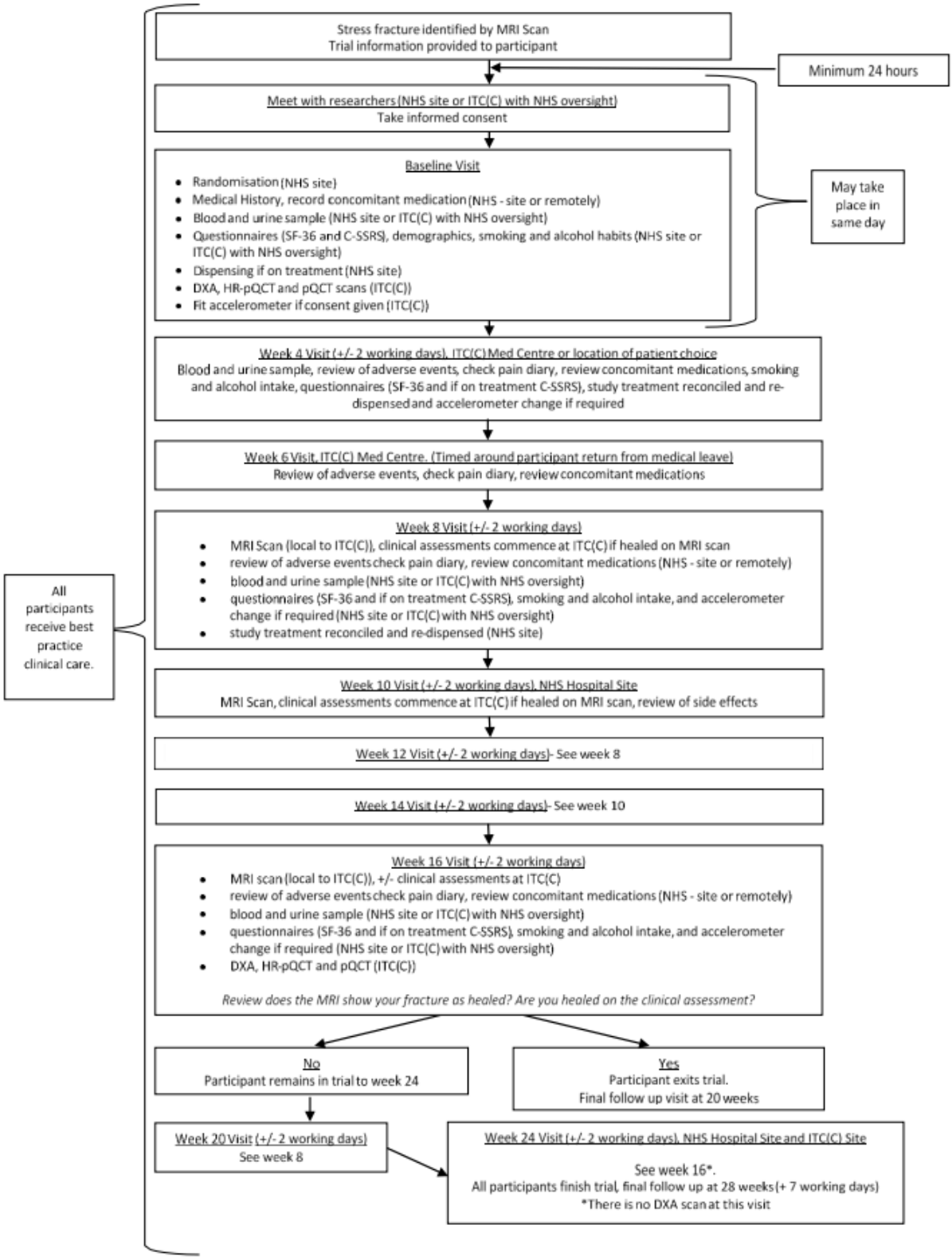


Figure 13-1: Trial diagram for the RETURN trial.

13.3.4 Site Selection

The ITC(C) was selected as the test site based on the number of recruits enlisting annually, recording the highest total number of BSIs of all training units within the MOD and prior research experience at the site, including within the permanent military staff, the clinical teams and the resources available.

A dedicated research team at ITC(C) – led by this PhD researcher, who was Principal Investigator (PI) for the site - was responsible for participant screening, bone imaging (DXA and HRpQCT) assessments, accelerometer fitting and clinical assessments. Participants' usual care team did not undertake trial roles to avoid potential coercion, however, delivery of standard care remained the responsibility of the permanent staff (doctors, physiotherapists, and exercise rehabilitation instructors) at ITC(C).

The Darlington Memorial Hospital was responsible for obtaining written informed consent, confirming eligibility of participants, reviewing follow up safety assessments, safety reporting, investigational medicinal product (IMP) prescribing and dispensing, and undertaking MRI scans. During the COVID-19 pandemic, to maintain the 'COVID secure' status of ITC(C), ITC(C) roles were expanded to include all the participant facing roles of the NHS host institution except for obtaining consent, confirming eligibility and MRI scanning.

Other trial activity, including blood and urine sampling, performing questionnaires (SF-36 and C-SSRS), recording smoking and alcohol habits, were conducted at either the NHS host institution or by the research team at ITC(C) at the convenience of the participant.

13.3.5 Randomisation

Eligible, consented participants were randomised on a 1:1 basis to one of the two trial arms using a using web-based randomisation process. This was blocked randomisation with block lengths of 2 to 6 in random order. Allocation was stratified by baseline vitamin D status (normal / insufficient or deficient) and whether one fracture site or more than one fracture site as indicated by MRI scan as part of the participants screening process.

13.3.6 Intervention

13.3.6.1 Control Arm: Standard Care

Participants in the standard care arm underwent a personalised rehabilitation plan. Participants were required to adhere to the standard care regimen for 16 weeks, with an extension to 24 weeks if the BSI remained unhealed.

13.3.6.2 Active Arm: Teriparatide Sub-Cutaneous Injection Plus Standard Care

Participants underwent standard care with a personalised rehabilitation plan. Participants were required to adhere to the standard care regimen for 16 weeks, with an extension to 24 weeks if the fracture remained unhealed.

In addition to standard care, participants in the intervention arm subcutaneously self-administered 20 micrograms of teriparatide (Gedeon Richter, Budapest, Hungary) (recombinant human PTH (rhPTH) (1-34)) in 80 microliters of diluent daily at the same time each day in the thigh or abdomen, for 16 weeks; treatment was extended to 24 weeks in the case of an unhealed BSI. RhPTH is produced in *E. coli* using recombinant DNA technology and is identical to the 34-N-terminal amino acid sequence of endogenous human PTH. The teriparatide was delivered by the reusable Terrosa multidose pen device containing a replaceable cartridge. Each cartridge of 2.4 mL contained 600 micrograms of teriparatide (corresponding to 250 micrograms per mL) and delivered 28 fixed doses of 80 microlitres containing 20 micrograms of teriparatide when used in combination with the Terrosa pen. Participant compliance was recorded by diary entries and counting the number of doses remaining in unused cartridges.

The pen (containing cartridge) was transported in Medactiv iCool Weekender travel cases with refreezable gel packs (Syringa, UK) when required and stored in a refrigerator (2°C - 8°C) when not in use.

The adverse reactions associated with the use of teriparatide in osteoporosis clinical trials and post-marketing exposure are summarised in table 13-1. The following convention has been

used for the classification of the adverse reactions: very common (≥1/10), common (<1/10 to ≥1/100), uncommon (<1/100 to ≥1/1,000), and rare (<1/1,000 to ≥1/10,000).

Table 13-1: Adverse reactions associated with the use of teriparatide in osteoporosis clinical trials.

Organ Class System	**Very Common** (≥1/10)	**Common** (<1/10 to ≥1/100)	**Uncommon** (<1/100 to ≥1/1,000)	**Rare** (<1/1,000 to ≥1/10,000)
Blood and lymphatic system disorders		Anaemia		
Immune system disorders				Anaphylaxis
Metabolism and nutrition disorders		Hypercholesterolaemia	Hypercalcaemia greater than 2.76 mmol/L, hyperuricaemia	Hypercalcaemia greater than 3.25 mmol/L
Psychiatric disorders		Depression		
Nervous system disorders		Dizziness, headache, sciatica, synocope		
Ear and labyrinth disorders		Vertigo		
Cardiac disorders		Palpitations	Tachycardia	
Vascular disorders		Hypotension		
Respiratory, thoracic and mediastinal disorders		Dysnopea	Emphysema	
Gastrointestinal disorders		Nausea, vomiting hiatus hernia, gastro-oesophageal reflux disease	Haemorrhoids	
Skin and subcutaneous tissue disorders		Sweating increased		
Musculoskeletal and connective tissue disorders	Pain in limb	Muscle cramps	Myalgia, arthralgia, back cramp / pain	
Renal and urinary disorders			Urinary incontinence, polyuria, micturition urgency, nephrolithiasis	Renal failure/impairment
General disorders and administration site condition		Fatigue, chest pain asthenia, mild and transient injection site events, including pain, swelling erythema, localised bruising, pruritis and minor bleeding at the injections site	Injection site erythema, injection site reaction	Possible allergic events soon after injection: acute dyspnoea, oro/facial oedema, generalised urticaria, chest pain, oedema (mainly peripheral)
Investigations			Weight increased, cardiac murmur,	

			alkaline phosphatase increased	

The most commonly reported adverse reactions in patients treated with teriparatide are nausea, pain in limb, headache and dizziness, which affect between 1 in 10 and 1 in 100 patients treated with teriparatide. Clinical trials licensed for using teriparatide report at least one adverse event in 82.8% of teriparatide-treated patients and 84.5% of placebo patients (Lilly, 2017).

13.3.6.3 Treatment Schedule

Participants were trained in the proper injection technique by the research team and were provided with a manufacturer designed User Manual, which described the process.

Participants were required to adhere to the treatment regimen for 16 weeks. In the event of an unhealed BSI on MRI scan, treatment was continued for 24 weeks. After 24 weeks all trial interventions ceased, if participants remained unhealed on MRI scan, their usual care team was informed. If a participant forgot, or was unable, to inject teriparatide at their usual time, they were instructed to administer the dose within 12 hours. After 12 hours, the dose was omitted, and the next dose was taken the following day. Participants were asked not to take a 'double dose' to compensate for any missed doses.

13.3.6.4 Dose Modifications

Dose modifications were made in response to pre-dose serum albumin adjusted calcium concentrations, described in table 13-2.

Table 13-2: Dose modifications in response to pre-dose serum calcium concentrations.

Pre- Dose Serum Albumin Adjusted Calcium	**Dose Modification**
Above the upper limit of normal	Exclude differential diagnosis; 1. Primary hyperparathyroidism 2. Vitamin D excess 3. Malignancy Ensure patient using device properly. Confirm serum adjusted calcium by repeat measurement. Re-check serum adjusted calcium after 7 days. Withhold drug until serum adjusted calcium returns to normal and restart every other day or every third day dosing, based on treating clinician clinical judgement in discussion with CI.
Within normal range	No change
Below 2 mmol/L	Continue teriparatide 20µg / day dose. Start supplementing with calcium 1500mg and vitamin D 400 IU twice daily.

13.3.6.5 Dose Discontinuations

Dose discontinuations were permitted under the following circumstances,

- Unacceptable treatment toxicity or an adverse event;
- Inter-current illness that prevented further treatment;
- Any change in the participant's condition that in the clinician's opinion justified the discontinuation of treatment;
- Intentional overdose;
- Participant withdrew consent.

13.3.7 Physical Activity

The GENEActiv wrist-mounted triaxial accelerometer (GA) (Activinsights Ltd, Cambs, United Kingdom) was used to compare the effect of teriparatide on physical activity. The working hypothesis was that if teriparatide improved BSI healing, physical activity would increase in the treatment group ahead of the control group.

The GA is a small (43×40×13mm), lightweight (16g), waterproof device that collects raw acceleration data (in three axis) in the range of ±8g. Participants were instructed to wear the GA continuously between visits. The devices were set to record at 20Hz. The 20Hz frequency was chosen to allow collection of the maximum number of data points the storage of the GA would allow over 28-days continuous wear. During this period, the GA recorded physical activity intensity and sleep/wake measurements.

Accelerometery data were downloaded with GENEActiv PC Software version 3.3. Accelerometer data were exported into freely available spreadsheet report templates (www.geneactive.org), and moderate to vigorous physical activity data and energy expenditure data were calculated. A validity study found GENEActiv to report near identical average total energy expenditure compared with doubly labelled water (doubly labelled water; 4112 ± 652 kcal·day^{-1} vs GeneActiv mean bias ± limits of agreement: −15 ± 851 kcal·day^{-1}) (Siddall *et al.*, 2019).

13.3.8 Radiological Investigations

HR-pQCT and DXA scans were undertaken at ITC(C) within the framework for the safe use of radiation outlined in the Ionising Radiation Regulations (IRR, 1999) and the Ionising Radiation (Medical Exposure) Regulations (IR(ME)R, 2018). Total exposure during the trial for each participant was <0.02 mSv for those who left the trial at 16-weeks, and <0.03 mSv for those who remained in the trial up to 24-weeks.

The non-dominant limb, defined as the contralateral limb of the primary stabilising side and the non-writing hand (upper limb), was scanned in preference. Where this was not possible due to the participants injury, the uninjured limb was scanned. Previous studies have found

no difference in the adaptations caused by military training between the dominant and non-dominant tibia (O'Leary *et al.*, 2019b) which supported this approach assessing bone adaptations following teriparatide treatment.

13.3.8.1 Tibial Volumetric Bone Mineral Density, Geometry and Microarchitecture

HR-pQCT (Xtreme CTII, Scanco Medical) was used to measure vBMD, bone microstructure, and estimated bone strength at the metaphyseal tibia (non-injured, non-dominant leg where possible) and metaphyseal radius (non-dominant arm) at baseline, week 16 and 24. The participant's limb was stabilised in an anatomically shaped carbon-fibre shell during the scan. HR-pQCT is sensitive to small movements so participants were asked to remain completely still during the scanning procedure. An anteroposterior scout view was used to acquire the region of interest. The metaphyseal site proximal to the tibial plafond was located using a manufacturer mask. The rationale for selecting this site was due to its ability to define the region of interest using an anatomic landmark. Data acquisition for each site took 3 minutes and exposed participants to less than 0.01 mSv. Total exposure during the trial for each participant was <0.02 mSv for those who left the trial at 16-weeks, and <0.03 mSv for those who remained in the trial to 24-weeks.

13.3.8.2 Areal Bone Mineral Density

DXA (Lunar iDXA™, GE Healthcare) was used to measure aBMD at the lumbar spine and left and right hip at baseline and week 16. Participants lay supine on the bed in a standardised position and were asked to remain still during the scan. Data acquisition for each site took approximately one minute and exposed participants to less than 0.1 mSv. The total protocol dose was less than 0.3mSv. The addition of the DXA was important to assess the distribution of skeletal changes, across axial and appendicular sites with teriparatide use. Within the protocol it was permitted for scans to be repeated once if a movement artefact affecting the image quality was detected during the visit.

Areal BMD, BMC, total area and Z-scores were exported for analysis. The Z-score— the number of SDs by which the BMD in an individual differs from the mean value expected for

age and sex—provides information about an individual's BSI risk compared to peers (Dimai, 2017).

13.3.9 Blood Analysis

All safety bloods were analysed at County Durham and Darlington Foundation Trust. White blood cells (WBC) and differentials, red bloods cells (RBC), platelets and haemoglobin (Hb) were measured using standard automated methods on a Sysmex XS1000i (Norderstedt, Germany). Urate, albumin, bilirubin, alanine transaminase (ALT), ALP, glucose, high density lipoproteins (HDL), total cholesterol, sodium, potassium, urea, creatinine, calcium, phosphate (PO_4) and magnesium were measured on a Siemens ADVIA Chemistry XPT (Erlangen, Germany). Low density lipoproteins (LDL) were calculated by subtracting HDL from total cholesterol. Calcium was adjusted for albumin as described by Gardner et al (Gardner *et al.*, 1981). PTH(1-84) was measured by immunoassay on a Siemens ADVIA Centaur (Erlangen, Germany).

13.3.10 Urine Collection and Handling

Single point urine samples were collected in 50mL sterile containers using no preservatives, during participant trial appointments, the time of day was not controlled for.

13.3.11 Urine Analysis

All safety urine samples were analysed at County Durham and Darlington Foundation Trust (Darlington, UK). Calcium and Phosphate were measured by spectrophotometric methods on a Siemens ADVIA Chemistry XPT (Erlangen, Germany).

Urinary pyridinoline, deoxypyridinoline and cyclic AMP were measured at the BioAnalytical Facility (Norwich, UK) using in house LCMS methods (Al Riyami, 2017; Tang *et al.*, 2016)

13.3.12 Protocol Development

13.3.12.1 Focus group

A focus group was organised to explore people's experience and views of army basic training and how they thought a clinical trial might work and what issues should be considered when developing the protocol. Participants were UEA students who either had experience of army basic training (two male mature students) or a previous BSI (six athletes, two females and four males). Participants were given an outline of the planned study and were asked i) what factors would affect their decision to participate, ii) factors that would improve chances of them volunteering to take part, iii) any other considerations they though the researchers should take into account when developing the protocol.

The results of the focus group where that six people would take part, one would not take part and one was borderline. An overview of response themes and supporting quotes are presented in table 13-3. A full transcript of the focus group is at appendix 2.

Table 13-3: An overview the protocol development focus group for the RETURN trial including response themes and supporting quotes.

