THE UNIVERSITY OF HULL

The effects of continuous, intermittent and mode of exercise on mechanical bone remodelling

being a Thesis submitted for the Degree of

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by

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Contents

Abstract

Bone health is known to deteriorate with age, which can increase the risk of osteoporotic fractures and subsequently all-cause mortality. Current life expectancies are higher than ever before and with our ageing population, osteoporosis and low bone density levels are an ever growing problem that command a lot of medical attention and resources. Women are at a greater risk than men due to increased rates of bone loss that occur in the early years following the menopause. Mechanical loading in the form of exercise is known to reduce the rates of postmenopausal bone loss although an optimal exercise programme is yet to be established. Furthermore, investigations conducted with animals have found intermittent mechanical loading to provide a greater stimulus for bone adaptation than continuous mechanical loading, this has not been investigated in human populations to date. The aim of this thesis was to establish a sufficient exercise mode for stimulating bone adaptation in postmenopausal women and investigate the effects of continuous and intermittent exercise on postmenopausal bone loss. This was attempted with a 12 month randomised controlled trial with postmenopausal women.

The first study gave a systematic review of the current literature that investigated continuous or intermittent exercise. The review found that as the studies were not designed to specifically analyse continuous or intermittent exercise, there were numerous problems regarding the control of previous exercise programmes with regards to defined exercise and rest intervals. This was due to the design of the included studies, as many of them were not specifically designed to analyse the different effects of continuous and intermittent exercise on bone mineral density (BMD). In addition, BMD outcomes were not reported in a standardized manner, which complicated the comparisons drawn. From this investigation, it was evident that well-controlled exercise interventions (using a single exercise), are

required for the comparison of the effect of continuous and intermittent exercise on BMD in human populations.

The second study investigated the feasibility of developing a non-motorised treadmill exercise intervention that included both continuous and intermittent exercise groups. Non-motorised treadmill (NMT) locomotion allows for the instantaneous quantification of ground reaction forces (GRF) and is well suited to both continuous exercise and intermittent exercise with the potential for the use of a range of intermittent running based protocols. In order to establish the osteogenic potential of this mode of exercise, it was necessary to quantify the mechanical loading parameters. This study found that loading parameters showed large reductions during NMT locomotion when compared to overground or motorised treadmill locomotion (24 to 29 %), which could potentially compromise the level of bone adaptation if this mode of exercise was used for intervention purposes.

The third study investigated the loading parameters of more traditional high impact exercises in a population of postmenopausal women. All exercises were performed under both continuous and intermittent conditions to assess for consistency during the two conditions. This project showed that countermovement jumps (CMJ) and box drops (BD) produced the highest loading parameters when compared to heel drops (HD) and stamping (STP) (d = 0.83 – 2.38), along with no statistical differences between continuous and intermittent conditions (continuous: 10.7 ± 4.8 g for CMJ, 9.6 ± 4.1 g for BD; intermittent 10.0 ± 5.0 g for CMJ, 9.5 ± 4.0 g for BD). CMJ, BD and HD exercises all appeared to generate a sufficient level of peak acceleration and acceleration gradient for osteogenic adaptation however. For consistency purposes and the fact that no equipment was required, CMJs were selected as the most appropriate home-based exercise for use in a 12 month intervention to reduce postmenopausal bone loss.

The fourth study investigated the effects of continuous and intermittent exercise on BMD in early postmenopausal women over the course of a 12 month randomised control trial. Unfortunately the study was underpowered and in addition, the findings showed no statistically significant differences in the bone response between groups. Only the control group experienced a statistically significant loss in both lumbar spine (-2.7% [95%CI: -3.9 to -1.4]) and femoral neck (-3.0% [95%CI: -5.1 to -0.8]) BMD, which exceeded the 95% least significant change at the lumbar spine and femoral neck in 57% of control group participants. There appeared to be no beneficial effect of continuous or intermittent exercise on BMD, hip structural analysis (HSA) parameters or muscular force characteristics when compared to a control group however. In conclusion, this thesis has identified that future research should further investigate the effects of continuous and intermittent exercise on BMD with appropriately controlled randomised control trials, with greater participant numbers. Whilst CMJ and BD provide adequate loading parameters, this does not translate into BMD adaptations. Continuous and intermittent CMJ exercises had no effect on reducing postmenopausal BMD loss at the lumbar spine and the femoral neck, although further investigation is required in an adequately powered study.