Question Posed	**Response Theme**	**Supporting Quotes**
Factors affecting an individual's decision to participate.	Personal/circumstantial pressure to participate which included, pressure to keep up with fellow cadets, fear of falling behind with training, personal commitment and investment of time in training process so far, mindset and education level of cadets - cadets are often young, may have a low education level and, due to pressure to fit in/keep up, are more inclined to say 'yes'.	Line 35 (C) *"it's basically a kind of 'keep up' environment otherwise you're continuously gonna drop back. I think this is why this study is actually quite important, because it will play on people's minds a lot. There's a term called "back squadders" - so if you do get injured or get a stress fracture, that's the last thing you want to do because it could put you in a medical military limbo where you're in rehab for 2 or 3 months whilst you see all your friends pass out through basic training…. So yeah, if you don't keep up, you're behind at all times really"* Line 119 (G) *"From my personal perspective, the fear of getting sort of both back squadded and then, if you're back squadded, subsequently removed from training especially if you have an injury, is very real. A lot of these troops have spent a long time trying just get to phase 1 training. They'll have gone through a very painful, very long selection process, a lot of admin/paperwork, taking up say 2-3 years work. So once they're there, and once they're on the training and say half way through training, the real incentive to take any chance of improving the chance of recovery, even if it's a 50:50 chance, I think, personally I would see that."*
	Veiled pressure from superiors to participate. Injured cadets have a bad reputation, if superiors are aware of who is and who is not participating (though logistical implications of	Line 132 (C) *"I have my own independence, I now have the chance, I can say 'no', I guess six and half years in the military, it's kind of 'you can't say no'. But looking back to if I was 18 years old, a lot of these lads if they're going to Catterick, they're 16 so they normally do two years' service at the Queens … doesn't go towards the military service. A lot of these lads are gonna be on a low education level, GCSEs or not even that - which is why I was taking notes on my phone. I think a lot of these people will*

	storing medication), this will 100% influence a cadet's decision on whether or not to participate. May well come across as being told to participate rather than volunteering.	*be what they call 'low internalization' - psychologically they feel that an example would be that 'I'm injured, I'm not as fit as everyone else' and I think that will have repercussion on the way they think, and that might push them to say 'yes'.* <u>Line 147 (A)</u> *"I think it [low education level of cadets] just makes them more inclined to 'go with the flow' rather than making an informed decision."*
	Stage of recovery, if stress fracture was already showing signs of recovery, they may decide not to participate.	<u>Line 65 (A)</u> *"If I had a stress fracture that was already showing improvement in recovery, that doing the study wouldn't warrant my time in terms of my stress fracture would recover before the study was up - if I already had a mode of recovering then it probably wouldn't be on the horizon…."*
	Severity of stress fracture and length of time with fracture, if the stress fracture was severe and had been present for a long time, they would probably wish to participate conversely if the stress fracture was severe, they would not wish to try something new, preferring tried and tested treatments.	<u>Line 68 (A)</u> *"But if it was a really serious one that was plaguing me all the time, and it had been there for a while and needed more intervention that what I was already having then I probably would."* <u>Line 112 (B)</u> *"Although if the fracture was bad enough, and I was really desperate, and like I really wanted to pass out as quick as possible, I'd probably say yes but it's difficult to put myself in that position when I'm not in and I'm not desperate to pass out or anything. But if it was bad enough and I really wanted it, I might do it. But if it was just a minor one, I wouldn't see it as worth it in case I get the placebo."* <u>Line 88 (E)</u>

		"It would probably be dependent on the severity of the injury as well. I'm not sure whether I would try something new. If it was quite a severe injury, I might stick to something safer."
	Reassurance about impact on other hormones.	<u>Line 73 (A)</u> ***"****I would have to be sure that taking the parathyroid hormone wouldn't change any of my other hormone levels."* <u>Line 155 (A)</u> *"Is it sensible to be giving someone hormones who's still at the age where their hormones are still settling I'd say or maturing?"*
	Impact of participating in study on free time, cadets get a small amount of free time.	<u>Line 51 (G)</u> *"It [free time] varies day to day. There will be time for you to square away your own personal administration so sorting things out like your uniform and stuff like… that you get to yourself but often that gets eaten into by other activities."*
	Daily injections, worry about injecting everyday (two participants), keeping to the injection schedule may be difficult, long-time commitment (five participants)	<u>Line 82 (H)</u> *"I think one of the main things I said I think I would be a bit worried about injecting myself everyday..."* <u>Line 109 (B)</u> *"I probably wouldn't do it, mainly because I wouldn't want to inject myself everyday, especially if it's a placebo."* <u>Line 167 (B)</u> *"It depends on how painful the injection is. How big is the needle? … I'd try it first. Try the injection, and then see if it was alright… If I could test one and see 'nah, I don't want that' or 'yes actually it's fine, it didn't hurt'.*

	Attending MRIs may result in missing crucial training sessions and training outcomes which may be viewed as negative impact of participating	<u>Line 308 (G)</u> *"…the way these training programmes work is similar to learning outcomes you have a list of training outcomes soldiers need to complete before they can complete that training. So depending on when they go for MRIs or if they're missing crucial training sessions that they miss that training outcome and can't get signed off on that, that's going to create impact obviously on the training programme be it either that they're having to catch up in their, not 'free time' but their other time, in which case that's a negative impact from their point of view, or it's affecting their training programme in some other way so just a consideration."*
	Odds of getting placebo, dislike odds of getting the placebo, if it was a minor injury would not participate.	<u>Line 109 (B)</u> *"I probably wouldn't do it, mainly because I wouldn't want to inject myself everyday, especially if it's a placebo. I'd be really, really pissed off at the end of it if I was injecting nothing into myself everyday. So if I knew I was getting the actual one, I might say yes, but I don't like the 50:50 odds of having to do it everyday and not getting anything out of it… But if it was just a minor one, I wouldn't see it as worth it in case I get the placebo."* <u>*Line 123 (G)*</u> *"So once they're there, and once they're on the training and say half way through training, the real incentive to take any chance of improving the chance of recovery, even if it's a 50:50 chance, I think, personally I would see that."* <u>*Line 160 (A)*</u> *"If it was with me with track, if they said to you 'you've got a stress fracture but in six months' time you can run a PB if you take this' I'd definitely jump on the bandwagon. So obviously I'm not in the forces, maybe that to them is the equivalent of what I've just described."*

	Wish to contribute to future research	Line 128 (G) *"I think I would want to contribute just because the future research."*
Factors that would improve chances of taking part	Greater chance of receiving PTH (3:2) vs placebo – one participant (previously 'no') said it would make them more likely to take part but would still depend on how painful the injection was. One participant (previously 'borderline') said this would not affect their decision.	Line 165 (B) ***"****Yes it would sway my decision if there was a greater chance of me getting the actual one"* Line 167 (B) (Host: Is that enough of a difference, do you think?) *"It depends on how painful the injection is. How big is the needle? … I'd try it first. Try the injection, and then see if it was alright… If I could test one and see 'nah, I don't want that' or 'yes actually it's fine, it didn't hurt'."* Line 172 (H) *"For me, that's not really enough. I'd be more likely to do it but its not enough for me to be like 'yeah, yeah I'm gonna jump on'."*
	Comparator group to be standard care with additional monitoring rather than injections - questioned methodological accuracy of study if you know which group you have been assigned to (previously 'yes'), better monitoring than they would receive otherwise (previously 'yes'). Interested to see the MRI results and to	Line 186 (H) *"It's just a waste of time"* Line 194 (A) *"Also decreases accuracy of the study, it's making something a bit more open that should not be disclosed."* Line 200 [Host explains there is no chance of receiving active PTH after intervention period for those in placebo group] Line 211 (C) *"Based on what you're saying, that would put me off. If there was no placebo group and it was that and what you've just described as the outcome then that would put me off because I'm not going to gain."*

	see if they are recovering (previously 'no')	Host: *Not to put words in your mouth, so you were actually borderline before but if it was sold to you like that…* (C) *If it was sold to me like that and I wouldn't ever get the drug if it worked then id be like 'no there's no point'.* <u>Line 191 (H)</u> *"You're possibly getting the support but I'd still see it as a waste of time if I know I'm getting the placebo."* <u>Line 187 (G or B)</u> *"You could argue that they're still getting better monitoring than they would otherwise."* <u>Line 223 (B)</u> *"I think if I was in that group I'd still do it because it'd just be interesting to have the MRI scans to see what's going on. It might show something else, it might find something else that's' undiagnosed or you can actually see the recovery, you can see the process."* <u>Line 227 (B)</u> *"For me, if I'm in that position, even if I say 'no' I've still got a stress fracture. If I say 'no' I'm not getting any MRI scans. IF I say 'yes, I'm in that' I still get a scan every so often I can see what's going on so it would still be quite beneficial for me if I was injured."*
Other considerations	Logistical constraints of being out on exercise and away from barracks without access to refrigeration was raised.	<u>Line 273 (G)</u> *"if they're on exercise in the field for example, keeping medication refrigerated or even simply the fact that they'll be potentially doing things at the time that during a normal day they'll be injecting where, for example, they might be doing something*

	Cadets go out on exercise even when injured.	*that means they don't have time to do that. Therefore, how from a logistics point of view, do you plan around the fact that they're not in a hardened accommodation environment all the time with access to refrigeration."* <u>Line 287 (C)</u> *"The only suggestion I can think of is there is a rehabilitation unit for the injured personnel anyway. Is having the fridge there that they report to every morning, they have their injection whether it's placebo or not. The only problem is, that yeah, they go out on exercise quite often for 5, 6, 7 days at a time and carrying that around is going to be problematic."* *Host: Would they still go out on exercise if they have a stress fracture. I mean if they're on reduced duties would they still be expected to go out?* <u>Line 293 (C)</u> *"Yeah they just lighten load or they don't do as many heavy marching duties. You might go out but instead of carrying 25 kilos you might have 20 or you might just not have your weapon. Or they might just say, so, the big one is the anterior stress fracture on the shin, is just loosen the shoelaces at the top to relieve the pressure and that's it and then carry on."*
	Representativeness of the population was raised.	<u>Line 243 (?)</u> *"Cadets will be young, up to the age 33."* Line 237 (?) *"Some ethnicities have denser bones and may be less prone to stress fracture and may have a different rate of recovery."*

13.3.13 Statistical Methods

13.3.13.1 Power Calculation

The target sample size was based upon a consideration of the between group difference in the primary endpoint (i.e. MRI indicated healing using the modified Fredericson BSI Grading System) at 8 weeks post-randomisation. A 'success' was defined as a grade of zero ('healed') or an improvement of two grades or more from baseline. In Almirol *et al*. 2016, 43% of individuals in the placebo arm (3 of 7) (Almirol *et al.*, 2016), met this definition. Assuming no more than a 20% drop-out rate over the first 8 weeks, 136 randomised participants (68 per group, 54 with 8-week MRIs) would provide 80% statistical power to detect a difference between groups if the teriparatide groups 'success' rate is 71% or more (in the Almirol group, 67%, 4 of 6, met this definition). This equates to an odds ratio of 3.23 or a relative increase in healing of around 65%.

13.3.13.2 Statistical Analysis

All analyses were based on the 'intention-to-treat' population. A logistic regression model, using maximum likelihood, was constructed to estimate the odds of radiological healing by week 8 (Primary outcome - index BSI improvement by two MRI grades or more, or reduction to grade zero) in the intervention group compared to standard care, expressed as an odds ratio (OR). Adjusted odds (using exact inference) of radiological healing at week 8 favoured the standard care group. An exact inference approach has also been presented for these data due to the relatively low number of participants (n = 26). The estimates from this approach differ little from the maximum-likelihood based approach.

13.4 Results

34 Army Infantry recruits (33 males, 1 female) with one or more lower body BSIs voluntarily participated in the study. 17 participants were randomly allocated to receive Army standard care, and 17 participants were randomly allocated to receive teriparatide in addition to Army standard care. This study did not recruit sufficient participants to reach power and as a result cannot be expected to detect (as statistically significant) any true effects of teriparatide. Participant progress through the trial is detailed in figure 13–2, following CONSORT guidance.

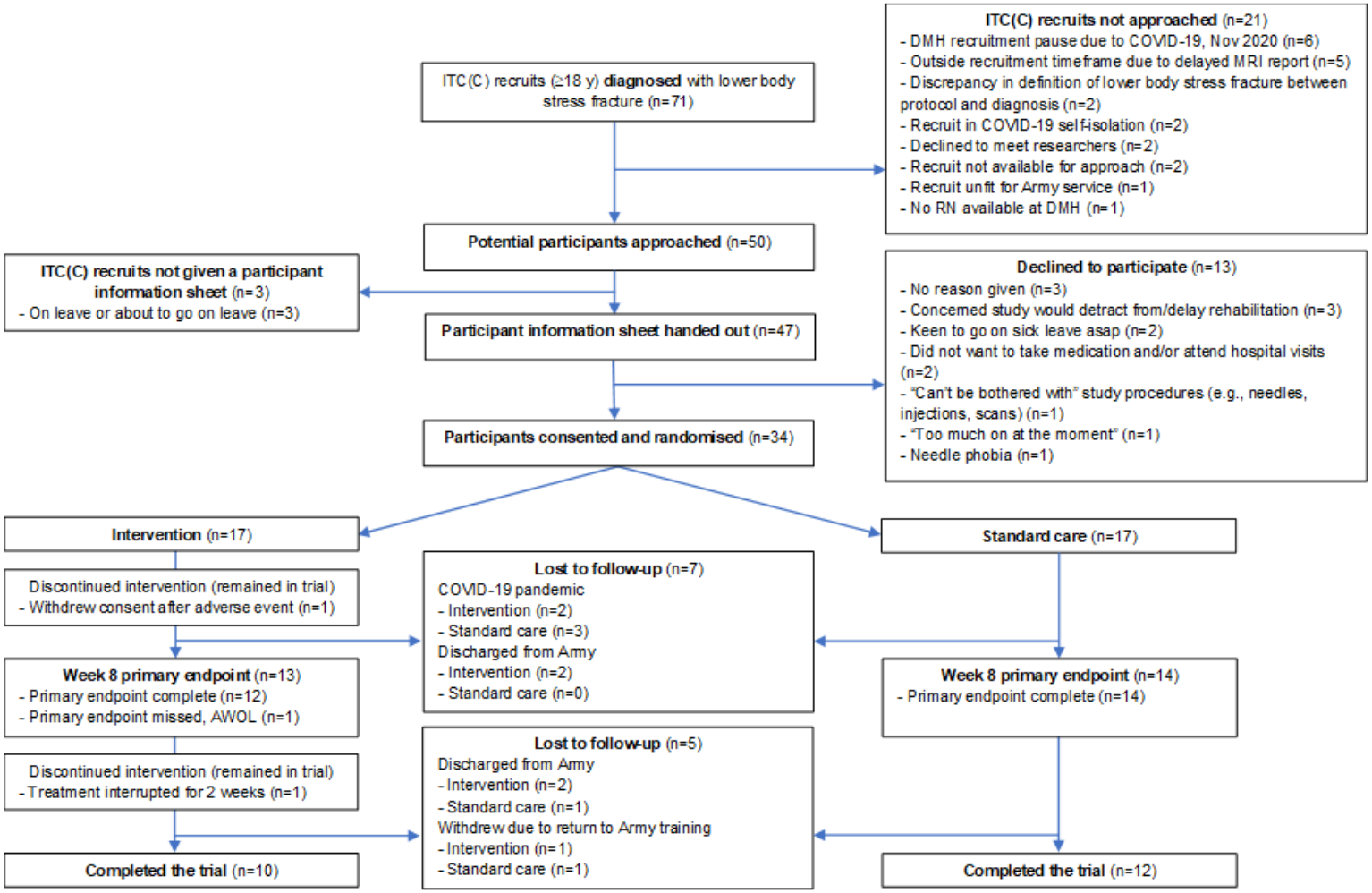


Figure 13-2: CONSORT flow diagram for the RETURN trial. ITC(C), Infantry Training Centre (Catterick); DMH, Darlington Memorial Hospital; MRI, magnetic resonance imaging; COVID-19, coronavirus disease 2019; RN, research nurse; AWOL, absent without leave.

13.4.1 Description of Participants by Randomised Group at Baseline

Baseline demographics, body composition or biochemistry were not significantly different between the treatment and control groups at baseline. A full description of these parameters is in table 13-4. One participant randomised to the standard care group declined to wear an accelerometer.

Table 13-4: Description of participants by randomised group at baseline.

	Intervention ***n* = 17**	**Standard care** ***n* = 17**	**Overall** ***n* = 34**
Age at consent (years): mean (SD)	21.2 (3.6)	21.4 (3.2)	21.3 (3.3)
Sex: *n* (%)			
- Male	16 (94.1%)	17 (100%)	33 (97.1%)
- Female	1 (5.9%)	0	1 (2.9%)
Ethnicity: *n* (%)			
- White	14 (82.4%)	16 (94.1%)	30 (88.2%)
- Asian/Asian British	2 (11.8%)	0	2 (5.9%)
- Multiple ethnic groups	0	1 (5.9%)	1 (2.9%)
- Other	1 (5.9%)	0	1 (2.9%)
Body mass (kg): mean (SD)	73.5 (9.1)	73.2 (7.1)	73.4 (8.1)
Height (cm): mean (SD)	173.9 (8.1)	176.5 (5.3)	175.2 (6.8)
Vitamin D status: *n* (%)			
- Not deficient (25(OH)D ≥30 nmol/L)	16 (94.1%)	14 (82.4%)	30 (88.2%)
- Deficient (25(OH)D <30 nmol/L)	1 (5.9%)	3 (8.8%)	4 (11.8%)
Number of alcohol units consumed in a normal week: median (IQR)	9 (0, 17)	3 (1, 18)	6 (0, 17)
Smoker: *n* (%)			
- Yes	7 (46.7%)	6 (42.9%)	13 (44.8%)
- No, but used to smoke	1 (6.7%)	3 (21.4%)	4 (13.8%)
- No	7 (46.7%)	5 (35.7%)	12 (41.4%)
Number of tobacco items[a] smoked in a normal day: median (IQR)	0 (0, 4)	0 (0, 3)	0 (0, 3)
Health-related quality of life (SF-36v2 total score): mean (SD)	116.1 (12.1)	111.0 (15.8)	113.6 (14.1)
BSI type: *n* (%)			
- Single	12 (70.6%)	12 (70.6%)	24 (70.6%)
- Multiple	5 (29.4%)	5 (29.4%)	10 (29.4%)
MRI grade of index BSI[b]: *n* (%)			
- 1	1 (5.9%)	0	1 (2.9%)
- 2	3 (17.7%)	2 (11.8%)	5 (14.7%)
- 3	5 (29.4%)	5 (29.4%)	10 (29.4%)
- 4a	6 (35.3%)	5 (29.4%)	11 (32.4%)
- 4b	2 (11.8%)	5 (29.4%)	7 (20.6%)
Site of index BSI[b]: *n* (%)			
- Neck of femur (left)	1 (5.9%)	1 (5.9%)	2 (5.9%)
- Neck of femur (right)	2 (11.8%)	4 (23.5%)	6 (17.7%)
- Femur (left)	3 (17.7%)	1 (5.9%)	4 (11.8%)
- Tibia (left)	3 (17.7%)	4 (23.5%)	7 (20.6%)

- Tibia (right)	7 (41.2%)	3 (17.7%)	10 (29.4%)
- Fibula (right)	0	1 (5.9%)	1 (2.9%)
- Foot/ankle (left)	0	2 (11.7%)	2 (5.9%)
- Foot/ankle (right)	1 (5.9%)	0	1 (2.9%)
- Pelvic girdle (right)	0	1 (5.9%)	1 (2.9%)

[a]Tobacco items are counted as zero for non-current smokers. [b]Index BSI defined as (in priority order): a) the BSI site with the highest Fredericson grade, b) the easiest site to grade (typically the largest bone), c) if same grade bilateral, the BSI on the dominant leg side.

13.4.2 Primary Outcome: Radiological Healing at Week 8

Adjusted odds (using exact inference) of radiological healing at week 8 favoured the standard care group, however, this was not statistically significant (adjusted OR: 0.590, 95% CI: 0.071, 4.350; *P* = 0.829; Table 13.5). An exact inference approach has also been presented for these data due to the relatively low number of participants (*n* = 26). The estimates from this approach differ little from the maximum-likelihood based approach.

Table 13-5: Radiological Healing at week 8.

Model[a]	**Intervention Grade 0 or ≥2 grade improvement *n* (%)**	**Standard care Grade 0 or ≥2 grade improvement *n* (%)**	**Odds ratio[b]**	**95% Confidence interval[c]**	***P* value**
***n* = 26**	***n* = 12**	***n* = 14**			
Group only	8 (66.7%)	11 (78.6%)	0.545	(0.095, 3.146)	0.498
Adjusted model, including group and stratification variables	8 (66.7%)	11 (78.6%)	0.521	(0.079, 3.447)	0.498
Adjusted model, including group, stratification variables, *using exact inference*	8 (66.7%)	11 (78.6%)	0.590	(0.071, 4.350)	0.829
Adjusted model, including group, multiple/single BSIs, baseline MRI grade	8 (66.7%)	11 (78.6%)	0.627	(0.101, 3.887)	0.616

[a]Logistic regression model used, modelling the odds of the index BSI MRI grade 0 or an improvement of 2 grades at week 8, adjusted for differences of vitamin D status at baseline, multiple or single BSIs at baseline (stratification variables), index BSI MRI grade at baseline and group. [b]Odds ratio (OR; the odds of the index BSI MRI grade 0 or an improvement of 2 grades or more at week 8 for participants in the intervention group, is (OR) times that of the odds of the index BSI MRI grade 0 or an improvement of 2 grades or more at week 8, for participants in the standard care group). [c]95% Wald or Exact confidence interval for odds ratio (if exact, stated in model description, otherwise Wald).

13.4.3 Secondary Outcomes

13.4.3.1 Time to Radiological Healing

A trend to shorter average time to radiological healing (index BSI MRI grade zero) was observed in the intervention group. However, this was not statistically significant (adjusted difference: -1.022, 95% CI: -3.553, 1.510; *P* = 0.411; Table 13.6).

13.4.3.2 Time to discharge from physical rehabilitation

A shorter time to discharge from rehabilitation in the standard care group was observed but this was not statistically significant (ratio of geometric means: 1.037, 95% CI: 0.723, 1.489; *P* = 0.834; Table 13.6).

13.4.3.3 Health-related quality of life

There was no difference between groups in health-related quality of life (SF-36v2 total score) at week 8 (ratio of geometric means: 1.012, 95% CI: 0.549, 1.867; *P* = 0.968; Table 13.6).

Table 13-6: Time to radiological healing, discharge from rehabilitation, health-related quality of life and pain.

Model[a]	**Intervention (mean (SD))**	**Standard care (mean (SD))**	**Untransformed**		**Transformed**[d]	
			Adjusted difference (95% CI)[b]	***P* value**	**Adjusted difference (95% CI)**[b]	***P* value**
***n* = 25**	***n* = 12**	***n* = 13**				
Time (weeks) to radiological healing (MRI grade zero)[c]	9.8 (2.2)	10.9 (3.8)	-1.022 (-3.553, 1.510)	0.411		
***n* = 23**	***n* = 11**	***n* = 12**				
Time (days) to discharge from rehabilitation[c]	127.4 (47.8)	121.7 (52.7)	2.470 (-42.871, 47.812)	0.910	1.037 (0.723, 1.489)	0.834
***n* = 32**	***n* = 15**	***n* = 17**				
Health-related quality of life (SF-36v2 total score) at week 8	139.5 (10.8)	132.9 (16.4)	4.179 (-5.760, 14.118)	0.396	1.012 (0.549, 1.867)	0.968
***n* = 31**	***n* = 14**	***n* = 17**				
Pain at week 8[c]	10.3 (19.6)	12.1 (19.2)	-2.538 (-17.125, 12.049)	0.724	0.898 (0.277, 2.915)	0.853

[a]Generalised Linear model used, adjusted for differences of vitamin D status at baseline, multiple or single BSIs at baseline (stratification variables), baseline value of outcome variable where available, and group. [b]95% Confidence interval for parameter estimates. [c]Baseline value of outcome variable not available. [d]All transformed models use a log transformation of the outcome. However, the SF36v2 total score model also uses a reflection of the outcome before the log transformation, so the interpretation of the direction of score is reversed. The adjusted difference given for the transformed models is the geometric mean ratio (converted back to the original scale).

13.4.3.4 Pain

There was no difference in pain scores between groups (ratio of geometric means: 0.898, 95% CI: 0.277, 2.915; *P* = 0.853; Table 13.7). A repeated measures analysis of pain from week 1–24 also showed no statistically significant difference between groups (Table 13.7).

Table 13-7: Pain week 1 to 24.

Model[a] *n* = 34	**Intervention** (mean ± SD) *n* = 17	**Standard care** (mean ± SD) *n* = 17	**Adjusted difference** (95% CI)[b]	***P* value**
Including group	13.3 ± 21.0	10.8 ± 15.8	2.742 (-5.556, 11.040)	0.505
Including group and stratification variables	13.3 ± 21.0	10.8 ± 15.8	1.561 (-6.764, 9.887)	0.704

[a]Repeated measures model used, with a first order autoregressive covariance structure, to account for a decaying within participant correlation between pain scores over time, adjusted for differences of vitamin D status at baseline, multiple or single BSIs at baseline (stratification variables) and group. A pain score was not collected at baseline, so this value is not available for use. [b]95% Confidence interval for parameter estimates.

13.4.3.5 Clinical healing

A trend to shorter average time to clinical healing was observed in the intervention group although was not statistically significant (adjusted difference: -1.101, 95% CI: -4.437, 2.235; *P* = 0.486; Table 13.8). For the proportion of participants clinically healed during the trial, there was no statistically significant difference between groups (exact OR: 0.855, 95% CI: 0.137, 4.881; *P* = 1.000; Table 13.9). An exact inference approach has been used due to the relatively low number of participants (*n* = 28).

Table 13-8: Time to clinical healing

Model[a] *n* = 19	**Intervention** (mean ± SD) *n* = 10	**Standard care** (mean ± SD) *n* = 9	**Adjusted difference** (95% CI)[b]	***P* value**
Time (weeks) to clinical healing	12.2 ± 2.2	12.6 ± 3.5	-1.101 (-4.437, 2.235)	0.486

[a]Generalised linear model used, adjusted for differences of vitamin D status at baseline, multiple or single BSIs at baseline (stratification variables), and group. Index BSI MRI grade at baseline was also added to the model, for consistency with the primary analysis. [b]95% Confidence interval for parameter estimates.

Table 13-9: Proportion of participants clinically healed.

Model[a] *n* = 28	**Intervention** *n* (%) *n* = 15	**Standard care** *n* (%) *n* = 13	**Odds ratio**[b]	**95% Confidence interval**[c]	***P* value**
Group only	10 (66.7%)	9 (69.2%)	0.889	(0.181, 4.375)	0.885
Adjusted model, including group and stratification variables	10 (66.7%)	9 (69.2%)	0.836	(0.151, 4.616)	0.837
Adjusted model, including group, stratification variables, *using exact inference*	10 (66.7%)	9 (69.2%)	0.855	(0.137, 4.881)	1.000

[a]Logistic regression model used, modelling the odds of clinically healed during the trial (up to week 24), adjusted for differences of vitamin D status at baseline, multiple or single BSIs at baseline (stratification variables), and group. Index fracture MRI grade at baseline was also added to the model, for consistency with the primary analysis. [b]Odds ratio (OR; the odds of being clinically healed during the trial for participants in the intervention group, is (OR) times that of the odds of being clinically healed during the trial, for participants in the standard care group). [c]95% Wald or Exact confidence interval for odds ratio (as given in model description).

13.4.3.6 Adverse events

Adverse events were reported by 14 participants (82%) in the intervention group and 14 participants (82%) in the standard care group (Table 13.10). No serious adverse events or unexpected adverse reactions were reported during the trial.

Table 13-10: Adverse events.

		Intervention *n* = 17	**Standard care** *n* = 17
Number individuals reporting adverse events: *n* (%)		14 (82%)	14 (82%)
Number of adverse events: *n*		62	43
Adverse events with number of occurrences	Grade 3	1 Vomiting	
		1 Nausea	
	Grade 2	2 Diarrhoea	1 Bone pain
		1 Headache	
		1 Lip infection	
		1 Tooth infection	
		1 Upper respiratory infection	
	Grade 1	11 Headache	10 Headache
		3 Musculoskeletal and connective tissue disorder - Other, specify	8 Musculoskeletal and connective tissue disorder - Other, specify
		3 Cough	4 Cough
		6 Sore throat	3 Sore throat
		8 Injection site reaction	2 Infections and infestations - Other, specify
		4 Skin and subcutaneous tissue disorders - Other, specify	2 Nasal congestion
		3 Flu like symptoms	1 Musculoskeletal deformity
		2 Dizziness	1 Dizziness
		1 Investigations - Other, specify	1 Blood bilirubin increased
		1 Middle ear inflammation	1 Sinus pain
		1 Muscle weakness upper limb	1 Allergic rhinitis
		1 Back pain	1 Sneezing
		1 Nasal congestion	1 Vomiting
		1 Neck pain	
		1 Neuralgia	
		1 Allergic rhinitis	
		1 Bruising	
		1 Stomach pain	
		1 Vomiting	
	No grade	1 Eye disorders - Other, specify	1 Ear pain
		1 Headache	3 Headache
		1 Nausea	1 Infections and infestations - Other, specify
			1 Pain

13.4.4 Exploratory Outcomes

13.4.4.1 Between Group Blood Biochemistry

Serum 1,25-dihydroxyvitamin D was higher for participants in the intervention group, compared with standard care at baseline, week 8 and week 16 (adjusted difference: 28.692, 95% CI: 13.391, 43.993; *P* < 0.001). There were no other statistically significant between group differences in blood biochemistry. The results are shown in table 13-11.

Table 13-11: Comparison of the effect of teriparatide on blood biochemistry between randomised groups.

Outcome *n* = 34	**Intervention** (mean ± SD) *n* =17	**Standard care** (mean ± SD) *n* = 17	**Adjusted Difference** (95% CI)	***P* value**
SRANKL[a] (pmol/L)	0.08 ± 0.08	0.09 ± 0.06	-0.007 (-0.048, 0.035)	0.748
DKK1 (pmol/L)	54.32 ± 16.11	55.20 ± 16.85	-1.495 (-12.739, 9.750)	0.788
OPG (pmol/L)	2.37 ± 0.92	2.13 ± 0.71	0.235 (-0.223, 0.694)	0.304
$1,25(OH)_2D$ (pmol/L)	139.09 ± 43.81	104.95 ± 25.98	28.692 (13.391, 43.993)	<0.001*
CTX (µg/mL)	0.62 ± 0.29	0.61 ± 0.26	-0.049 (-0.187, 0.090)	0.477
PINP (µg/mL)	145.56 ± 60.90	126.57 ± 59.42	-4.527 (-35.365, 26.311)	0.767
SOST (pmol/L)	37.53 ± 10.73	38.21 ± 9.57	-0.130 (-6.369, 6.109)	0.966

[a] Five participants at baseline and four participants at week 8 had SRANKL <0.01 pmol/L, which means these values were undetectable, so they were imputed as 0.005 pmol/L (midpoint of the undetectable range).
*Denotes significant result.

13.4.4.2 Within Group Blood Biochemistry

N-terminal propeptide of type I collagen increased in intervention participants at week 16 compared to baseline (mean difference: 49.54, 95% CI: 12.70, 90.97; *P* = 0.041). Serum $1,25(OH)_2$ D3 increased in standard care participants at week 16 compared to baseline (mean difference: 24.22, 95% CI: 11.43, 37.01; *P* = 0.012). SOST decreased in standard care

participants at week 16 compared to baseline (mean difference: -6.73, 95% CI: -11.85, -1.61; *P* = 0.041). There were no other statistically significant within group differences in blood biochemistry. The results are shown in table 13-12 and for analytes with significant results, intervening time points are shown in table 13-13.

Table 13-12: Comparison of the effect of teriparatide on blood biochemistry between randomised groups.

	Intervention					Standard Care				
	Baseline (Mean ± SD)	**Week 16** (Mean ± SD)	**Mean Difference**	**95% CI**	***P* Value**	**Baseline** (Mean ± SD)	**Week 16** (Mean ± SD)	**Mean Difference**	**95% CI**	***P* Value**
SRANKL[a] (pmol/L)	0.08 ± 0.07	0.06 ± 0.06	-0.02	-0.04, 0.03	0.559	0.09 ± 0.05	0.07 ± 0.06	-0.02	-0.07, 0.03	0.295
OPG (pmol/L)	2.39 ± 0.84	2.60 ± 1.07	0.21	- 0.70, 0.42	0.582	2.10 ± 0.56	2.30 ± 0.90	0.20	-0.26, 0.66	0.511
$1,25(OH)_2D$ (pmol/L)	116.94 ± 25.71	139.98 ± 50.19	21.64	- 19.66, 62.94	0.182	92.63 ± 22.02	116.85 ± 24.45	24.22	11.43, 37.01	0.012*
CTX (µg/mL)	0.59 ± 0.18	0.56 ± 0.23	-0.03	– 0.14, 0.08	0.769	0.67 ± 0.22	0.52 ± 0.29	-0.15	-0.31, 0.01	0.136
PINP (µg/mL)	114.07 ± 30.80	163.61 ± 68.82	49.54	12.70, 90.97	0.041*	118.46 ± 52.53	126.82 ± 68.09	8.36	-5.92, 22.64	0.727
SOST (pmol/L)	37.70 ± 10.82	38.38 ± 9.08	0.68	-3.49, 4.85	0.861	42.02 ± 8.53	35.29 ± 8.05	-6.73	-11.85, -1.61	0.041*

[a] Five participants at baseline and four participants at week 8 had SRANKL <0.01 pmol/L, which means these values were undetectable, so they were imputed as 0.005 pmol/L (midpoint of the undetectable range).

*Denotes significant result.

Table 13-13: Intervening timepoint analysis for analytes showing significant differences in comparison of blood biochemistry within randomised groups.

	Baseline (Mean ± SD)	Week 4 (Mean ± SD)	Mean Difference	95% CI	*P* Value	Week 8 (Mean ± SD)	Mean Difference	95% CI	*P* Value	Week 12 (Mean ± SD)	Mean Difference	95% CI	*P* Value
Intervention													
P1NP (µg/mL)	114.07 ± 30.80	140.52 ± 47.59	24.96	7.15, 42.77	0.089	168.32 ± 62.57	52.08	19.44, 84.72	0.010*	163.42 ± 67.72	49.59	17.00, 82.18	0.032*
Standard Care													
1,25(OH)2D (pmol/L)	92.63 ± 22.02	103.16 ± 20.92	10.53	-4.91, 25.97	0.201	108.85 ± 24.46	13.04	0.06, 26.02	0.062	114.76 ± 20.14	19.96	1.40, 38.52	0.010*
SOST (pmol/L)	42.02 ± 8.53	37.35 ± 7.39	-4.68	-9.17, 0.19	0.107	-36.30 ± 10.19	-6.15	-12.09, 12.82	0.118	-36.83 ± 6.38	-6.88	-13.09, 0.67	0.081

*Denotes significant result.

13.4.4.3 Areal Bone Mineral Density: Lumbar Spine and Neck of Femur

Spine BMD increased significantly in the treatment group compared to control over 16 weeks (*P* = 0.002). There was no change in BMC, Z-score or total area at the spine over 16 weeks. The results are shown in table 13-14.

At the non-dominant neck of femur, shaft BMD and total area increased significantly in the treatment group compared to control over 16 weeks (*P* = 0.048 and *P* = 0.047 respectively). The results are shown in table 13-14.

Table 13-14: aBMD, BMC, total area and z-scores at the spine (L1-L4) and non-dominant femoral neck in treatment and standard care groups at baseline and week 16. Data are mean ± standard deviation, mean difference and 95% confidence intervals of the mean. Probability was calculated by ANCOVA and statistical significance was accepted at 0.05.

		Treatment				**Standard Care**				
				Difference Between Baseline and Week 16				**Difference Between Baseline and Week 16**		
Spine		**Baseline**	**Week 16**	**Mean Difference**	***95% CI***	**Baseline**	**Week 16**	**Mean Difference**	***95% CI***	***P***
		(mean ± SD)	(mean ± SD)			(mean ± SD)	(mean ± SD)			
BMD (g/cm2)	Total	1.23 ± 0.45	1.28 ± 0.16	0.03	-0.04, -0.03	1.19 ± 0.13	1.19 ± 0.11	0.01	-0.02, 0.01	0.002*
BMC (g)	Total	74.34 ± 12.90	78.78 ± 14.83	2.69	-3.36, -2.02	70.12 ± 12.02	70.73 ± 10.22	0.61	-2.11, 0.89	0.360
Area (cm2)	Total	60.31 ± 6.53	61.56 ± 7.24	0.61	-0.96, -0.26	58.47 ± 5.80	58.80 ± 4.42	0.34	-0.78, 0.10	0.065
Z-Score		0.06 ± 1.13	0.43 ± 1.27	0.26	-0.35, -0.18	-0.17 ± 1.02	-0.14 ± 0.89	0.03	-0.17, 0.110	0.404
Non-Dominant Neck of Femur										
BMD (g/cm2)	Shaft	1.37 ± 0.18	1.39 ± 0.18	0.02	-0.03, 0.00	1.29 ± 0.12	1.30 ± 0.10	0.00	-0.02, 0.01	0.048*
	Total	1.17 ± 0.15	1.19 ± 0.15	0.02	-0.03, 0.00	1.10 ± 0.08	1.11 ± 0.07	0.01	-0.02, 0.01	0.094
BMC (g)	Shaft	20.90 ± 3.00	21.16 ± 2.92	0.27	-0.53, 0.00	19.61 ± 2.05	19.68 ± 1.89	0.07	-0.37, 0.23	0.157
	Total	40.50 ± 6.20	40.79 ± 5.87	0.30	-0.86, 0.27	38.63 ± 5.01	38.97 ± 5.00	0.34	-0.98, 0.29	0.923
Area (cm2)	Shaft	15.25 ± 0.93	15.27 ± 0.95	0.02	-0.14, 0.10	15.16 ± 1.08	15.20 ± 1.07	0.04	-0.17, 0.10	0.849
	Total	34.61 ± 2.64	34.40 ± 2.65	- 0.20	-0.01, 0.42	35.01 ± 2.81	35.15 ± 2.87	0.14	-0.41, 0.13	0.047*
Z-Score		0.41 ± 1.12	0.54 ± 1.08	0.13	-0.23, -0.04	-0.08 ± 0.62	-0.04 ± 0.58	- 0.04	-0.14, 0.05	0.070

13.4.4.4 Bone Density, Geometry and Microarchitecture: Ultradistal Radius and Tibia

One week-16 radial scan (one treatment participant) was excluded from analysis as they had common regions below 80%. Two baseline radial scans, both treatment participants were excluded from analysis as the scans were grade 3.

Bone geometry and microarchitecture did not significantly change at the radius or tibia between treatment and control groups following 16 weeks of treatment. The results are shown in table 13-15.

Table 13-15: vBMD, bone microarchitecture and geometry in treatment and standard care groups at Baseline and Week 16. Data are mean ± standard deviation, mean difference and 95% confidence intervals of the mean. Probability was calculated by ANCOVA and statistical significance was accepted at 0.05.