Keywords: Exercise; Bone Mineral Density; Osteoporosis Prevention; Postmenopausal Women

Dissemination

The work from this thesis has been disseminated in peer reviewed journal articles, along with national and international conference proceedings.

Peer Reviewed Journal Article

Montgomery, G., Abt, G., Dobson, C., Smith, T. and Ditroilo, M. (2016) 'Tibial impacts and muscle activation during walking, jogging and running when performed overground, and on motorised and non-motorised treadmills', Gait & Posture, 49, pp. 120–126.

Conference Presentations and Proceedings

Montgomery, G., Abt, G., Dobson, C., Fagan, M., Smith, T., Aye, M. and Ditroilo, M (2016) 'The effect of continuous and intermittent countermovement jumping on bone mineral density in postmenopausal women', (Abstract), Osteoporosis International, 27 (Suppl 2), S609–S685

Montgomery, G., Abt, G., Dobson, C., Fagan, M., Smith, T., Aye, M. and Ditroilo, M (2016) 'The effect of continuous and intermittent exercise on bone mineral density in postmenopausal women: a six-month randomised control trial', (Abstract), Bone Research Society Annual Conference 2016.

Montgomery, G., Evans, W., Zoffoli, L., Abt, G., Dobson, C., Fagan, M., Smith, T. and Ditroilo, M. (2015) 'The osteogenic index of four common continuous and intermittent exercises used in osteoporosis prevention in an at-risk population', (Abstract), XXV Congress of the International Society of Biomechanics, 605-606.

Montgomery, G., Abt, G., Dobson, C., Fagan, M., Smith, T. and Ditroilo, M. (2014) 'Muscle activation during walking, jogging and running when performed over-ground, on a motorised and non-motorised treadmill', (Abstract), Journal of Sport Sciences, 32, Supplement 2, 20, BASES Conference 2014

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Abbreviations

Abbreviations

2D	Two dimensional
3D	Three dimensional
95% CI	95% confidence interval
aBMD	Areal bone mineral density
ACC	Accelerometry
BD	Box drops
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
bpm	Beats per minute
CMJ	Countermovement jump
CMRR	Common mode rejection ratio
CON	Control group
CT	Computed tomography
CTS	Continuous group
CV%	Coefficient of variation %
dB	Decibels
DPA	Dual photon absorptiometry
DXA	Dual energy X-ray absorptiometry
EMG	Electromyography
EU	European Union
FRAX™	Fracture risk assessment tool
Fx	Medial-lateral acceleration
Fy	Anterio-posterior acceleration
Fz	Vertical acceleration
g/cm^2	Grams per centimetre squared
g/cm^3	Grams per centimetre cubed
GL	Gastrocnemius lateral head
GPa	Gigapascals
GRF	Ground reaction force
HD	Heel drop
HRT	Hormone replacement therapy
HSA	Hip structural analysis
INT	Intermittent group
ISCD	International Society of Clinical Densitometry
kg/m^2	Kilograms per metre squared
L1 – L4	First lumbar vertebrae to the fourth lumbar vertebrae
L2 – L4	Second lumbar vertebrae to the fourth lumbar vertebrae
m	Metres