	Treatment				**Standard Care**				
	Baseline (mean ± SD)	**Week 16** (mean ± SD)	**Mean Difference**	**95% CI**	**Baseline** (mean ± SD)	**Week 16** (mean ± SD)	**Mean Difference**	**95% CI**	***P***
Radius									
Total vBMD (mg HA/cm^3)	310.95 ± 31.93	310 ± 34.41	0.80	-4.23, 5.83	351.18 ± 46.66	353.36 ± 49.79	2.18	-8.66, 4.30	0.823
Trabecular vBMD (mg HA/cm^3)	202.49 ± 19.90	204.54 ± 20.07	2.05	-4.28, 0.18	229.45 ± 25.40	232.25 ± 28.18	2.79	-6.07, 0.49	0.666
Trabecular Area (mm^2)	314.12 ± 77.59	315.15 ± 77.77	1.03	-2.44, 0.37	286.17 ± 58.47	285.83 ± 58.23	0.35	-1.74, 2.43	0.259
Compact Bone Density (mg HA/cm^3)	820.88 ± 71.37	813.84 ± 77.97	7.04	-4.50, 18.58	831.41 ± 34.23	830.71 ± 36.10	0.70	-7.81, 9.21	0.376
Trabecular Number (1/mm)	2.26 ± 0.25	2.33 ± 0.25	0.06	-0.16, 0.04	2.17 ± 0.19	2.28 ± 0.26	0.11	-0.23, 0.01	0.607
Trabecular Thickness (mm)	0.08 ± 0.01	0.07 ± 0.01	<0.01	-0.00, 0.00	0.09 ± 0.01	0.09 ± 0.01	0.00	0.00, 0.01	0.571
Trabecular Spacing (mm)	0.37 ± 0.05	0.36 ± 0.04	0.01	-0.01, 0.03	0.38 ± 0.04	0.36 ± 0.05	0.02	0.00, 0.04	0.698
Cortical Area (mm^2)	54.91 ± 11.69	53.20 ± 13.40	1.71	-0.34, 3.76	63.76 ± 12.24	63.75 ± 12.46	0.01	-2.07, 2.09	0.383
Cortical vBMD (mg HA/cm^3)	827.40 ± 74.14	823.72 ± 74.39	3.68	-8.01, 15.37	836.89 ± 39.09	837.14 ± 40.60	0.25	-9.83, 9.33	0.554
Cortical Perimeter (mm)	80.39 ± 9.76	80.47 ± 9.53	0.08	-0.00, 0.00	79.71 ± 7.20	79.90 ± 7.22	0.19	-0.38, 0.00	0.642
Cortical Porosity (%)	0.02 ± 0.02	0.02 ± 0.02	<0.01	-0.00, 0.04	0.02 ± 0.01	0.03 ± 0.01	0.00	-0.01, 0.00	0.312
Cortical Thickness (mm)	0.83 ± 0.13	0.81 ± 0.14	0.02	-0.00, 0.01	0.92 ± 0.15	0.92 ± 0.14	0.00	-0.02, 0.02	0.142

Cortical Pore Diameter (mm)	0.14 ± 0.01	0.14 ± 0.01	<0.01	-0.00, 0.01	0.15 ± 0.01	0.15 ± 0.01	0.00	0.00, 0.01	0.302
Stiffness (kN·mm)	96598.05 ± 17895.18	94465.46 0 ± 19797.39	2132.59	-2313.59, 6578.77	110653.31 ± 13048.41	109308.14 ± 12184	1345.17	-2310.38, 5000.72	0.702
Estimated Failure Load (kN)	-4968.21 ± 903.92	-4894.13 ± 978.89	74.08	-276.58, 128.42	-5599.54 ± 634.84	-5555.33 ± 621.69	44.22	-207.67, 119.24	0.784
Tibia									
Total vBMD (mg HA/cm^3)	346.99 ± 61.07	351.05 ± 64.91	4.06	-7.06, -1.06	340.85 ± 45.48	342.00 ± 44.60	1.15	-2.86, 0.56	0.070
Trabecular vBMD (mg HA/cm^3)	224.08 ± 36.93	226.58 ± 38.41	2.50	-4.23, -0.77	220.65 ± 27.92	221.42 ± 27.82	0.77	-1.94, 0.40	0.085
Trabecular Area (mm^2)	680.28 ± 191.36	678.54 ± 192.66	1.73	-0.24, 3.71	671.32 ± 161.62	671.30 ± 160.07	0.02	-1.89, 1.92	0.194
Compact Bone Density (mg HA/cm^3)	897.68 ± 40.99	900.37 ± 39.26	2.68	-8.19, 2.82	889.95 ± 24.40	893.39 ± 23.27	3.44	-9.31, 2.43	0.971
Trabecular Number (1/mm)	2.13 ± 0.21	2.25 ± 0.19	-0.11	-0.22, -0.01	2.10 ± 0.35	2.13 ± 0.28	-0.03	-0.14, 0.08	0.129
Trabecular Thickness (mm)	0.09 ± 0.01	0.08 ± 0.01	0.00	0.00, 0.01	0.09 ± 0.01	0.09 ± 0.01	0.00	0.00, 0.01	0.480
Trabecular Spacing (mm)	0.38 ± 0.04	0.36 ± 0.04	0.02	0.00, 0.04	0.40 ± 0.07	0.39 ± 0.06	0.01	-0.01, 0.03	0.164
Cortical Area (mm^2)	132.91 ± 22.14	133.27 ± 22.31	-0.36	-1.22, 0.50	133.27 ± 17.77	133.26 ± 16.50	0.01	-1.23, 1.25	0.119
Cortical vBMD (mg HA/cm^3)	909.18 ± 48.58	913.88 ± 49.55	-4.70	-12.16, 2.76	894.92 ± 28.11	897.56 ± 28.76	-2.64	-9.27, 3.99	0.622
Cortical Perimeter (mm)	111.57 ± 13.24	121.38 ± 37.89	-9.82	-31.31, 1.67	111.82 ± 10.98	111.87 ± 11.1	-0.05	-0.3, 0.2	0.314
Cortical Porosity (%)	0.04 ± 0.01	0.04 ± 0.01	0.00	0.00, 0.00	0.04 ± 0.01	0.04 ± 0.01	0.00	0.00, 0.00	0.760
Cortical Thickness (mm)	1.28 ± 0.22	1.27 ± 0.22	0.00	-0.01, 0.02	1.28 ± 0.21	1.28 ± 0.20	0.00	-0.01, 0.01	0.550
Cortical Pore Diameter (mm)	0.15 ± 0.01	0.15 ± 0.01	0.00	0.00, 0.01	0.16 ± 0.02	0.16 ± 0.01	0.00	-0.01, 0.01	0.093
Stiffness (kN·mm)	273155.48 ± 46497.62	270509.14 ± 45076.13	2646.33	-4078.4, 371.07	265742.15 ± 22351.54	267523.27 ± 30001.55	-1781.12	-8422.37, 4860.12	0.321
Estimated Failure Load (kN)	-13689.61 ± 2324.69	-13604.17 ± 2251.29	-85.44	-345.88, 174.99	-13341.08 ± 1276.4	-13398.98 ± 1601.88	57.90	-216.01, 331.8	0.406

13.4.4.6 Physical Activity

Physical activity, measured by accelerometery and categorised as 'sedentary', 'moderate' to 'vigorous' activity and estimated energy expenditure per day, did not differ between the treatment and standard care group. Whilst results were consistent between groups, they showed high interindividual variability at each time point (Table 13-16).

Table 13-16: Physical activity comparison between treatment and control groups at 4 weekly intervals from week 1 to 16. Data are mean ± standard deviation. Probability was calculated by mixed design (split-plot) ANOVA and statistical significance was accepted at 0.05.

	Treatment				Standard Care				*P*
	Weeks 1 - 4 (mean ± SD)	Weeks 5 - 8 (mean ± SD)	Weeks 9 – 12 (mean ± SD)	Weeks 13 – 16 (mean ± SD)	Weeks 1 - 4 (mean ± SD)	Weeks 5 - 8 (mean ± SD)	Weeks 9 – 12 (mean ± SD)	Weeks 13 – 16 (mean ± SD)	
Sedentary Time (hrs)	19.0 ± 1.9	18.4 ± 2.1	18.4 ± 2.3	18.0 ± 2.2	18.3 ± 2.4	18.5 ± 2.2	18.3 ± 2.5	18.0 ± 2.5	0.978
Moderate to Vigorous (hrs)	3.6 ± 1.8	4.2 ± 1.7	4.1 ± 1.9	4.5 ± 1.8	4.2 ± 2.0	4.1 ± 1.9	4.2 ± 2.2	4.4 ± 2.1	0.953
Estimated Energy Expenditure (kcal)	3240 ± 723	3328 ± 738	3513 ± 752	3689 ± 724	3350 ± 722	3517 ± 674	3334 ± 844	3440 ± 819	0.246

13.4.5 Effect of Female Inclusion

Removing the one female participant from the results did not alter the statistical significance of any of the results presented in this chapter.

13.5 Discussion

This clinical trial is the largest investigating the use of teriparatide in the treatment of BSIs to date. Recent legislation changes have opened ground close combat roles to women for the first time, and women will need to complete infantry training that is more physically arduous than standard military basic training. These increased physical demands are predicted to result in increased rates of BSIs in women and it is the aspiration of the British Army to offer the best clinical treatment available.

Despite recruiting more than double the number of participants previously studied (Almirol *et al.*, 2016), the RETURN study did not recruit sufficient participants to reach power and as a result cannot be expected to detect (as statistically significant) any true effects of teriparatide. Recruitment was reduced for two reasons; i) all training at Infantry Training Centre Catterick was stopped in March 2020 when the UK went into the first lockdown of the COVID-19 pandemic, on return to training BSI rates were much lower than previously reported, ii) British Army recruitment reduced during the period of investigation, so the throughput of trainees was lower than expected.

The incidence of lower body BSI injuries (*n* = 71; 2.8 per month) at ITC(C) during the January 2020 – May 2022 study recruitment period (25 months, not including April–July 2020 when recruitment was closed due to the COVID-19 pandemic) was lower than the anticipated 8.0 per month originally estimated when the trial was designed. This lower-than-expected incidence of injury was likely due in part to COVID-19 related changes to training which included reduced numbers of recruits in training and recruits undergoing periods of COVID-19 self-isolation. The sample of potentially eligible participants was, therefore, lower than anticipated. Eight potential participants were not approached due to a recruitment pause caused by the COVID-19 pandemic in November 2020 (*n* = 6) and recruits being in COVID-19 self-isolation (*n* = 2). The first 5 participants were lost to follow-up before the week 8 primary outcome due to COVID-19 pandemic restrictions (March 2020). The study did not recruit the numbers predicted to result in significance in the power calculation (n = 136) and so the true effect of teriparatide on short term BSI healing and long-term future BSI rate, within this

population, remains unknown. All treatment estimates made in the results section are done so with a substantial degree of uncertainty as illustrated by the accompanying confidence intervals.

The results of PHAB (Chapter 12) suggest that the use of female data (Almirol *et al.*, 2016) as the basis for the power calculation for RETURN was not appropriate since females experience an attenuated response to PTH therapy compared with men, and the majority of participants in the RETURN trial were men (33 males, 1 female). If this study was repeated, males and females should be considered separately to evaluate difference in the clinical response to teriparatide. This was an intention to treat trial and as such all data was included in the analyses, however given the results of the PHAB trial (Chapter 12), showing that males and females respond differently to PTH (1-34) data were reanalysed excluding the single female participant. Exclusion of the female participant did not elicit different results to the original analysis discussed below.

There were no statistically significant differences between the intervention and standard care groups for the primary outcome (improvement in radiological healing by two Fredericson MRI grades or more, or reduction to grade zero, 8 weeks after randomisation) or secondary outcomes (time to radiological healing, discharge from physical rehabilitation or clinical healing; proportion clinically healed; health-related quality of life; or pain). Thus, this study presents no clinical evidence of a beneficial effect of teriparatide. No subgroup analyses by BSI site or compliance to the intervention have been performed due to the small sample size.

The use of the Fredericson classification to assess initial severity of BSI and subsequent healing was selected as the method of analysis for the primary outcome as it was perceived to be objective and there was precedent for its use (Almirol *et al.*, 2016; Kijowski *et al.*, 2012), although the use of serial MRI scans to monitor healing of BSIs was novel. All MRI scans were assessed independently by two consultant musculoskeletal radiologists blind to the randomisation outcome, as per the protocol assessors were notified of between-assessor grading discrepancies and asked to agree a consensus grade. This happened on 43 occasions (44% of all scans). The number of between-assessor grading discrepancies was much higher

than expected and may have contributed to imprecise results. The Fredericson grading system was found to work well as a staging system in long bones but was less well suited to follow up, when the consultant radiologists reported heterogeneous patterns of healing, in particular differentiating evolving granulation of tissue and bone marrow oedema proving a source of repeated discrepancy. The difference between chronic oedema and new vascularisation was difficult to differentiate.

The assessors concluded the Fredericson grading system is not suited to the assessment of healing BSIs. It has previously been suggested that grade 2, 3 and 4a BSIs have similar degrees of periosteal and bone marrow oedema and similar return-time to sports activity, which may have compounded a lack of sensitivity to detect healing (Kijowski *et al.*, 2012). A more robust and precise method of assessing bone healing with increased sensitivity would be required if a similar research question were to be investigated in the future. At present there does not seem to be another validated method of assessing BSIs. CT scanning may help assess healing of complete cortical fractures but would not be able to assess marrow-related changes. Positron Emission Tomography and gamma scintigraphy techniques are not specific enough for BSI and wouldn't allow the radiologist to grade BSIs appropriately *(personal communication[3]).*

The administration method (sub-cut injection) and storage requirements (fridge) for teriparatide presented challenges in this participant cohort. Self-injection was daunting for participants and made them less likely to take part in the trial, significant training and mentoring was required especially in the early stages to maintain participants on the trial, this was difficult to provide participants were at home on sick leave. Logistically maintaining the cold-chain was difficult as participants were accommodated in 10-person dorm rooms and did not have any way to secure their medicines in a fridge.

[3] Personal communication via email James McKay to Katharine Eastman 18 Sept 23.

The self-administered daily injections of teriparatide used in the study appear to be safe. No serious adverse events or unexpected adverse reactions were reported during the trial. An independent Data Monitoring Committee, tasked with reviewing safety data throughout the trial, did not raise any safety concerns.

Differences between the treatment and standard care group were identified in the biochemistry analyses, the PTH (1-34) treated group had an elevated 1,25 $(OH)_2$ D3 compared to standard care at all time points, including baseline despite vitamin D sufficiency / insufficiency being considered as part of the randomisation protocol. As part of standard care all trial participants found to be vitamin D deficient (serum 25(OH)D levels below 30 nmol/L) were prescribed a vitamin D supplement in line with Defence policy on the 'Management of Vitamin D Deficiency'. The difference identified between the treatment and standard care group may reflect the wide range of vitamin D levels with the participants deemed to be vitamin D 'sufficient'. The higher levels of 1,25 $(OH)_2$ D3 in the treatment group may also have been due to differences in seasonal recruitment as more participants on the treatment group were recruited in the spring, compared to the standard care group who were recruited in the autumn and winter. The difference between the treatment and standard care group at later time points may have been an indication of the pharmacological activity of the PTH (1-34) as it stimulates the metabolism of 25(OH)D to 1,25$(OH)_2$D3 via the 1α hydroxylase in the kidneys.

Within the standard care group, 1,25$(OH)_2$D3 increased significantly between baseline and weeks 12 and 16, whilst remaining stable in the treatment group; again this difference may have been due to the variation in seasonal recruitment. It was unlikely to be due to difference in supplementation or differences in dietary intake since participants all ate from the same cookhouse whilst participating in the study.

As expected, the PTH (1-34) treated group had an increase in bone formation marker and by-product of collagen synthesis, P1NP, at weeks 8, 12 and 16 this is a direct result of the action of the PTH (1-34). Although P1NP had increased at 4 weeks within the PTH (1-34) treated group, this was not significant, which suggests that the kidney response (as described in Chapter 12) is more sensitive to PTH in the short term, compared to the bone response which requires the activation of different second messenger pathways and subsequently osteoblasts.

There was also a trend for increased P1NP between treatment and standard care groups, but this did not reach significance, this is likely because of the small numbers in each of the groups and a wide variability in the analytes as demonstrated by the wide confidence intervals. The wide variability may also have been in part due to adherence to treatment, as this was difficult to monitor effectively especially when participants were on leave from training.

Participants in the standard care group had lower sclerostin in week 16 compared to baseline, which may be an effect of fracture healing as sclerosteosis and diseases that result in high bone mass have little or no SOST; so it could be argued that reducing SOST may result in better bone formation via the Wnt signalling pathway (Moester *et al.*, 2010) although further investigation into the SOST response to fracture healing and PTH (1-34) stimulus is needed to draw any firm conclusions.

Further evidence of PTH (1-34) pharmacological activity was observed as the areal lumbar spine BMD significantly increased following 16 weeks of teriparatide treatment compared to control (spine BMD 2.44% vs 0.00% *P* = 0.002). There are no published data in a similar patient cohort, but data in teriparatide-treated osteoporotic men showed significant increases in areal BMD and whole body BMC at the spine after 3 and 6 months of treatment compared to placebo (Orwoll *et al.*, 2003). Increases in BMD following teriparatide treatment are greatest at trabecular sites such as the lumbar spine (Orwoll *et al.*, 2003; Neer *et al.*, 2001). Typically BMD at the lumbar spine increases by 10 to 14% following teriparatide treatment over 1 to 3 years in osteoporotic patients (Hodsman *et al.*, 2005), the shorter duration of treatment in this study may explain the smaller increases reported here although the patient cohort is also different.

Although less commonly reported than BMD, Z – scores, describe how the bone density of the individual compares to an average of an age, sex, gender and ethnicity matched reference population (Carey *et al.*, 2007; Carey *et al.*, 2009) and are a useful comparator in this cohort of young patients. At the lumbar spine, Z scores did not differ between treatment and control groups (*P* = 0.404).

A diagnosis of low bone mass for age results if the Z -score is < - 2.0. Z scores are well within the reference range for all groups at all timepoints in this study, which is expected as this cohort were physically active military recruits who had met physical occupational standards for arduous military roles, and physical activity is the strongest predictor of BMD in men at both the spine and hip (McGuigan *et al.*, 2002).

BMD at the shaft of the non-dominant neck of femur significantly increased following 16-weeks of teriparatide treatment compared to control (shaft BMD 1.46% vs 0.78% *P* = 0.048) and the total area at the non-dominant neck of femur also significantly increased with 16 weeks of teriparatide treatment compared to control (total Area -0.06% vs 0.40% *P* = 0.047).

This contrasts with findings in osteoporotic patients where BMD gains at trabecular sites appear to be achieved at the expense of cortical bone (Kurland *et al.*, 2000; Body *et al.*, 2002;

Neer *et al.*, 2001; Orwoll *et al.*, 2003). This may be an age affect as the population studied are younger than osteoporotic patient cohorts, more active and have a different diet (recruits are provided with meals from a cookhouse) provided as part of their employment, which may negate the requirement to take bone from cortical sites (Parfitt, 1976). This is an important finding as it indicates that future studies on the use of PTH 1-34 in such clinical applications should not be concerned about any decreases in BMD at the neck of femur in this population in the short term.

The 'real world' significance of these findings is questionable as the changes are small, the *p* values approaching 0.05 and they are not supported by the more precise vBMD measurements at the distal radius or distal tibia, where there was no difference in any of the measurements between treatment and control between baseline and 16 weeks. Previous studies have shown little effect of teriparatide at sites mainly comprised of cortical bone and have reported a small reduction of 1-2% in radial BMD in osteoporotic patients at the distal radius (Kurland *et al.*, 2000; Body *et al.*, 2002; Neer *et al.*, 2001; Orwoll *et al.*, 2003). The clinical significance of this reduction is less clear, but it is thought to be a combination of effects including increased endocortical remodelling, increased remodelling space within the cortical haversian systems, and an increase in measured area due to periosteal bone apposition that occur following PTH administration (Hodsman *et al.*, 2005).

The participants in the RETURN trial are unusual as they transition from a period of intense, loaded training to 2-6 weeks (duration dependent on symptoms) of non-weight bearing before they start their rehabilitation. A small study (n=12, 34.8 ± 7.7 years, 10 males / 2 females) investigating the effect of non-weight bearing at the ultra-distal tibia in patients waiting for surgery found decreased vBMD (-1.2%), and trabecular thickness (-5.4%) (both $P < 0.05\%$) in a six-week non weightbearing interval in the injured leg (Kazakia *et al.*, 2014) (RETURN scanned the uninjured for preference, non-dominant if possible). True non-weight bearing in a Military training establishment is difficult to achieve due to lifestyle differences as an example, recruits' accommodation can be 1 km from the cookhouse where they are required to attend 3 times a day for meals. Once inside the cookhouse, recruits reported they discarded their crutches to collect their food on a tray.

Physical activity levels based on estimation of sedentary time, moderate and vigorous physical activity and energy expenditure using accelerometery were not different between treatment and control groups. The hypothesis that physical activity would increase more so in the treatment than control group has not been shown. Teriparatide treatment for a BSI does not affect physical activity during 16 weeks of treatment, suggesting that teriparatide does not increase the rate that a recruit progresses through rehabilitation. The structure of the rehabilitation programme was such that there was no minimum time a recruit needed to spend in each of the levels ('earlies', 'intermediates' and 'lates'), they would progress to the next level as soon as they could achieve required testing standards. This has the potential to affect the results of the study as recruits could pass these tests whilst tolerating and not reporting pain with the aim of returning to training earlier to progress their career. This is also a possible cause of the repeat injuries that are anecdotally reported. Appendix 3 details a summary of British Army BSI Management Procedures for Infantry Recruits.

No prior data exist on the usual physical activity levels and energy expenditure of recruits undertaking rehabilitation programmes but, it was expected that physical activity would increase as participants progress through the incremental rehabilitation programme over the 16 weeks. This is not observed and whilst there is no difference in the activity between treatment and control groups, there is considerable variation at different time points. During weeks 1 – 4 following BSI diagnosis, treatment pathways do differ as some patients may be capable of starting rehabilitation straight away and others require more prolonged periods of rest. Severity of BSI and the time taken to get an MRI scan compound this variation. The heterogeneity in the accelerometer data support this.