mm	Millimetres
mm^2	Millimetres squared
mm^3	Millimetres cubed
mm^4	Millimetres 4^{th} power
$mmol \cdot L^{-1}$	Millimoles per litre
MPa	Megapascals
MRI	Magnetic resonance imaging
ms	Milliseconds
$m \cdot s^{-1}$	Metres per second
$m \cdot s^{-3}$	Metres per second cubed
MT	Motorised treadmill
MVIC	Maximum voluntary isometric contraction
n	Sample size
NA	Not available
NHS	National Health Service
Nm	Newton metres
$N.m^{-2}$	Newtons per metre squared
$nmol \cdot L^{-1}$	Nanomoles per litre
NMT	Non-motorised treadmill
NS	Not significant
OG	Overground
OI	Osteogenic index
pQCT	Peripheral quantitative computed tomography
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTH	Parathyroid hormone
RF	Rectus femoris
RM	Repetition maximum
RMSQ CV%	Root mean square coefficient of variation %
QUS	Quantitative ultrasound
RTD	Rate of torque development
RTD50	Rate of torque development after 50ms from force onset
RTD100	Rate of torque development after 100ms from force onset
RTD150	Rate of torque development after 150ms from force onset
SD	Standard deviation
SE	Standard error
SL	Soleus
ST	Semitendinosus
STP	Stamp
TA	Tibialis anterior
vBMD	Volumetric bone density

Symbols

Symbols

d	Cohen's *d* effect size
g	Acceleration due to gravity
Hz	Hertz
˚	Degrees
MΩ	Milliohm
N	Newtons
R^2	Coefficient of determination
με	Microstrain

1. Introduction

The optimization of bone health throughout the lifecycle depends upon a number of lifestyle factors, the most widely supported factor being the amount and intensity of mechanical loading in the form of physical activity. Peak bone mass is reached around the ages of 20 – 45 years (Mein et al, 2004; Pedrazzoni et al, 2003), after which a steady decline in bone mass is experienced due to higher rates of bone resorption that are associated with the ageing process. Rates of annual bone loss are relatively consistent in men but in early postmenopausal women BMD can be lost at 1 – 2% per year (Shipman et al, 1999; Finkelstein et al, 2008). This can predispose many women to low levels of BMD, which are strongly related to future fracture risk (Kanis et al, 2001; Kanis et al, 2009).

Low levels of BMD lead to osteoporosis, which is a disease characterised by structural deterioration of bone tissue, leading to bone fragility and an increased risk of fractures of the hip, spine, and wrist (National Institutes of Health, 2015). Osteoporosis is becoming increasingly problematic in modern society due to the ageing population and the increase in sedentary lifestyles (Davies et al, 2011; Bennie et al, 2013; United Nations Department of Economic and Social Affairs, 2017). Currently, in the European Union (EU), 46% of women and 22% of men over the age of 50 will experience an osteoporotic fracture in their lifetime (Hernlund et al, 2013). It is estimated that 15% of women in the UK over the age of 50 have osteoporosis (Gauthier et al, 2011). The treatment of osteoporotic

fractures costs the National Health Service (NHS) £3,496 million each year and is rising annually, this figure is expected to increase 25% by 2025 (Svedbom et al, 2013). Hip fractures are particularly traumatic incidents, which lead to a 20-24% mortality rate within one year of fracture incidence (Cooper et al, 1993; Leibson et al, 2002; Klop et al, 2014), with hip fracture patients having a 2.78 times higher risk of all-cause mortality when compared to non-hip fracture patients within one year of fracture, this increased risk remains elevated for many years thereafter (Katsoulis et al, 2017).