The GENEActiv wrist-mounted triaxial accelerometer has previously been shown to correlate strongly with the gold-standard method of measuring energy expenditure—doubly labelled water—which obviates the need to obtain daily urine samples (Gifford *et al.*, 2021). While accelerometery is reported to underestimate total energy expenditure (Murakami *et al.*, 2016), the same method was used in each group.

There is a trend towards decreasing sedentary time, increasing moderate and vigorous time and energy expenditure over time for participants in both the treatment and control groups, which is expected as participants progress through the rehabilitation programme and return to normal levels of training. There is a lot of variation in these data, the cause of which is not clear; all participants were undergoing the trial at different times, so periods of leave were different. All participation in the trial took place during the COVID-19 pandemic and following a positive COVID-19 test participants were subject to quarantine periods in a single room for 10 to 14-days, again this was not concurrent for participants. Four participants had visits delayed due to COVID-19 isolation (1 intervention, 3 standard care), but unfortunately if a participants positive COVID-19 test but it did not affect a study visit it was not recorded so further analysis any associated effects could not be made.

13.6 Conclusion

This clinical trial is the largest investigating the use of teriparatide in the treatment of BSIs to date. Whether teriparatide can enhance BSI healing in young, otherwise healthy adult males remains unclear. The recruitment was much lower than anticipated, largely due to the COVID-19 pandemic. There were no clinically significant differences between treatment with 20 µg of teriparatide by SC injection daily and standard care. No sub-group analyses were performed due to small sample size. Teriparatide instigated anabolic activity both in blood biochemistry (P1NP significantly increased within the treatment group) and radiologically. Areal lumbar spine BMD increased in the treatment group, possibly at the expense of cortical bone (femur and tibia) where no changes were observed. This contrasts with findings in osteoporotic patients where early BMD gains at trabecular sites appeared to be achieved at the expense of cortical bone. There was no significant difference in physical activity between the two groups.

No serious adverse events or unexpected adverse reactions were reported during the trial and the use of teriparatide in this patient cohort appears safe. If this study were to be repeated a more sensitive accurate and reproducible method of assessing BSI healing should be investigated. Any future study should also be expanded to include a larger sample and consider males and females as separate populations.

14 Overall Discussion

14.1 Project Overview

This thesis has presented four studies that explored the risk factors for bone stress injuries (BSIs) in British Military infantry trainees, and the efficacy of recombinant parathyroid hormone (PTH (1-34)) for their treatment. Trainees in basic military training are susceptible to lower body BSIs due to the physically intense nature of their training (Blacker, Wilkinson and Rayson, 2009; Sharma *et al.*, 2015). Older age, female sex, extremes of body mass index, poor cardiovascular fitness, low muscle strength, white ethnicity, smoking and low vitamin D are all potential risk factors for injury (Neely, 1998; Burgi *et al.*, 2011; Ruohola *et al.*, 2006; Lappe, Stegman and Recker, 2001). A prospective study investigated whole body and local bone properties using dual X-ray absorptiometry (DXA), high resolution peripheral quantitative computed tomography (HR-pQCT), and biochemical markers of bone metabolism to predict BSI risk in recruits (Chapter 10). A 9-fold increased risk of BSIs was observed in the 'elite' Parachute Regiment compared with 'non-elite' Line Infantry, and no intrinsic predictive risk factors were identified, suggesting that injury propensity is likely training load and risk factors are population-specific.

Within the British Military, bone stress injury treatment is currently conservative, the current mainstay of treatment is surgical fixation, if indicated, and a programme of rehabilitation. All trainees with a BSI are withdrawn from training and undergo a period of rehabilitation; they re-enter training if injury is healed or are medical discharged from service if the injury fails to heal. Following a BSI, trainees routinely undergo rehabilitation for more than 80 days. Teriparatide (PTH (1-34)) was identified as a potential treatment to expedite and improve BSI healing, the hypothesis being treatment would reduce the amount of time trainees spent in rehabilitation, reduce their chances of being discharged from service and reduce chances of a subsequent stress fracture. Teriparatide is an anabolic drug licensed for the treatment of osteoporosis; it is not licensed for the treatment of BSIs or fracture healing but has been used for this indication in a randomised controlled pilot study (n=13) (Almirol *et al.*, 2016) and case reports (described in Appendix 4). A meta-analysis was performed to understand the current evidence for teriparatide in all-cause fracture healing (Chapter 11). Meta-analysis of

published data supported the use of PTH analogues to improve functional outcomes across a range of fracture types with no additional incidence of adverse events compared with bisphosphonates or standard care. Parathyroid hormone did not improve fracture healing rate or reduce of pain, but it was concluded that the low-quality and heterogeneity of trial designs justified further investigation as there was no evidence that PTH treatment caused harm or impeded fracture healing. As a result, a randomised controlled clinical trial was developed to investigate the use of teriparatide within British Military infantry trainees, the first using teriparatide for treatment of BSI in military personnel (Chapter 13).

This clinical trial - Efficacy of Teriparatide Use in the Return of Recruits to Normal duty (RETURN) - was hosted at the Infantry Training Centre (ITC) Catterick, which specialises in the provision of Ground Close Combat training and reports the highest number of BSIs within the British Army. Due to its nature, Ground Close Combat training has a well-documented risk of BSIs which are more prevalent in females undertaking this training (HQ Army, 2016). Females were not expected to be recruited into RETURN in high enough numbers significant enough to enable meaningful sub-group analysis. Therefore, a further study, Pharmacokinetic and Pharmacodynamic Analysis and Evaluation of Teriparatide (PTH 1-34) Formulations Between Sexes (PHAB) (Chapter 12) was performed to investigate the sex differences in pharmacokinetics and pharmacodynamics in a population matched to a military population in training, thus enabling the results of the RETURN trial to be extrapolated to females and inform Defence treatment policy for BSIs.

14.2 Key Findings

14.2.1 Bone Stress Injury Associations with Bone Geometry and Biochemical Markers of Bone Metabolism

British Military infantry trainees who suffered a BSI during basic military training had no pre-injury differences in demographics, body composition, physical performance, or biochemistry compared with their peers who did not go on to suffer a BSI. Cortical area ($P = 0.029$), stiffness ($P = 0.012$), and estimated failure load ($P = 0.011$) were significantly lower in BSI cases compared with controls.

In binary logistic regression analysis, training regiment was the only variable associated with BSI incidence ((OR 9.3 [95%CI, 2.6, 33.4]) Parachute *versus* Line Infantry, $P \leq 0.001$) when training course, age at start of military training, total body mass, lean body mass, height, leg aBMD, and total 25(OH)D were considered. Adding physical performance (2.4-km run time, maximum dynamic lift strength, and peak power output) (Model 2) identified both training course and 2.4-km run time as associated with BSI incidence; for every 1 second increase in run time, there was a 5.5% increase in BSI risk (1.06 [95%CI, 1.02, 1.10), $P \leq 0.04$). Adding total area, cortical vBMD, trabecular thickness and cortical pore diameter (Model 3), did not change these findings; training course and 2.4-km run time remained the only associated factors.

Exploratory analysis of parachute regiment trainees as a sub-group found uninjured participants had significantly higher total lean mass ($P = 0.025$) and leg aBMD ($P = 0.012$) compared with BSI cases.

14.2.2 Efficacy of Parathyroid Hormone in Fracture Healing: A Meta-Analysis

Eleven studies were met the inclusion criteria and were included in the meta-analysis which included 1,452 patients, 91.8% (n=1,333) of whom were females. The mean age of patients was 72 years, one study, Almirol *et al.* evaluated PTH analogues for lower extremity stress fractures in young female adults (n=13) with a mean age of 32 (± 5.8) years and 31 (± 3.4) years in treatment and control groups, respectively. Fracture sites in the included studies

were vertebrae (n=789, 3 trials) (Shigenobu *et al.*, 2019; Zhao *et al.*, 2016b; Hadji *et al.*, 2012), femur (atypical) (n=13, 1 trial) (Greenspan *et al.*, 2018), hip (n=343, 4 trials) (Malouf-Sierra *et al.*, 2016; Chesser *et al.*, 2016; Bhandari *et al.*, 2016; Kanakaris, West and Giannoudis, 2015), tibia (n=13, 1 trial) (Almirol *et al.*, 2016), humerus (n=40, 1 trial) (Johansson, 2016) and radius (n=102, 1 trial) (Aspenberg *et al.*, 2010).

Radiological assessment of fracture healing was used in nine trials and was the primary outcome in three trials (Greenspan *et al.*, 2018; Johansson, 2016; Aspenberg *et al.*, 2010). Four trials used plain film radiographs (Greenspan *et al.*, 2018; Malouf-Sierra *et al.*, 2016; Bhandari *et al.*, 2016; Johansson, 2016) and one trial used magnetic resonance imaging (MRI) (Almirol *et al.*, 2016). Two trials used a combination of X-Ray and computed tomography (CT) (Aspenberg *et al.*, 2010; Kanakaris, West and Giannoudis, 2015). Kanakaris *et al* not describe the radiological method used (Kanakaris, West and Giannoudis, 2015).

The criteria used to grade the scans and the detail to which it was described differed greatly between the articles. Radiological healing was categorised as two outcomes — fracture healing rate (healing at set time points) and fracture healing time (days). There was no difference in the fracture healing rate when PTH analogues were compared with comparator and control groups (OR 0.96, 95% CI 0.57 to 1.61, $P = 0.87$).

Pain was reported as an outcome in nine of the 11 trials, eight of which used an 11-point visual analogue scale (VAS) (0 = no pain, 10 = greatest pain imaginable) (Malouf-Sierra *et al.*, 2016; Chesser *et al.*, 2016; Zhao *et al.*, 2016b; Bhandari *et al.*, 2016; Johansson, 2016; Hadji *et al.*, 2012; Aspenberg *et al.*, 2010; Shigenobu *et al.*, 2019). Two trials (n=78) published sufficient data for meta-analysis (Chesser *et al.*, 2016; Zhao *et al.*, 2016b). PTH analogue-treated groups reported less pain compared with comparator / controls in the trials that were suitable for meta-analysis (MD -4.55, 95% CI -7.47 to -1.63, $P = 0.002$).

Nine trials reported functional outcomes (Malouf-Sierra *et al.*, 2016; Shigenobu *et al.*, 2019; Chesser *et al.*, 2016; Zhao *et al.*, 2016b; Bhandari *et al.*, 2016; Johansson, 2016; Kanakaris, West and Giannoudis, 2015; Hadji *et al.*, 2012; Aspenberg *et al.*, 2010), and three of these

used multiple assessment methods (Shigenobu *et al.*, 2019; Chesser *et al.*, 2016; Hadji *et al.*, 2012). In meta-analysis, the trials that published sufficient data showed a statistically significant improvement in functional outcome for participants treated with PTH analogues (MD -1.59, 95% CI -1.97 to -1.21, $P \leq 0.00001$).

Three trials reported biochemical markers of bone formation and resorption (Almirol *et al.*, 2016; Shigenobu *et al.*, 2019; Zhao *et al.*, 2016b). All trials demonstrated significant increases in serum N-terminal propeptide of type I procollagen (P1NP) following PTH analogue treatment vs placebo (Almirol *et al.*, 2016) and vs bisphosphonates (Zhao *et al.*, 2016b; Shigenobu *et al.*, 2019), and no significant change in CTX, resulting in a greater anabolic window. Insufficient data on biochemical markers of bone turnover were included for meta-analysis.

Eight trials reported adverse events, and five (1182 patients) provided enough data for meta-analysis. There was no statistical difference between the PTH analogue treated groups and comparators / controls (OR 0.74, 95% CI 0.45 to 1.02, $P = 0.07$). Four trials (1023 patients) reported treatment discontinuation of PTH analogues as an outcome measure, there was no statistical difference between PTH analogue treated groups and comparators / controls (OR 1.13, 95% CI 0.72 to 1.77, $P = 0.58$).

Sub-analysis was performed on the comparator groups as six trials used placebo (Almirol *et al.*, 2016; Bhandari *et al.*, 2016; Aspenberg *et al.*, 2010) or standard care (Greenspan *et al.*, 2018; Chesser *et al.*, 2016; Johansson, 2016) and four used a bisphosphonate (Shigenobu *et al.*, 2019; Malouf-Sierra *et al.*, 2016; Zhao *et al.*, 2016b; Hadji *et al.*, 2012). One trial had both a standard care arm and a bisphosphonate arm so was included in both analyses (Kanakaris, West and Giannoudis, 2015), findings were consistent with the overall evaluations. Meta-analysis of published data supports the use of PTH analogues to improve functional outcomes across a range of fracture types with no additional incidence of adverse events compared with bisphosphonates and standard care. PTH did not improve fracture healing rate or reduce pain but no evidence that PTH treatment causes harm or impeded fracture healing or impedes fracture healing. Prior to the work presented in this thesis, only one pilot study considering

the use of teriparatide in stress fracture healing had been performed in a young patient cohort (Almirol *et al.*, 2016). The duration of treatment in this study was 8 -weeks and the authors concluded that optimum duration of treatment for stress fractures exceeded this. The finding of this meta-analysis supported further investigation of PTH analogues, particularly teriparatide in the treatment of stress fractures in military personnel given the potential benefits of reducing the time spent in rehabilitation for all military personnel diagnosed with stress fractures.

14.2.3 Pharmacokinetic and Pharmacodynamic Analysis and Evaluation of Teriparatide (PTH 1-34) Formulations Between Sexes: The PHAB Study

Thirty-three participants completed the PHAB study with a further three providing complete day one data. Their overall age was 22.2 [7.68] years. Males were older and had greater BMI's than females (P = 0.026 and P = 0.022 respectively). There was no significant difference in alcohol intake, smoking or ethnicity between groups.

The concentration over time (assessed by the Area Under the Curve) of teriparatide following a single 20 µg dose was higher in males compared to females (22385.0 [17554.0 to 27216.0] vs 14078.0 [9817.3 to 18339.0], P = 0.016) although there was no difference in the maximum concentration (C_{max}) to time of maximum concentration (T_{max}).

Following the same single 20 µg dose of teriparatide, the area under the curve from time zero to last measurable concentration (AUC_{0-t}) of plasma cyclic Adenosine Mono Phosphate (cAMP) was significantly lower in females (P = 0.007), as were the albumin adjusted calcium measurements over time (P = 0.0079). There was no statistical difference in ALP (P = 0.1046) and serum PO_4 (P = 0.4552) between males and females.

Urinary cAMP (a combination of nephrogenous plus filtered plasma load) was significantly higher in females compared to males ($P \leq 0.001$). Females have significantly lower urine Ca (P = 0.009) and PO_4 concentrations ($P \leq 0.001$). These results remained unchanged when the PO_4 excretion was corrected for creatinine. This suggests greater second messenger generation

but lesser end organ response. Females appear less responsive to PTH (1-34) stimulus as they conserve Ca and PO_4, but further investigation is required to elucidate the exact mechanism.

A second 20µg dose of teriparatide was administered after a 24-hour washout period (day 3 of experimental testing visit), there were no statistically significant differences for any of the analytes, examined. There was no difference between CTX and P1NP either between sexes or between day one (baseline) and three (approx. 46 hours post first dose), this was probably because there was too little PTH administered (one dose) to elicit a response, as shown in RETURN, P1NP increases were only seen after 8 weeks of treatment with PTH (1-34).

No severe adverse events were reported during the study. Three adverse events were reported following teriparatide dosing, one injection site reaction, one report of dizziness and one vasovagal episode. All adverse events were self-limiting and resolved before the participant left the clinical research facility. The participant experiencing the vasovagal episode was withdrawn from the study.

14.2.4 Efficacy of Teriparatide Use in the Return of Recruits to Normal duty (RETURN): A Randomised Controlled Trial on the Treatment for Bone Stress Injuries

Thirty-four Army Infantry recruits (33 males, 1 female) with one or more lower body BSIs voluntarily participated in the RETURN study. 17 participants were randomly allocated to receive Army standard care, and 17 participants were randomly allocated to receive teriparatide in addition to Army standard care. Twenty-six participants reached the primary endpoint.

Analysis of the primary outcome, improvement in radiological healing - assessed using Fredericson grading of MRI, by two grades or more, or reduction to grade zero - 8 weeks after randomisation and treatment initiation favoured the standard care group, however, this was not statistically significant (OR: 0.590, 95% CI: 0.071, 4.350; *P* = 0.829). Similarly there was a trend towards the standard care group being discharged from rehabilitation earlier, but again this was not statistically significant (ratio of geometric means: 1.037, 95% CI: 0.723, 1.489; *P* = 0.834). As per the protocol assessors (two consultant radiologists) were notified of

between-assessor grading discrepancies and asked to agree a consensus grade, between-assessor grading discrepancies were much higher than expected (44% of all scans). The assessors reported heterogeneous patterns of healing, in particular differentiating evolving granulation of tissue and bone marrow oedema as the source of repeated discrepancy.

In the treatment arm, there was a trend toward improved time to radiological healing (index fracture MRI grade zero) (OR: -1.022, 95% CI: -3.553, 1.510; *P* = 0.411) and improved clinical healing (adjusted difference: -1.101, 95% CI: -4.437, 2.235; *P* = 0.486).

There was no difference in health-related quality of life (SF-36v2 total score) (ratio of geometric means: 1.012, 95% CI: 0.549, 1.867; *P* = 0.968) or pain scores (ratio of geometric means: 0.898, 95% CI: 0.277, 2.915; *P* = 0.853) between the two arms.

Adverse events were reported by 14 participants (82%) in the intervention group and 14 participants (82%) in the standard care. No serious adverse events or unexpected adverse reactions were reported during the trial.

Spine BMD increased significantly in the treatment group compared to control over 16 weeks (*P* = 0.002), although, there was no change in BMC, Z-score or total area at the spine over 16 weeks. At the non-dominant neck of femur, shaft BMD and total area increased significantly in the treatment group compared to control over 16 weeks (*P* = 0.048 and *P* = 0.047 respectively). Bone geometry and microarchitecture did not significantly change at the radius or tibia between treatment and control groups following 16 weeks of treatment.

14.4 Synthesis of Findings

This thesis set out to investigate BSIs in basic military training and the potential role of Recombinant Parathyroid Hormone (1-34) in their treatment. Within this British Miliary trainee cohort, intrinsic risk factors, including ultradistal tibial density, geometry, and microarchitecture, were not associated with lower body BSI during arduous infantry training. The 9-fold increased risk of BSIs in the Parachute Regiment compared with Line Infantry suggests that training type dominates all other previously reported risk factors for BSI, other than female sex although, females were not eligible to undertake infantry training at the time of the investigations described in Chapter 10, so this risk is less well understood in Infantry cohorts. The use of PTH analogues to improve fracture healing across a range of fracture types was reviewed by meta-analysis, which supported the use of PTH analogues to improve functional outcomes across a range of fracture types with no additional incidence of adverse events compared with bisphosphonates and standard care. The hypothesis that PTH improves fracture healing rate or reduction of pain was not proven, but the low-quality and heterogeneity of trial designs justifies further investigation as there is no evidence that PTH treatment caused harm or impeded fracture healing. Only one of the studies included in this review considered stress fractures, which was in a young female patient population (Almirol *et al.*, 2016). These findings supported the further investigation of PTH analogues, particularly teriparatide in the treatment of stress fractures in military personnel given the potential benefits of reducing the time spent in rehabilitation for all military personnel diagnosed with stress fractures. The pilot data from Almirol *et al*. 2016 was used as the basis of the power calculation for the RETURN trial (Chapter 13), however, the results of the PHAB study (Chapter 12) cast doubt on the appropriateness of this approach since females have a different response to PTH administration compared with males.