Physical activity is known to improve BMD status in young, adult and older populations of men and women (Valdimarsson et al, 2006; Nikander et al, 2010; Krustrup et al, 2010; Marques et al, 2011a). Exercise interventions involving high mechanical loading and high muscular forces have shown to be particularly efficacious in improving BMD status (Judex and Carlson, 2009; Robling, 2009; Hinton et al, 2015). Bone has a high mechanosensitivity in the years leading to peak bone mass and therefore must be stimulated through high impact physical activity in younger populations to ensure optimal bone development (Burrows, 2007). Walking and running are common forms of physical activity in younger through to older populations but are usually performed on treadmills to enable testing in a closely monitored environment for the purpose of exercise research (Stamatakis and Chaudhury, 2008; Elsworthy et al, 2015). The loading characteristics of internally paced non-motorised treadmill locomotion could

provide a safe and useful means of investigating the effects of continuous and intermittent exercise on bone adaptation using the instantaneous GRF (Tofari et al, 2015), but this requires a full evaluation of the loading parameters. Postmenopausal women are at particular risk of developing osteoporosis due to the severe reduction in oestrogen levels, which can increase their likelihood of experiencing a fracture (Compston et al, 2013). Postmenopausal bone loss can be reduced at sites prone to fracture (femoral neck and spine) with appropriate targeted loading exercise programmes (Howe et al, 2011). Optimal modes of exercise and optimal exercise programmes have yet to be established for this population however (Xu et al, 2016).

Intermittent mechanical loading has shown to generate greater levels of bone adaptation than continuous mechanical loading in animal studies (Robling et al, 2001; Srinivasan et al, 2007). This phenomenon is thought to arise partly due to the desensitisation of bone tissue to repeated mechanical loading stimuli (Turner and Robling, 2003). Inserting a brief rest interval between mechanical loads is thought to re-sensitise the bone tissue somewhat and create a greater osteogenic stimulus (Srinivasan et al, 2015). It is currently unknown as to whether this mechanism occurs in human populations and it could potentially increase the osteogenic potential of exercise programmes aimed at maintaining BMD levels in postmenopausal women.

Therefore, the aim of this thesis was to establish the most effective modes of

exercise from a selection of commonly used exercises for both stimulating improvements in BMD in young adults and also for reducing postmenopausal BMD loss. The most effective exercise, which displayed consistency between continuous and intermittent conditions was used in an intervention to evaluate the effects of continuous and intermittent exercise on postmenopausal BMD.

1.1 Thesis Outline

Chapter 2 gives a review of the literature for current concepts relating to bone adaptation and mechanical loading. The potential mechanisms that determine bone adaptation and how this is measured are outlined along with the effects that bone loading has in both animal and human populations.

Chapter 3 provides a systematic review of the effects of continuous and intermittent exercise on BMD in human populations. This assesses the findings from a number of well-controlled exercise interventions with a randomised controlled trial design. The review identifies avenues for future research and gives recommendations for the reporting of exercise interventions designed to improve BMD status.

Chapter 4 is the first of three experimental studies and provides information on the loading characteristics of overground, motorised treadmill and non-motorised treadmill locomotion with a view to designing future continuous and intermittent

running protocols and assessing the effects of both continuous and intermittent exercise on bone in a human population. Walking and running are advocated for their beneficial effects on bone tissue for all ages. A range of locomotion velocities were examined for their mechanical loading parameters across three different conditions to establish the most beneficial forms of human locomotion exercise for a population of young adults.

Chapter 5 is the second of three experimental studies and determines the loading characteristics of a number of home-based high impact exercises performed by early postmenopausal women. This was undertaken in order to find exercises with adequate loading characteristics for the reduction of postmenopausal bone loss when used in a future intervention study. In addition, all exercises were performed in a continuous and intermittent condition to detect any differences in loading parameters when the inter-cycle rest interval was manipulated. This was performed to identify suitably consistent exercises when completed in continuous and intermittent conditions, which could be used in an intervention study and ensure that loading characteristics would be similar between both conditions.

Chapter 6 is the final experimental study and assesses the effects of continuous and intermittent high impact exercise on BMD in early postmenopausal women during a 12 month randomised control trial. Changes in BMD, geometric bone parameters and muscular adaptations are explored.

Chapters 7, 8 and 9 provide an overview of the thesis findings. Recommendations

for future research are given and conclusions are made.