The urinary response to PTH stimulus in healthy females when compared to males is analogous to that of pseudohypoparathyroid type two patients, who have a greater cAMP response but a poorer phosphate response to PTH. The diagnosis of pseudohypoparathyroid disease is made by monitoring the total phosphate output in response to PTH; in type one pseudohypoparathyroidism, there is no cAMP or phosphaturic response however in type two pseudohypoparathyroidism a cAMP response is seen but no phosphaturic response

(Mantovani *et al.*, 2018). The findings of this study, although carried out in healthy participants may have implications for the future research and treatment of hypoparathyroid patients. The majority of hypoparathyroid patients are female (Powers *et al.*, 2013), and a greater percentage of patients might be getting a poorer response from treatment. In RETURN, no differences were identified in tolerability between the two groups which supports further study, and males and females must be considered separately in future work. The effects of age should also be explored; the majority of work presented in this thesis is in young participants (all < 36 years), a hypoparathyroid patient cohort will include younger patients, where an osteoporosis cohort would be older. There may also be a difference in response to teriparatide between pre– and post– menopausal females due the different hormone milieu.

Until 2016, there was a ban on women serving in close combat roles within the British Military, when this was lifted, recruitment of women into these roles was slow initially, which is reflected in the low uptake of women into RETURN. The trial steering committee took the decision to close RETURN early in 2022 due to lack of recruitment. The lack of recruitment was a direct result of a reduction in BSI rate. There were two main reasons for this, British Army recruitment was reduced, so the throughput of trainees was lower than expected and all training at Infantry Training Centre Catterick was stopped in March 2020 when the UK went into the first lockdown of the COVID-19 pandemic, on return to training BSI rates were much lower than previously reported. The lower BSI rates were attributed to the changes and mitigations implemented to re-start training whilst the COVID-19 pandemic was ongoing, this including periods of isolation following a positive COVID-19 test (which was common). This built in 10 to 14-day rest periods into the training schedule giving recruits the opportunity to rest and recover and as a result overuse injury rates (including BSIs) were reduced. The low number of participants completing RETURN made it unlikely that it would elucidate any clinically significant findings (Chapter 13). There is evidence that teriparatide treatment instigates anabolic activity both in blood biochemistry (P1NP significantly increased within the treatment group) and radiologically at the spine and neck of femur at 16 weeks, which suggests teriparatide has utility in the treatment of BSIs, although no changes were observed in cortical bone where BSIs typically develop in recruits. This is supported by the findings of

the meta-analysis (Chapter 11) which found PTH improved functional outcomes across a range of fracture types.

Teriparatide appears to be having an anabolic effect on the axial skeleton in trabecular bone as an increase in aBMD was observed at the spine. The effect of teriparatide in the appendicular regions of the skeleton, which are predominantly cortical bone, is less clear. No changes were observed at the ultra -distal radius or tibia although small increases were seen in the aBMD of shaft and total area of the neck of femur. This contrasts with findings in osteoporotic patients for whom teriparatide induced BMD gains at trabecular sites are associated with losses at cortical sites (Kurland *et al.*, 2000; Body *et al.*, 2002; Neer *et al.*, 2001; Orwoll *et al.*, 2003).

This may be an age affect as the population studied are younger, more active and have meal provided as part of their employment, which may offset the redistribution of bone from cortical to trabecular sites (Parfitt, 1976). Future studies on the use of PTH 1-34 in such clinical applications should not be concerned about any decreases in BMD at the neck of femur or tibia in this population in the short term.

14.5 Future Work

This thesis has demonstrated that bone stress injuries continue to be difficult to predict and manage in military populations. Young healthy males undertaking the most intense military training are particularly susceptible. Further work should be undertaken to evaluate BSI risks within this cohort. Associations with bone geometry and biomechanical markers of bone metabolism within a female cohort should also be investigated. In addition to the parameters considered in Chapter 10, the difference in hormone milieu experienced as part of the menstrual cycle and type (if any) of contraception should also be considered.

Teriparatide remains a promising therapy for the treatment of BSIs in young exercising adults but further study is required to elucidate its true value, the findings of the PHAB study (Chapter 12) show a different pharmacodynamic response (in Ca and PO_4) by females compared to males in response to a single dose of 20 µg of teriparatide suggesting that teriparatide may be more effective in females compared to males. Any future work investigation the use of teriparatide in BSI's should consider males and females separately.

The PHAB study focused on the Ca, PO_4 and cAMP related responses to PTH, measurement of other responses including the response of G-protein coupled receptors, conformational changes that take place on these receptors and intracellular calcium changes and their contribution to the differences in response were beyond the scope of this study. Future work to elucidate the exact mechanism of the different pharmacodynamic responses to PTH by males and females should examine intracellular calcium and kinase responses, especially in bone. This could potentially include taking kidney and bone cells from male and female animal models and comparing the second messengers intracellularly.

The results reported in the RETURN trial (Chapter 13) in this trial provide data for a more robust power calculation, however the number required is likely to be bigger than the original target for RETURN. If this study is to be undertaken in the British Army, it will require multiple sites to ensure that target recruitment is met. Further work is also required to establish the optimum duration of treatment, which probably exceeds 8 weeks. The development of oral

(Ish-Shalom *et al.*, 2015; Ish-Shalom *et al.*, 2021) and long-acting formulations (Zhao *et al.*, 2019; Bi *et al.*, 2016; Shimizu *et al.*, 2016) of PTH analogues will introduce new anabolic therapies that may be more user friendly in the Army environment and result in better responses to treatment. Where possible future investigations of teriparatide in this patient cohort should consider oral formulations of teriparatide.

Throughout this work, vitamin D supplementation was given in line with current Army policy at the time, in RETURN (Chapter 13) recruits were issued 800iU of vitamin D per day but anecdotally adherence was limited. Recruits presenting with a BSI had their vitamin D concentrations reviewed and were prescribed high dose supplementation if found to be deficient. Calcium supplementation was not given at any point. Given the well documented role of calcium and vitamin D supplementation in the prevention of bone stress injuries (Lappe *et al.*, 2008; Tenforde *et al.*, 2010; Knechtle *et al.*, 2021), the effect of supplementation in healing should also be investigated. This may be of particular significance in male BSI sufferers if being treated with teriparatide as the PHAB results (Chapter 12). PHAB found that males excreted more calcium and phosphate following a dose of teriparatide than females.

During the performance of this work other pharmacological agents have been developed for the treatment of osteoporosis which may have utility in the treatment of BSI's. One agent that may be of use is sclerostin inhibitor romosozumab, as this agent is relatively new there is no data supporting its role in BSI, but as it promotes bone remodelling processes, mechanistically it shows promise. The dosing schedule (monthly SC injections) would also be easier to administer and more palatable to patients.

The lack of reliability of inter-radiologist grading of BSIs using the Frederickson scale, both at the point of diagnosis and to grade healing was a key draw back of the RETURN trial (Chapter 13). Development of robust method to diagnose BSI's and subsequently evaluating their healing in such a way that enables grading of granulation of tissue and bone marrow oedema and removes the rate of discrepancies that were seen in the RETURN trial is key before further evaluation of any intervention into stress fractures is undertaken.

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16 Appendix 1: MODREC Approved Consent Forms for Studies Reported in Chapters 9, 10, 11 and 12.

16.1 MODREC Approved Consent Form for Intra Operator Reliability of High Resolution Peripheral Quantitative Computed Tomography and Dual Energy X-Ray Absorptiometry Scans Study (METHODS).

CONSENT FORM

Title of Study : Intra-operator reliability of, and sex differences in, measurements of bone macro- and microstructure: Sex differences in bone strength index, and the relationship with bone size, shape and density.

MoDREC Reference : 982/MoDREC/19

Please Initial or Tick Boxes

- The nature, aims and risks of the research have been explained to me. I have read and understood the Information for Participants and understand what is expected of me. All my questions have been answered fully to my satisfaction. ☐
- I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately without having to give a reason. I also understand that I may be withdrawn from it at any time, and that in neither case will this be held against me in subsequent dealings with the Ministry of Defence. ☐
- I understand that if I withdraw, any data already provided by me will be used to help with certain aspects of the study, unless specifically requested otherwise. ☐
- I understand that the screening process to decide if I am suitable to be selected as a participant will include completing a medical screening questionnaire (including pregnancy status) and I consent to this. ☐
- I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 2018. ☐
- I agree to volunteer as a participant for the study described in the information sheet and give full consent. ☐
- The consent given here is specific to this study, as described in the Information for Participants, and shall not be taken to imply my consent to participate in any subsequent study or deviation from that detailed here. ☐
- I understand that in the event of my sustaining injury, illness or death as a direct result of participating as a volunteer in Ministry of Defence research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached. ☐
- I understand the compensation arrangements that have been provided. ☐

- OPTIONAL I consent to photographs/ recordings of the Study procedures and equipment, and understand that I will not be identufable. This might include (but is not limited to), the right to use them in their printed and online publicity, social media, press releases and funding applications ☐

Participant's Statement :

I .....

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information for Participants about the project, and understand what the research study involves.

Signed : **Date :**

Investigator's Statement :

I .....

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the Participant.

Signed : **Date :**

Authorising Signatures

The information supplied above is to the best of my knowledge and belief accurate. I clearly understand my obligations and the rights of research participants, particularly concerning recruitment of participants and obtaining valid consent.

Signature of Chief Investigator

..... **Date :**

Name and Contact Details of Independent Medical Officer:
Dr James Lyon
Tel: 01276 412234
Email: James.Lyon892@mod.gov.uk
Deputy Senior Medical Officer, Royal Military Academy Sandhurst

Name and Contact Details of Chief Investigator :
Dr Thomas O'Leary
Tel: 01264 887644
Email: Thomas.OLeary100@mod.gov.uk
Higher Scientific Officer, Women in Ground Close Combat, Army Headquarters

16.2 MODREC Approved Consent Form for Pharmacokinetic and Pharmacodynamic Analysis and Evaluation of Teriparatide (PTH 1-34) Between Sexes (PHAB).

Date and Version 30/04/2021 – Version 1.2

2017/MODREC/21 IRAS:245407

Participant Identification Number for This Trial:

Participant Hospital Identification Number:

Consent Form

Study Title: **P**harmacokinetic and P**H**armacodynamic **A**nalysis and Evaluation of Teriparatide (PTH 1-34) **B**etween Sexes

Short Title: PHAB

Name of Researcher: Katharine Law

Initial Each Box

1. I confirm that I have read and understood the information sheet dated 30/04/2021, version 1.2 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered to my satisfaction. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that the screening process to decide if I am suitable to be selected as a participant may include completing a medical screening questionnaire and/or a physical examination by a medical officer and I consent to this. ☐
4. I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the General Data Protection Regulation (GDPR) (EU) 2016/679 and the Data Protection Act 2018. ☐
5. I understand that relevant sections of data collected during the study, may be looked at by responsible individuals from the Norfolk and Norwich University NHS Foundation Trust or regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my research records. ☐
6. I understand that if I withdraw, or am withdrawn, from the study, all data collected up to the date of withdrawal will be retained for use in the study. ☐
7. I give permission for a copy of this consent form to be sent to, and kept confidentially and securely at the University of East Anglia. ☐

Completed informed consent for filing instructions;
1 copy to participant
1 copy to site master file
1 copy to trial master file (Prof. Fraser at UEA)

8. I agree to my GP/medical team being informed of any serious findings about my health and wellbeing and agree to provide their details for this purpose. ☐

9. I understand that in the event of my sustaining injury, illness or death as a direct result of participating as a volunteer in Ministry of Defence research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached. ☐

10. I understand the compensation arrangements that have been provided. ☐

11. I give permission for my contact details to be shared with members of the local research team and agree that they can contact me to schedule my follow-up visits. ☐

12. OPTIONAL: I agreed for my anonymised individual patient data being published open source for the benefit of others (e.g. on ClinicalTrials.gov). ☐

13. OPTIONAL: I agree for my samples to be stored securely in an anonymous form for use in future studies pending ethical approval. ☐

14. OPTIONAL: I wish to receive trial updates via email during my participation in the above referenced study. ☐

Participant's Statement:

I .....

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information for Participants about the project. I understand what the research study involves and give my full informed consent to participate.

Signed: **Date:**

Investigator's Statement:

I .....

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the Participant.

Completed informed consent for filing instructions;
1 copy to participant
1 copy to site master file
1 copy to trial master file (Prof. Fraser at UEA)

Signed: **Date:**
Authorising Signatures

The information supplied above is to the best of my knowledge and belief accurate. I clearly understand my obligations and the rights of research participants, particularly concerning recruitment of participants and obtaining valid consent.

Name and Contact Details of Principal Investigator:

Professor William Fraser

Address: University East Anglia, Office 2.31, Bob Champion Research & Education Building, Norwich. NR4 7TJ.

Email: W.Fraser@uea.ac.uk

Tel: 01603 59 7174

If you have concerns about the conduct of this research or the care you received in the Norwich Clinical Research Facility please contact the Patient Advice and Liaison Service (PALS) on 0800 073 0741 or you can visit the PALS website at: http://www.pals.nhs.uk/

Completed informed consent for filing instructions;
1 copy to participant
1 copy to site master file
1 copy to trial master file (Prof. Fraser at UEA)

16.3 MODREC Approved Consent Form for Association of Vitamin D and Iron Status with Injury Risk, Health and Physical Performance in British Army Recruits (ALLIPP Study

CONSENT FORM FOR PARTICIPANTS IN RESEARCH STUDIES

Title of Study: Association of Vitamin D and Iron status with Injury Risk, Health and Physical Performance in British Army Recruits

Ministry of Defence Research Ethics Committee Reference: 165/Gen/10

Please initial each box

- **The nature, aims and risks of the research have been explained to me. I have read and understood the Information for Participants and understand what is expected of me. All my questions have been answered fully to my satisfaction.** ☐
- **I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately without having to give a reason. I also understand that I may be withdrawn from it at any time, and that in neither case will this be held against me in subsequent dealings with the Ministry of Defence.** ☐
- **I understand that the lifestyle and dietary process to decide if I am suitable to be selected as a participant may include completing a medical lifestyle and dietary questionnaire and/or a physical examination by a medical officer and I consent to this.** ☐
- **I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.** ☐
- **I agree to volunteer as a participant for the study described in the information sheet and give full consent.** ☐
- **This consent is specific to the particular study described in the Information for Participants attached and shall not be taken to imply my consent to participate in any subsequent study or deviation from that detailed here.**
- **I understand that in the event of my sustaining injury, illness or death as a direct result of participating as a volunteer in Ministry of Defence research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached.** ☐

Participant's Statement:

I _____

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information for Participants about the project, and understand what the research study involves.

Signed **Date**

Witness **Name**

Signature

Investigator's Statement:

I _____

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the Participant.

Signed **Date**

AUTHORISING SIGNATURES

The information supplied above is to the best of my knowledge and belief accurate. I clearly understand my obligations and the rights of research participants, particularly concerning recruitment of participants and obtaining valid consent.

Signature of Chief Investigator

…… **Date**

Chief Investigator:

Dr Julie Greeves

Department of Occupational Medicine

HQ Army Recruiting and Training Division
Upavon, Wilts
SN6 9BE
Email: ARTD-OccMed-SSO (Greeves Julie, SSO) Tel: 94344 8193

Independent Medical Officer(s):

Infantry Training Centre Catterick
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Tel: 01748 872610

Army Training Centre Pirbright
Col H Goshai Medical Officer
Alexander Barracks
Tel: 01483 798251

Royal Military Academy Sandhurst
Col Bruce Baker
Senior Medical Officer
RMAS
Tel: 01276 412234

16.4 MODREC Approved Consent Form for Research on Efficacy of Teriparatide Use in the Return of Recruits to Normal Duty (RETURN).

ASC
ANALYSIS SUPPORT CONSTRUCT

NORWICH CTU

PARTICIPANT CONSENT FORM

Research on Efficacy of Teriparatide Use in the Return of recruits to Normal duty

(The* RETURN *Project)

Ministry of Defence Research Ethics Committee Reference : 932MoDREC18

	Please Initial Boxes
1) The nature, aims and risks of the study have been explained to me. I have read and understood the Participant Information Sheets and understand what is expected of me. All my questions have been answered fully to my satisfaction.	☐
2) I understand that if I decide at any time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately without having to give a reason. I also understand that I may be withdrawn from it at any time, and that in neither case will this be held against me in subsequent dealings with the Ministry of Defence.	☐
3) I understand that the screening process to decide if I am suitable to be selected as a participant may include completing a medical screening questionnaire and/or a physical examination by a medical officer and I consent to this.	☐
4) I consent to the processing of my personal information for the purposes of this research study. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the General Data Protection Regulation (GDPR) (EU) 2016/679 and the Data Protection Act 2018.	☐
5) I understand that relevant sections of data collected during the study, may be looked at by responsible individuals from Norfolk and Norwich University Hospital NHS Trust, Norwich Clinical Trials Unit, or regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my health records.	☐
6) I understand that if I withdraw, or am withdrawn, from the study, all data collected up to the date of withdrawal will be retained for use in the study.	☐

ASC
ANALYSIS SUPPORT CONSTRUCT

NORWICH CTU

7) I agree to provide researchers with my MOD Number so they can access my physical fitness and medical records during my active participation in the study and for 5 years afterwards. ☐

8) I give permission for a copy of this consent form to be sent to, and kept confidentially and securely by the Norwich Clinical Trials Unit. ☐

9) I agree for the research team to notify my General Practitioner and Chain of Command of my participation in this study. ☐

10) I agree to my medical team being informed of MRI scan date and grade, and of any serious findings about my health and wellbeing. ☐

11) This consent is specific to the particular study described in the Participant Information Sheets attached and shall not be taken to imply my consent to participate in any subsequent study or deviation from that detailed here. ☐

12) I understand that in the event of my sustaining injury, illness or death as a direct result of participating as a volunteer in Ministry of Defence research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached. ☐

13) I understand the compensation arrangements that have been provided. ☐

14) I give permission for my contact details to be shared with members of the local research team and agree that they can contact me to schedule my follow-up visits. ☐

15) I agree for my samples to be stored securely in a linked-anonymised form for use in future studies pending ethical approval. ☐

16) OPTIONAL: I am happy to wear an accelerometer during my participation in the trial, so my activity levels can be monitored. ☐

17) OPTIONAL: I wish to receive trial updates via email during my participation in the above referenced study. ☐

Participant's Statement:

I .....

agree that the research project named above has been explained to me to my satisfaction and I agree to take part in the study. I have read both the notes written above and the Information for Participants about the project. I understand what the research study involves and give my full informed consent to participate.

Signed: **Date:**

Witness **Name:**

Signature:

Date:

Investigator's Statement:

I .....

confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the Participant.

Signed: **Date:**

Additional statement (required for consent taken remotely):

I .....

confirm that I verbally received the Participant's consent on the _____ (insert date when consent was undertaken remotely).

Signed: **Date:**

Authorising Signatures

The information supplied above is to the best of my knowledge and belief accurate. I clearly understand my obligations and the rights of research participants, particularly concerning recruitment of participants and obtaining valid consent.

Name and Contact Details of Independent Medical Officer:

Dr David Hindmarsh

Email: david.hindmarsh632@mod.gov.uk

Tel: 01748 873345

Name and Contact Details of Chief Investigator:

Professor William Fraser

Address: University East Anglia, Office 2.31, Bob Champion Research & Education Building, Norwich. NR4 7TJ.

Email: W.Fraser@uea.ac.uk

Tel: 01603 59 7174

17 Appendix 2: Transcript of the Potential Participant Focus Group to aid the Development of the RETURN Protocol

Teriparatide in Stress Fracture Focus Group Transcript

Project: Analysis and Evaluation of the Efficacy of Parathyroid Hormone for Stress Fracture Healing During Military Training

Date: 24/04/2018, 19:00-20:00

Venue: Roger Banister Conference Room, Sportspark, UEA

Facilitator: Nick Leavey

Notes: Helen Risebro

Participants: A, B, C, D, E, F, G, H (8 in total)

Welcome and Ground Rules (start)

Nick Leavey

Introductions (1:49)

C: I'm about to graduate, studying physical education. I did an access course to get here so I'm a mature student, I'm 29 and I've previously done 6.5 years service in the army beforehand.