2. Background

2.1 The Skeletal System

The human skeleton comprises of 206 bones, which can be divided into five subcategories; long, short, flat, irregular and sesamoid. Typically long and irregular bones experience the majority of the skeletal weight-bearing and movement related tasks via the lower limbs and vertebral column. Human bone is required to provide strength, support and mobility. There are two types of bone tissue, which make up its macrostructure, cortical bone and trabecular (cancellous) bone. These tissues are orientated to withstand high mechanical loads from ground reaction and also muscular forces. Cortical bone is more densely compact and is arranged in sheet like layers around the outer edges of the bone surface (Zimmermann and Ritchie, 2015). The microstructure element of cortical bone is made of Haversian systems and osteons where cylindrical layered bone structures surround blood vessels. Osteons are separated by highly mineralised structures called cement lines but adjoined by Volkmann canals, which provide a vascular channel for the distribution of blood and nutrients throughout the cortical bone tissue (Vaughan et al, 2013). The sub-microstructure consists of lamellae, which are smaller cylinders of woven bone within the osteons, whilst the nanostructure consists of bundles of mineralised collagen fibrils (Zimmermann and Ritchie, 2015). The sub-nanostructure consists of

calcium based bone mineral crystals (primarily hydroxyapatite), which are interspersed amongst type 1 collagen molecules that are initially bound together by immature intermolecular enzymatic cross links, which then become mature non-reducible cross links (Zimmermann and Ritchie, 2015). The cross links serve to stabilize the arrangement of collagen molecules. (Fig. 2.1.) (Rho, et al 1998).