D: I'm 3rd year studying nursing, I was a track athlete before *(inaudible)*

G: I'm a medical student but I also work as a recruit commander (*inaudible) o*n the reserve unit.

B: I'm an athlete and I'm the fastest man in ____ (local area)!

F: I'm second year English literature student and I'm an athletics student as well. Did it in school and now its nice to be back.

A: I'm in my fourth year. I did a Foundation year as well so I'm actually 3rd year biological sciences. I too sprint on the track.

E: I'm E and I'm first year PE student, I do athletics and hoping to become a PE teacher when I graduate.

H: I'm H, foundation student in computer science and I do athletics too.

PowerPoint Presentation by Nick Leavey - Questions (3:35)

A (11:39)**:** *Inferred: Do they know which group they are in?*

B (12:30): What is the incentive for people to take part in the study?

B?C? (13:00)**:** You don't get paid? <<Nick L explains>> but there's no payment.

A? (16:07): Out of the people who are actually receiving the PTH, are they all actually receiving the same dosage of PTH?

Nick L (16:40): Has anyone had an MRI before? (yes: A raised hand)

Description of Military Life/Training - prior to group discussion (20:00)

Nick L: Before we get into some group discussions, we're really lucky obviously that we have got Nick and G here who have actually got some experience of being in the military and what it's like to go through that phase 1 training. Not to put you guys on the spot, but if you wouldn't mind, for the benefit of everyone else in the room, if you could give a brief description of what it's actually like to go through that training process – what a general day might look like, what might happen if you get an injury and what might be expected of you in that circumstance?

C (20:30): Yeah umm...A general day is kind of like hell really. You're up at sort of 4:30 every morning making your bed and you're eating your food really quickly and you're doing continuous exercise, and weapons drills, and you're out in all sorts of weather. And it's basically a kind of 'keep up' environment otherwise you're continuously gonna drop back. I think this is why this study is actually quite important, because it will play on people's minds a lot. There's a term called "back squadders" - so if you do get injured or get a stress fracture, that's the last thing you want to do because it could put you in a medical military limbo where you're in rehab for 2 or 3 months whilst you see all your friends pass out through basic training. So it's tough, it's 14 weeks of continuous arduous exercise, it gets harder every single week and the weight gets a lot harder and heavier that you're carrying. So yeah, if you don't keep up, you're behind at all times really.

Helen: You said was it "back squad"?

C: Yeah "back squadder". Each week you go through, all your bosses and your superiors look at you as a single person out of a troop of 40 men or 40 women and they'll determine whether you should go through to the next stage, so whether you're fit enough, strong enough, fast enough. Whether you can accurately fire a weapon, if you can't do everything they're asking each week then you'll go back, including injury. If they think you're limping in runs they'll drop you back 3 or 4 weeks.

Nick L (22:07): …You're day is very structured…You do get a very small amount of free time, each day is that right?

G: It varies day to day. There will be time for you to square away your own personal administration so sorting things out like your uniform and stuff like… that you get to yourself but often that gets eaten into by other activities.

Nick L: So I can imagine free time is quite hard to come by. Are you protective of your free time in that regard?

G: Yeah, but you're also used to getting it taken away, so…

Nick L: You will be expected to do 5 MRI scans…they could well eat into your free time.

Group Activity 1 (23:54)

- Using the pen and paper provided, please consider all of the information provided and write down whether or not you think you would participate in this study, if you were an eligible military cadet who had been diagnosed with a stress fracture.
    - What was the most important factor for you, when considering if you would participate or not?

Participants given 5 minutes to write notes on above questions. Vote by show of hands if you would take part in the study (27:05)**:**

Yes	**No**	**Maybe**
6 (A, E, F, C, D, G)	1 (B)	1 (H)

H: I said I'd be intrigued

A: My 'yes' is quite conditional though…My 'yes' has conditions. If I had a stress fracture that was already showing improvement in recovery, that doing the study wouldn't warrant my time in terms of my stress fracture would recover before the study was up - if I already had a mode of recovering then it probably wouldn't be on the horizon….But if it was a really serious one that was plaguing me all the time, and it had been there for a while and needed more intervention that what I was already having then I probably would.

Nick L: Because you've started, are there any other factors that are important to you or would influence you?

A: I would have to be sure that taking the parathyroid hormone wouldn't change any of my other hormone levels. Because I know that hormone supplementation can sometimes mess things up…well not mess things up, but have a knock on effect on other areas and I'd like to know what's going on.

Nick L: Ok that's understandable, so you'd want more input from a clinician, a doctor you'd want to speak to them more about potential side effects.

A: 'Cos usually within the body if you're adding a hormone it will have an effect on another hormone.

….

H: I'd be all up for the study if it does do progress bone… but I think one of the main things I said I think I would be a bit worried about injecting myself everyday and maybe having the time to keep up the schedule. I just don't want to mess anything up, if I started then half way through having to pull out, I wouldn't want to mess up the study.

….

E: Yeah similar to H, in terms of time constraints and stuff. Being able to dedicate time to it. Whether I could commit to 6 months. It would probably be dependent on the severity of the injury as well. I'm not sure whether I would try something new. If it was quite a severe injury, I might stick to something safer.

…

F (30:33)**:** Really worthwhile, really important but the same thing, the time inconsistency. I just wonder how you'll account for not putting in an injection one day or any other inconsistency around that very long period of time.

…(explanation by Nick L, 30:50)…

F: How would you go about monitoring that then?

…(explanation by Nick L, 31:29)…

A (31:50)**:** Obviously, not everybody shares the same day to day life. If everyone of us in the room had a stress fracture, we would probably all rehabilitate a little bit differently in terms of exercise and in terms of treatment. How would you differentiate between the reason for their recovery being better than someone else's was because of the drug and not because of their regime?

…(explanation by Nick L, 32:20)…

B (33:48)**:** A' question raised a question for me, how do you go about the differences between people - when they've got the same injury but, if they're someone like me, supposed to rest I won't rest, some people will actually rest. You might get someone who is on the drug but they're still training. How do you account for that?

…(explanation by Nick L, 34:20)…

B: I probably wouldn't do it, mainly because I wouldn't want to inject myself everyday, especially if it's a placebo. I'd be really, really pissed off at the end of it if I was injecting nothing into myself everyday. So if I knew I was getting the actual one, I might say yes, but I don't like the 50:50 odds of having to do it everyday and not getting anything out of it. Although if the fracture was bad enough, and I was really desperate, and like I really wanted to pass out as quick as possible, I'd probably say yes but it's difficult to put myself in that position when I'm not in and I'm not desperate to pass out or anything. But if it was bad enough and I really wanted it, I might do it. But if it was just a minor one, I wouldn't see it as worth it in case I get the placebo.

D: I just wondered why you are trialling for 6 months?

…(explanation by Nick L, 36:10)…

G (36:57)**:** From my personal perspective, the fear of getting sort of both back squadded and then, if you're back squadded, subsequently removed from training especially if you have an injury, is very real. A lot of these troops have spent a long time trying just get to phase 1 training. They'll have gone through a very painful, very long selection process, a lot of admin/paperwork, taking up say 2-3 years work. So once they're there, and once they're on the training and say half way through training, the real incentive to take any chance of improving the chance of recovery, even if it's a 50:50 chance, I think, personally I would see that.

D: I think I would want to contribute just because the future research. Perhaps could be something new that changes so many people's lives…but because of the fact that I wouldn't contribute, is just time and dedication, to be able to commit to the set time - cos you have to take the medication on time for this so what if I missed that so…

C (38:24): I'm kind of on the fence. I wouldn't now based on who I am, what I've learnt over the years. I have my own independence, I now have the chance, I can say 'no', I guess six and half years in the military, it's kind of 'you can't say no'. But looking back to if I was 18 years old, a lot of these lads if they're going to Catterick, they're 16 so they normally do two years service at the Queens … doesn't go towards the military service. A lot of these lads are gonna to be on a low education level, GCSEs or not even that - which is why I was taking notes on my phone. I think a lot of these people will be what they call 'low internalization' - psychologically they feel that, an example would be that 'I'm injured, I'm not as fit as everyone else' and I think that will have repercussion on the way they think, and that might push them to say 'yes'.

…Nick L…

A: Nick raised quite a big thing that I think, with regards to, I don't mean to be rude, but a lot of people entering the army at 16, they won't be the sharpest tools in…wherever you keep your tools. (laughter)….A lot of the people I know who I went to school or who I as friends with who entered the army, were quite ill behaved in school or they didn't joule(?) with what they were doing at school…. But that knock on effect of them being effectively less smart influenced their decision but doesn't push them in one way or another, I think it just makes them more inclined to 'go with the flow' rather than making an informed decision. Like they wouldn't think how we're thinking now, they're not going to think 'how would it help me?' or 'how would I know that it's the drug that's helped me?' or they wouldn't think why they wouldn't take it, they'd just go for it, it might be a pressure sort of thing, rather than them understanding the actual process.

A?? (40:45): Would you be able to do it on someone who is 16, or would they have to be over 18?

…Nick L…

A: Is it sensible to be giving someone hormones who's still at the age where their hormones are still settling I'd say or maturing?

…Nick L…

Nick L: Any other thoughts?

A?: One more thing is that, if it's something that you really want to do, you've got that desire to succeed. If it was with me with track, if they said to you "you've got a stress fracture but in

six months' time you can run a PB if you take this" I'd definitely jump on the bandwagon. So obviously I'm not in the forces, maybe that to them is the equivalent of what I've just described.

Group Activity 2 (42:40)

- If the trial was designed differently so you had a **greater** chance of receiving the Active PTH treatment rather than the Placebo- would this influence the likelihood of you participating in the study?
  - Example- there was actually 2/3 chance of receiving PTH instead of the Placebo- as opposed to a 1/2 chance.

B: Yes it would sway my decision if there was a greater chance of me getting the actual one.

Nick L: Is that enough of a difference, do you think?

B (43:35)**:** It depends on how painful the injection is. How big is the needle?

Nick L: It's a really fine, narrow needle…it's an injection, so you will feel it obviously**.**

B: I'd try it first. Try the injection, and then see if it was alright…Nick L/Helen..If I could test one and see 'nah, I don't want that' or 'yes actually it's fine, it didn't hurt'.

Nick L: Anyone else?...

H: For me, that's not really enough. I'd be more likely to do it but its not enough for me to be like 'yeah, yeah I'm gonna jump on'.

D (44:43: I wondered if you could administer the medication in other ways, rather than injection?

…Nick L…

A: This might sound really bad, but if you're the sort of person who doesn't wanna to stick a needle in you, because you're either worried or whatever, I don't think you should be in the army.

C: I was gonna kind of agree. A lot of these people have gone through a lot worse, or they will do. I don't think they're the type of characters that are gonna be phased by a small jab. They have to have lots of jabs to go all over the world so injecting themselves once every couple of days I don't think that will have an effect on their thoughts too much.

…

Group Activity 3 (46:50)
❖ Would using a **treatment as usual** only control group (no placebo injections necessary), influence your decision to participate in the study? ❖ If allocated to the Placebo Group, you would however still be asked to attend all 6 x Follow-Ups and 5 x MRI Scans for 6-months.

H: It's just a waste of time (laughing).

G (or B?): You could argue that they're still getting better monitoring than they would otherwise.

…Nick L…

H: You're possibly getting the support but I'd still see it as a waste of time if I know I'm getting the placebo.

…Nick L…

A: Also decreases accuracy of the study, it's making something a bit more open that should not be disclosed.

Another thing I was thinking of was, if you allocate someone…someone's sworn off and they know they're not having the placebo and they're just having TAU and they invest their time into the study - is this drug licenced to someone to be prescribed at the minute, to be after the trial?

Nick L: …only osteoporosis …

A: So if someone signs up to the trial hoping that they'd get this PTH, and it turns out throughout the trial the PTH works and all these people that got allocated into that group recovered and then you got allocated into the TAU and you hadn't recovered - wouldn't the people who'd been in the placebo group say 'Well, look I want the hormone now because it was only by luck that I got this, whereas all the people on the other side of the study have gone into the study in the same position but they've come out of it with a healed stress fracture and I've got nothing'? Would they get anything for participating in the study because they've got nothing out of it?

…**Nick L**…

A: 'Cos that could decrease the need for a placebo because then they'd get the TAU cos then they know that if the actual drug works then they get the drug anyway.

…**Nick L**…

C? (50:40): Based on what you're saying, that would put me off. If there was no placebo group and it was that and what you've just described as the outcome then that would put me off because I'm not going to gain.

Nick L: Not to put words in your mouth, so you were actually borderline before but if it was sold to you like that…

C?: If it was sold to me like that and I wouldn't ever get the drug if it worked then id be like 'no there's no point'.

D (51:10)**:** How would you do the allocation?

…**Nick L**…

A?: You know you said the participants don't know what drug they're getting, how many of the clinicians and people carrying out the treatment know what they're getting? Is it double blinded?

…**Nick L**…

B: I think if I was in that group I'd still do it because it'd just be interesting to have the MRI scans to see what's going on. It might show something else, it might find something else that's' undiagnosed or you can actually see the recovery, you can see the process.

…**Nick L** (subsequent MRIs would have been nice, it's a load off your mind)…

B: For me, if I'm in that position, even if I say 'no' I've still got a stress fracture. If I say 'no' I'm not getting any MRI scans. IF I say 'yes, I'm in that' I still get a scan every so often I can see what's going on so it would still be quite beneficial for me if I was injured.

G: Also you are getting time away from training which is quite nice.

Nick L: interesting as I thought MRIs scans might put people off

A?: Usually on NHS it takes months as well, you have to wait ages for an MRI

Group Activity 4 (53:53)
❖ Are there any other aspects of the trial that you think could be changed, which would improve the likelihood of you agreeing to participate in this trial?

A: Not to do with the likelihood of me agreeing to participate but maybe to do with accuracy of the trial, is how many people did you say?

…**Nick L** …

A: What's the demographic in terms of ethnicity and there's variable factors that can influence bone recovery so everyone's bones recover, more or less, depending on diet and stuff, you should be able to recover within a certain window, but I know that there are certain groups of people who have denser bones who are less likely to get stress fractures and stuff like that – is all the people gonna be the same…

…Nick L/Helen…

D: What is the age range or does it matter? I feel the older are at more risk of…

…Nick L…

Nick L: What age?

C: Cadets are 16-18. But it's normally 18-21, you get the odd late 20 early 30, just the odd one or two.

G: 33 is the cut off.

C: yeah I think its 33.

B: What A said about the different people? How diverse are the people that are in the training? The people that you're aiming at, that you're recruiting from that group - how diverse is that group?

G?: Less than civilian population (?*inaudible)*

B: its not going to be representative

…**Nick L**…

C (57:22): How is the research team going to approach the injured personnel that come through in ways of ramifications if they say 'yes' or 'no' and they go back to their camp, are people including their bosses and superiors going to know that they're doing a drug trial? Because that's going to impact their thoughts.

…**Nick L**…The physio will know. In terms of superior officers, not something we'd thought of. Imagine they'd know….

G (58:24): Because the injections are refrigerated, I imagine the chain of command would find out one way or the other.

…**Nick L**…Especially if they're having a personal fridge. So the drug has to be kept in the fridge.

C: That will have an impact on their thoughts, 100%. Because these people have already...no offence to them, but they already have a bad reputation when you're injured you tend to see a lot of people going back to rehab and a lot of those people tend to get back squadded or they go home…so if that got back to their bosses I think that there would be repercussions on whether they would say yes or no at the earlier stages.

G: Form an ethics perspective, while in the plan it might be completely their choice as to whether they'll do it but with the army being what it is it may well come across to the soldiers that they are being told to volunteer for something which is different to actually volunteering.

…**Nick L/Helen**…

G: Just from a logistics point of view, and I have discussed this with Nick the other day, if they're on exercise in the field for example, keeping medication refrigerated or even simply the fact that they'll be potentially doing things at the time that during a normal day they'll be injecting where, for example, they might be doing something that means they don't have time to do that. Therefore, how from a logistics point of view, do you plan around the fact that they're not in a hardened accommodation environment all the time with access to refrigeration.

…**Nick L**…

C: So how long would they be taking the injections for?

…**Nick L**…

B: How accommodating would the army be to the trial? Would they let have time off for it? Would they let them have time for taking the injection? Or would it be like it has to work around their training? Or does it come before their training?

…Nick L…Chosen barracks due to research experience.

Helen:

C (1:02:13)**:** The only suggestion I can think of is there is a rehabilitation unit for the injured personnel anyway. Is having the fridge there that they report to every morning, they have

their injection whether it's placebo or not. The only problem is, that yeah, they go out on exercise quite often for 5, 6, 7 days at a time and carrying that around is going to be problematic.

Nick L: Would they still go out on exercise if they have a stress fracture. I mean if they 're on reduced duties would they still be expected to go out?

C: Yeah they just lighten load or they don't do as many heavy marching duties. You might go out but instead of carrying 25 kilos you might have 20 or you might just not have your weapon. Or they might just say, so, the big one is the anterior stress fracture on the shin, is just loosen the shoe laces at the top to relieve the pressure and that's it and then carry on.

A? (1:03): Would all the samples be kept together? So what if they got mixed up? It doesn't give away which one it is. If they're all in one fridge at one barracks.

…**Nick L**…

B? A?: If they're doing it themselves they have access to it all themselves and its all kept together, someone gives them their specific one.

D: What if they swap?

B: You'd have to have someone else there who knows, who gives them all individual, someone responsible for giving the right one, otherwise you've got to trust that they take the right one everyday.

…Discussion about colour coding….

G (1:05:10): One more consideration is this, the way these training programmes work is similar to learning outcomes you have a list of training outcomes soldiers need to complete before they can complete that training. So depending on when they go for MRIs or if they're missing crucial training sessions that they miss that training outcome and can't get signed off on that, that's going to create impact obviously on the training programme be it either that they're having to catch up in their, not 'free time' but their other time, in which case that's a negative impact from their point of view, or it's affecting their training programme in some other way so just a consideration.

1:06 Conclusions

18 Appendix 3: Summary of British Army Bone Stress Injury Management Procedures for Infantry Recruits.

References

ITC Guidelines for the Management of Stress reaction/fracture May 16.

Centre for Lower Limb Rehabilitation Defence Medical Rehabilitation Centre: EXERCISE INDUCED LEG PAIN QUALITY STANDARD INDICATOR AND BEST PRACTICE GUIDANCE April 2018

Pre – Rehabilitation

1. Action on initial presentation to Doctor / Medic

a. **Suspected Neck of Femur (NOF) Fracture**: All hip and groin pain referred to a doctor to exclude possibility of NOF bone stress injury (BSI), with a low threshold for obtaining an MRI scan.

b. **Crutches**: If a BSI is suspected crutches should be issued in accordance with symptoms whilst awaiting confirmation of diagnosis. Crutches should be issued by the medic and the patient should be taught how to use them including how to go up and down stairs safely. It must be explained to the recruit and documented that they must use crutches as directed and that relative rest and offloading of the affected bone is required for the healing process. Failure to comply will likely result in longer time to return to training (RTT).

c. **Advice on weightbearing**: Patients should be directed to weight bear in accordance with orthopaedic advice (Table 1).

d. **MT BSIs**: should be put into a pneumatic boot as an alternative or in addition to crutches as symptoms direct.

e. **Light duties**: should be issued by the medical officer (MO)/medic to modify symptomatic activities. In the case of severe pain or for safety/duty of care issues, consider bedding down at the Medical Reception Station (MRS).

f. **Analgesia**: NSAIDs should not be prescribed as they can increase healing time.

g. **Recall leave:** optional whilst waiting for MRI. Recruit to maintain non-impact CV fitness and basic military skills where possible and safe to do so; a recruit should remain with their platoon and continue in training with appropriate light duties disposal. Appropriate levels of specific, restricted weight bearing PT can be prescribed.