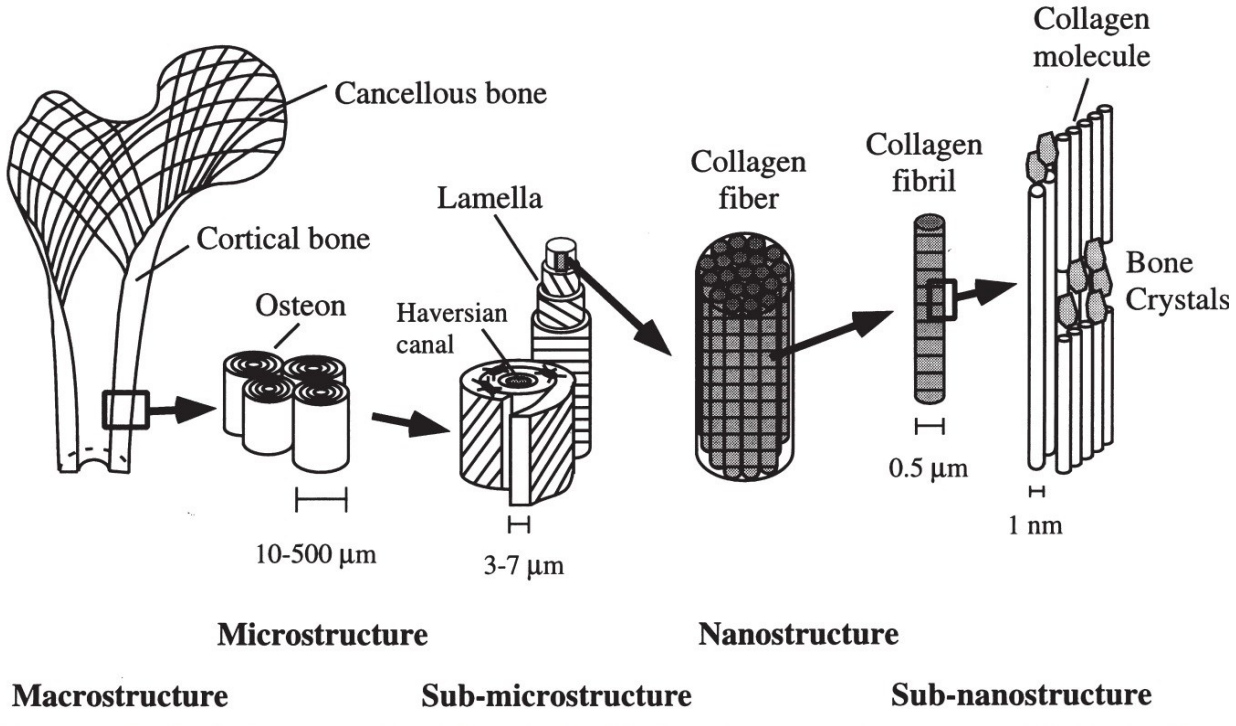

Fig. 2.1. Hierarchical structural organization of cortical bone (Rho et al, 1998)

Trabecular bone forms a lattice type structure throughout the inner surfaces of the bone and displays a porous structure, which is arranged for structural rigidity dependent upon the habitual loading patterns and also reducing the mass component of bone. Trabecular bone micro and sub-microstructure consists of

primary lamellae that are orientated parallel to the trabecular surface, it can also contain hemiosteons, which represent incomplete osteons that are not circular and therefore obtain blood supply from the adjacent marrow cavity (Burr and Allen, 2013). As in the lamellae of cortical bone, trabecular lamellae are also constructed from bundles of collagen fibrils (Burr and Allen, 2013).

2.2 Bone Remodelling

Bone remodelling is a continual process, which is controlled by osteoblasts and osteoclasts respectively. Osteoblasts are derivations of mesenchymal stem cells and differentiation depends on specific gene expression resulting from the activation of transcription factors binding with deoxyribonucleic acid (Lerner, 2012). Osteoclasts are derived from myeloid haematopoetic stem cells, which require the ligand macrophage colony stimulating factor for proliferation and the fusion of mononuclear progenitor cells. This occurs due to the activation of the RANK receptor by RANKL and the decrease in osteoprotegerin (Edwards and Mundy, 2011).

Osteocytes are also derived from mesenchymal stem cells, firstly osteoblasts are formed before becoming embedded in osteoid, the embedded osteoblasts then mineralise, which then develop into mature osteocytes that are encapsulated in the mineralised bone tissue (Bonewald, 2011). Osteocytes grow dendritic

processes that reach to the mineralising surfaces or local vascular supply and are important structures for the mechanotransduction of signaling processes to the cell body that induce bone remodeling (Burra et al, 2010). Osteocytes can activate osteoblast and osteoclast activity, the dendritic processes of osteocytes is a site that sees RANKL expression, which stimulates osteoclast differentiation. In addition, osteocyte apoptosis that is associated with osteoporosis, can also upregulate osteoclast activity through the RANKL pathway and reduce the structural integrity of bone tissue (Bonewald, 2011). Upon osteocyte apoptosis, mechanosensitivity is also reduced along with cell signaling that can then lead to reduced osteoblast activity (Bellido, 2014). A number of factors have been suggested as being preventative for osteocyte apoptosis including oestrogen, oestrogen receptor modulators and mechanical loading that stimulates canalicular fluid flow throughout the lacunocanalicular network and allows for adequate osteocyte oxygenation and nutrient delivery (Dallas et al, 2013). Osteocytes are known to increase osteoblast activity in response to mechanical loading via signaling that is transferred through the dendritic processes. Mechanical loading increases the fluid flow shear stress in bone, which in turn is thought to activate the Wnt/β-catenin pathway leading to increases in osteoblast formation (Dallas et al, 2013). Osteocytes produce insulin-like growth factor 1, prostaglandin E2, prostacyclin and nitric oxide in response to mechanical loading, all of which have an anabolic effect on osteoblast activity whereas the lack of mechanical loading causes osteocytes to produce sclerostin, which inhibits osteoblast activity through

interfering with the Wnt/β-catenin pathway (Schaffler et al, 2014).