Table 18-1: Weight bearing guidelines for recruits with a suspected or confirmed bone stress injury.

Site of Fracture	**Weight Bearing Guidelines**
Neck of Femur (NOF)	Toe Touch Weight Bearing (TTWB) with crutches from point of suspicion for 6 weeks. Protected weight bearing (WB) for a further 4-6 weeks (i.e. gradually increase weight through the limb and wean off crutches in accordance with symptoms.) Non-weight bearing (NWB) is not necessary. All NOFs will be under orthopaedic management and guidance should be sought on an individual basis.
Tibia	Proximal medial tibial bone stress injuries and those affecting the antero-medial tibial cortex should be totally NWB for 4-6 weeks and then treated symptomatically.
Foot and ankle	Off load for 6 weeks – should not heel/toe walk, so heel walking only or NWB. Metatarsal fractures to use pneumatic boot. Often secondary to biomechanical issues so should all have biomechanical screening and podiatry input. Can do low impact rehabilitation.
Long bone (femur and mid-distal tibia)	Treat symptomatically, Unit sick leave (USL) in accordance with symptoms.

2. Diagnosis

a. Bone stress injury diagnosis is made by MRI Scan.
b. MRI is the investigation of choice for all suspected BSIs except tibial or foot symptoms with a 4 week history of shin or foot pain and pain on walking which may initially be referred for an X-ray.

c. MRI report should specify the grade of BSI (Table 18-2) and thus be identified as low or high risk for non-union and managed accordingly.

Table 18-2: Fredericson's classification of bone stress injury grading.

Grade of Fracture	**Description**
Grade 0	Normal
Grade I	Periosteal oedema only
Grade 2	Bone marrow oedema visible on T2 weighted images
Grade 3	Bone marrow oedema visible on T1 & T2 weighted images
Grade 4a	Multiple focal areas of intracortical signal abnormality
Grade 4b	Linear areas of intracortical signal abnormality

3. Unit Sick Leave (USL)

a. To be authorised as appropriate.
b. Early rehabilitation is appropriate for recruits on crutches who still have some symptoms on weight bearing.
c. The length of USL should reflect the severity of the injury and symptoms. Bilateral BSI, those graded 4b (Fredericson's classification) or those with extensive bony oedema may require longer.
d. Home circumstances and patient compliance with crutches should be considered when arranging USL.
   A maximum of 4 weeks USL should be given before reviewing the patient.
e. Prolonged periods of USL (over 4 weeks) should be avoided as early rehabilitation is crucial to recovery. Where 6 weeks USL is suggested by orthopaedics – this should be queried as an early referral to rehabilitation platoon will protect the recruit and rehabilitation and maintenance of CV fitness can begin.

4. Orthopaedic Referral

Orthopaedic Referral should be made for all NOF BSIs and those at high risk of non-union (see list below):

a. All femoral neck fractures
b. Tibial shaft – anterior cortex and proximal tibial
c. 5th metatarsal – diaphyseal-metaphyseal junction
d. Navicular
e. 2nd metatarsal – proximal section

f. Talus
g. Medial malleolus
h. Sesamoids

Low risk BSIs are managed symptomatically within primary care

5. Review of Medical Grading

a. On confirmation of injury, a recruit is medically downgraded. Once rehabilitation is complete and a recruit is fully fit they are graded as fit to RTT.
b. Recruits are considered for discharge on medical grounds after 84 consecutive days out of effective training.
c. Sacral BSIs (grades 4a & 4b) should be medically downgraded. Grades 1 to 3 with minimal symptoms to be given consideration to retain but seek military orthopaedic opinion.
d. Recruits who have undergone orthopaedic surgery e.g. DHS, should be referred to rehabilitation but should be considered for early medical board as they are unlikely to return to full fitness within the necessary time guidelines.
e. If internal fixation is required, the recommendation is that the patient should be discharged with a one year period of relative rest/light exercise advised. It should be regarded as a life-altering injury, due to the potential for further injury if exercising hard.
f. Bilateral (symptomatic) BSIs may be considered for early medical board.
g. If a recruit has a recurrence of a BSI or sustains a second BSI (occurs in approx. 10%) after already having been through rehabilitation the recruit should be referred for a medical board.
h. Week of training reached should not influence the decision of whether to refer BSI patients to rehab or for medical board. If the recruit fails to recover in rehab within the specified timescales his future will be determined by the unit in conjunction with the doctor and rehab team at the UHC.

<u>Rehabilitation</u>

6. Management of rehabilitation

a. On confirmation of BSI, Vit D levels are to be measured and the patient treated if deficient.

b. Recruits with confirmed stress reaction or fracture should be taken out of training and referred for extended rehabilitation in a rehabilitation platoon.
c. Referral to a rehabilitation platoon should be done at the time of sending on USL for appropriate recruit administration and monitoring. The exception to this is if there is any doubt over whether the recruit is appropriate for extended rehab e.g. possibility of early medical board or waiting for orthopaedic guidance. In these cases the doctor / physiotherapist should review on return from USL before referring to the rehabilitation platoon.
d. Recruits on crutches can still begin appropriate early rehabilitation.
e. Weaning off crutches should be under the direction of a physio in accordance with reduction of symptoms. Grade 4b BSIs may require longer periods of partial weight bearing (PWB).
f. Symptoms should be monitored throughout the rehabilitation process to determine progression/regression.
g. Rehabilitation should be progressed according to symptoms and functional testing.
h. Consider shock absorption orthotics.

7. Exercise Intervention

a. Early rehabilitation (pre-recovery) should concentrate on upper body, non-impact CV work and swimming/deep water running.
b. Maintain cardiovascular fitness with cross-trainer/non-impact activity.
c. Maintain strength as pain allows.

8. Return to Training (RTT)

a. The final functional test before RTT is successful completion of a weighted/distance march with no symptoms and at a level appropriate to the week of training they are returning to.
b. Review by an exercise rehabilitation instructor, physiotherapist and doctor is required to determine a recruit is ready to RTT.

19 Appendix 4: Case Reports of Teriparatide for Fracture Healing

Ser	Author	Treatment	Fracture / Issue	Details	Patient Demographic
1	Mailoo, 2019 (Mailoo *et al.*, 2019)	Teriparatide and Vitamin D	Mid Femur Fracture	This retrospective study suggests that teriparatide may enhance fracture healing and improve the union rate in OVCF.	One 71 year old woman
1	Iwata, 2017 (Iwata *et al.*, 2017)	Teriparatide Vs Bisphosphonate	Osteoporotic vertebral compression fracture.	This retrospective study suggests that teriparatide may enhance fracture healing and improve the union rate in OVCF.	98 (38 on Teriparatide)
2	Kasukawa, 2017(Kasukawa *et al.*, 2017)	Teriparatide	Sacral Insufficiency Fractures	Retrospective study. Seven elderly women with SIFs had significant improvement in pain and demonstrated bone union or sclerotic changes at fracture sites.	12 Women (aged 66- 83)
3	Almirol, 2016(Almirol *et al.*, 2016)	Teriparatide	Lower extremity stress fractures	Prospective placebo controlled.	14 Women Premenopausal (aged 21-45)

				According to MRI, 83.3% of the TPTD- and 57.1% of the placebo-treated group had improved or healed stress fractures (p = 0.18).	
4	Aspenberg, 2016(Aspenberg *et al.*, 2016)	Teriparatide Vs Residronate	Pertrochanteric Hip Fracture	Prospective, active controlled. Teriparatide was associated with less pain and a shorter time to complete the TUG test between 6 and 26 weeks compared with risedronate. Other fracture-recovery outcomes were similar. The results should be interpreted with caution as these were secondary end points	224 Participants 77% female (aged 77 ± 8 years)
5	Harada, 2016(Harada *et al.*, 2016)	Teriparatide	Anderson Type II otonoid fracture	Case report.	Elderly patient. No further details available.

6	Huang, 2016(Huang *et al.*, 2016b)	Teriparatide	Cementless bipolar hemiarthroplasty	Retrospective. Teriparatide significantly reduces the subsidence of the cementless femoral stem in elderly patients in the early post-operative period, but this benefit does not reflect better functional outcomes and HRQoL	52 patients (demographics not described)
7	Huang, 2016(Huang *et al.*, 2016a)	Teriparatide (3 arms, 1= nil pre-op, calcium and vit D post op, 2= nil pre-op, teriparatide post-op, 3= alendronate pre-op, teriparatide post-op)	Osteoporotic intertrochanteric fractures with DHS surgery	Retrospective study. A significantly shorter time-to-union was found in the teriparatide-treated groups. QoL, scores were significantly better in teriparatide-treated groups at 3 and 6 months. Similar inter-group differences were noted when comparing the pain scores, the ability to get	189 Participants

				around the house, the ability to get out of the house, and the ability to go shopping at 3 and 6 months. Complications and mortality were also markedly reduced in the teriparatide-treated groups.	
8	Kastirr, 2016(Kastirr *et al.*, 2016)	Teriparatide	Delayed union lower leg fracture	Full consolidation of 5delayed union fracture was achieved 4 months after initiating teriparatide treatment.	Male 49 years
9	Nozaka, 2016(Nozaka *et al.*, 2016)	Ultrasound alone vs Ultrasound + Teriparatide	Lower limb fracture	The mean time to union was 111.9 days (range, 94–175 days) for IEF alone and 72.1 days (range, 68–141 days) for the IEF combination; it was significantly shorter with the IEF combination.	38 (aged over 60) Abstract only.

10	Stanciu, 2016(Stanciu and Popa, 2016)	Teriparatide	Sacrum and pubic ramus fractures	Callus formation in the left pubic ramus and pain free, unassisted walking were observed in a patient one and two months after injury occurrence, respectively.	155 Men aged 65 + 585 Women aged 65 +
11	Yang, 2016(Yang *et al.*, 2016)	Teriparatide	Osteoporotic vertebral fracture	6 of the 12 patients whose fractures were examined with MRI after 6 months of therapy demonstrated complete bone healing.	12 x Female 73 +/- 4.8 years
12	Coppola, 2015(Coppola, Del Buono and Maffulli, 2015)	Teriparatide	Open fixation of traumatic fracture with non-union	All 4 patients demonstrated integration around the bone graft and adequate formation of bone callus at the site of the non-union.	Male, 36 years Male, 33 years Male, 28 years Male, 30 years
13	Huang, 2015(Huang *et al.*, 2015)	Teriparatide	Unstable pertrochanteric fractures post DHS	Teriparatide improves radiographic outcome and yields better clinical	211 patients. (aged 65-92)

				outcomes at 3 and 6 months postoperatively.	
14	Kim, 2015 (Kim *et al.*, 2015)	Teriparatide	Femoral fracture	Callus formation was noted in 3 patients after 2 weeks of teriparatide; with abundant callus formation 8 weeks post treatment.	Female, 88 years Female, 96 years Male, 78 years
15	Mancilla, 2015(Mancilla *et al.*, 2015)	Teriparatide	Delayed or nonunion fracture of the tibia or femur	Complete union of the fracture was achieved between 3 to 9 months in 5 of the 6 patients.	19 – 64 years Full text requested
16	Matsumoto, 2015 (Matsumoto, Ando and Sasaki, 2015)	Teriparatide	Delayed union of lumbar spine fracture with diffuse idiopathic skeletal hyperostosis (DISH)	Callus formation was observed 3 weeks after teriparatide initiation, with further bone formation and resolution of lumbar instability at 6 months.	Male, 70 years
17	Uemura, 2015(Uemura *et al.*, 2015)	Teriparatide	Bone Healing of Nonunion After Ulnar Shortening Osteotomy for Smokers	Partial bone union began to be observed on radiographs after the first 4 weeks of teriparatide	Male, 62 years Female, 42 years Abstract only.

				administration and successful bone healing without additional surgical interventions was achieved after 10 (for male) and 6 (Female) months of treatment with teriparatide	
18	Fattah, 2014(Biro *et al.*, 2014)	Teriparatide	Spinal fracture with ankylosing spondylitis	Complete fracture healing with 6 months of teriparatide treatment without any complications.	Male, 50 Years
	Nozaka, 2014(Nozaka *et al.*, 2014)	Teriparatide combined with low intensity pulsed ultrasound	Femoral shaft fracture	Bone formation to support osseous union was achieved 6 months after combination treatment.	Male, 56 Years
19	Bednar, 2013(Bednar, 2013)	Teriparatide	Type III odontoid process fracture	Complete fracture site healing was evident with 6 months of teriparatide treatment.	Full details unavailable.

20	Borges, 2013(Borges, Freitas and Bilezikian, 2013)	Teriparatide	Transtrochanteric fracture	Dense callus formation was evident after 1 month of teriparatide treatment.	Female, 84 years
21	Franco, 2013(Cortés Franco *et al.*, 2013)	Teriparatide	Nonunion of cervical fracture with ankylosing spondylitis	Consolidation was noted 2 months after initiating teriparatide in a patient with pseudoarthrosis at the fracture site.	Male, 52 years.
22	Malhotra, 2013(Malhotra, Meena and Digge, 2013)	Teriparatide	Tensile type of stress fracture neck of femur	Complete healing of the fracture line was evident after 3 months of teriparatide treatment.	Female, 62 Years
23	Ochi, 2013(Ochi *et al.*, 2013)	Teriparatide	Nonunion of periprosthetic fracture	Once weekly administration of teriparatide for 6 months resulted in successful bone fusion.	Female, 74 Years
24	Tamai, 2013(Tamai, Takamatsu and Kazuki, 2013)	Teriparatide	Nonunion after ankle arthrodesis and femoral shaft fracture	Union was obtained at both the fracture and nonunion sites within 3 months of initiation of teriparatide.	Female, 25 Years

25	Lee, 2012(Lee, Ha and Koo, 2012)	Teriparatide	Femoral nonunion	Successful union was achieved by 2 patients after 3 months of teriparatide treatment. Another patient had successful union after 9 months of teriparatide treatment.	Male, 38 years Female, 64 years Male, 29 years
26	Moon, 2012(Moon *et al.*, 2012)	Terparatide	Pelvic Insufficiency fractures	We confirmed that pain relief and callus formation were relatively faster when analgesic was administered with PTH 1-34.	Female, 76 years Female, 82 years
27	Raghavan, 2012(Raghavan and Christofides, 2012)	Teriparatide	Metatarsal stress fractures	Callus formation was noted after 4 weeks of teriparatide in 2 patients. In addition, new bone formation was evident in 1 of the patients.	Female, 35 Years Female, 40 Years
28	Wu, 2012(Wu *et al.*, 2012)	Teriparatide	Insufficiency fracture of combined pubic rami and sacrum	At 3 months' followup, the pain had subsided completely, with abundant callus	Female, 73 years

				formation on rami fractures. The fractures showed good consolidation at the end of 18 months.	
29	Díez Ulloa, 2011(Díez and Ulloa, 2011)	Teriparatide and halo jacket	Delayed union of cervical fracture with ankylosing spondylitis	Patient with no consolidation after 2 months with a halo jacket was started on teriparatide and achieved consolidation 2 months later.	Male, 60 years
30	Gomberg, 2011(Gomberg *et al.*, 2011)	Teriparatide, Calcium and Vitamin D	Bilateral subtrochanteric stress fractures	Based upon the chronology of fracture healing in our patient and published evidence that teriparatide heals stress fractures in a rat model, we think that teriparatide was probably primary in this patient's positive response to therapy, with calcium, vitamin D therapy, and alendronate discontinuation	Female, 63 years

				playing secondary roles.	
31	Paridis, 2011(Paridis and Karachalios, 2011)	PTH 1-84	High energy, two level, comminuted fracture (pertrochanteric and middiaphyseal), of right femur in a road traffic accident	At the end of the second month, satisfactory radiological callus formation was observed and the patient was allowed to walk full weight bearing. At one year follow up, the patient regained painless, full range, motion of the adjacent joints and walked with a mild limp due to a minor leg length discrepancy.	Male, 48 years
32	Peichl, 2011(Peichl *et al.*, 2011)	PTH 1-84	Pelvic fracture healing in post-menopausal women	In elderly patients with osteoporosis, PTH 1-84 accelerates fracture-healing in pelvic fractures and improves functional outcome.	65 patients Abstract only
33	Zati, 2011(Zati *et al.*, 2011)	Teriparatide	Loosened hip prosthesis	Teriparatide treatment	Male, 61 years

				following loosening of a hip prosthesis increased bone mineral density of cancellous and cortical bone.	
34	Aspenberg, 2010(Aspenberg *et al.*, 2010)	Teriparatide	Dorsally angulated distal radial fracture	The primary hypothesis that teriparatide 40 microg would shorten the time to cortical bridging was not supported. The shortened time to healing for teriparatide 20 microg compared with placebo still may suggest that fracture repair can be accelerated by teriparatide.	102 Women. (45-85 years).
35	Aspenberg, 2010(Aspenberg and Johansson, 2010)	Teriparatide	Dorsally angulated distal radial fracture Post hoc sub group analysis of above study.	Teriparatide appeared to improve early callus formation in distal radial fractures.	27 participants.

36	Chintamaneni, 2010(Chintamaneni, Finzel and Gruber, 2010)	Teriparatide	Sternal fracture nonunion	Nonunion in the body of the sternum was successfully treated with teriparatide.	Male, 67 years
37	Oteo-Alvaro, 2010(Oteo-Alvaro and Moreno, 2010)	Teriparatide	Atrophic humeral shaft nonunion	A direct relationship was established between the drug and healing. This is the first reported case of diaphyseal nonunion in long bones resolved with teriparatide; because of the different activity of teriparatide on trabecular and cortical, it suggests that teriparatide could accelerate healing in nonunions in this type of bone.	Male, 32 years
38	Schalin-Jäntti, 2010(Schalin-Jäntti *et al.*, 2010)	Teriparatide	Femur fractures	PTH 1-84 improves pain, mobility, and fracture repair in adult HPP, even after repeat treatment.	2 x female, 56, 54 years.

39	Rubery, 2010(Rubery and Bukata, 2010)	Teriparatide	Type III odontoid fractures with delayed union	Union of fractures and resolution of neck pain in 3 patients following teriparatide treatment.	Full details unavailable.
40	Bukata, 2009(Bukata *et al.*, 2009)	Teriparatide	Difficult to heal fractures, various sites.	Observed success rate, particularly in a cohort with a high percentage of patients with nonunions, delayed unions, and significant medical comorbidities, certainly warrants prospective clinical trials of 1-34 PTH as an adjuvant for fracture healing in difficult to heal fracture situations.	145 patients.

41	Yu, 2008(Yu *et al.*, 2008)	Teriparatide and internal fixation	Hip fracture	Enhanced callus formation was evident after daily teriparatide treatment for 1 month.	Female, 62 Years
Unsuccessful Fracture Healing with Teriparatide					
42	Denehy, 2016(Denehy *et al.*, 2016)	Teriparatide	Proximal humeral fracture	One patient receiving teriparatide prior to fracture and continuing for a year after, demonstrated complete healing within 4 months of discontinuation.	Female, 43 Years
43	Bhandari, 2016(Bhandari *et al.*, 2016)	Teriparatide	Neck of femur fracture healing	The small sample size limited this study's power to detect potential differences, and the results are exploratory. With the patients available, teriparatide did not decrease the risk of revision surgery, improve	159 Men and postmenopausal women Aged 50 + years

				radiographic signs of fracture healing, or decrease pain compared with the placebo. The adverse event data observed were consistent with the teriparatide safety profile	
44	Johansson, 2016(Johansson, 2016)	Teriparatide	Proximal Humerous Fractures	This study did not show that teriparatide had any positive effect on the treatment of proximal humerus fractures, either radiographically or regarding function and pain.	40 Post menopausal women.
45	Malouf-Sierra, 2016(Malouf-Sierra *et al.*, 2016)	Teriparatide Vs Residronate	Pertrochanteric Hip Fracture post surgery	No improvement in fracture recovery or healing outcomes. *Study not powered to measure this.	224 patients 77% women (aged 77±7.7 years)