Osteoblasts and osteoclasts form basic multicellular units (BMU) which control the modelling and remodelling processes in bone tissue. Bone modelling is the formation of new bone tissue whereas bone remodelling is the replacement of existing bone tissue in either the same orientation or in a more optimally aligned orientation to better withstand mechanical stresses and strains (Fig. 2.2.). Osteoblast cells control bone tissue formation whereas osteoclast cells control bone tissue resorption (Lerner, 2012). Throughout the maturation process osteoblast activity outweighs osteoclast activity resulting in a positive BMU balance and therefore a net BMD gain up until peak bone mass is attained somewhere between the ages of 20 to 45 years (Mein et al, 2004; Pedrazzoni et al, 2003). Around the stage of peak bone mass, the osteoblast and osteoclast activity equal one another, a normal BMU balance is achieved and BMD is maintained. With ageing, a net loss in BMD for both men and women is characterized by a negative BMU balance caused by an imbalance in bone remodelling in which osteoclast activity surpasses osteoblast activity (Looker et al, 1998; Finklestein et al, 2008). This imbalance increases the rates of bone resorption in relation to bone formation. This is usually more problematic for postmenopausal women as the reduction in oestrogen causes an accelerated loss in BMD (Cauley, 2015).

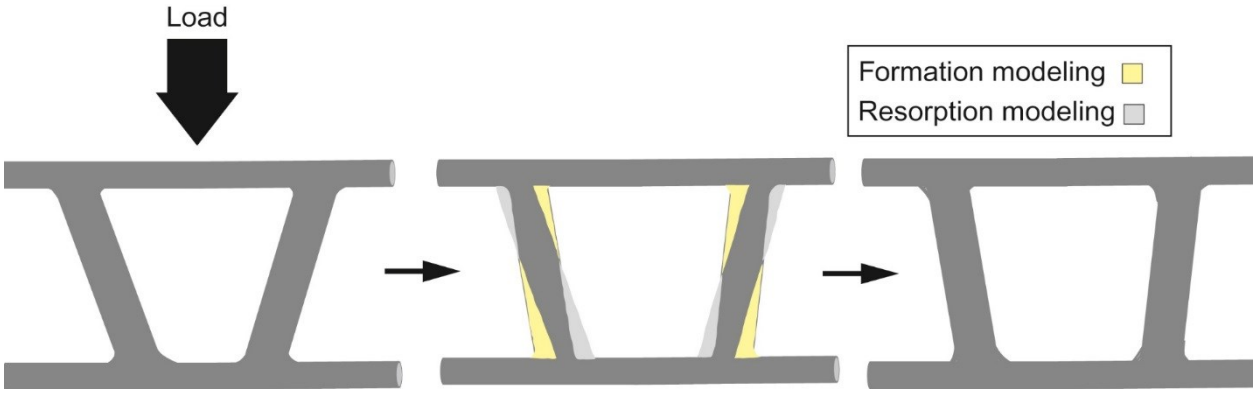

Fig. 2.2. Schematic of remodeling responses to loading in trabecular bone (Burr and Allen, 2013)

There are two types of bone formation, intramembranous ossification and endochondral ossification. Intramembranous ossification is initiated by mesenchymal stem cells typically during embryo development and the very early stages of life or during bone repair (Berendsen and Olsen, 2015). This is where stem cells differentiate into osteoblasts from which bone formation and calcification of bone matrix then occurs (Lerner, 2012). Endochondral ossification is the process that transforms hyaline cartilage to calcified bone tissue. This too begins with mesenchymal stem cells, which in this case, differentiate into chondroblasts, which then form hyaline cartilage tissue and becomes increasingly calcified (Berendsen and Olsen, 2015). From the differentiation of chondrocytes, an influx of osteoblast progenitors is stimulated, which travel into the cartilage. The osteoblast progenitors differentiate into osteoblasts and then form bone tissue. With the increase in ossification and the

mineralization process comes an increase in arteries and capillarisation to form Haversian canals in the bone sub-microstructure (Zimmermann and Ritchie, 2015).

2.3 The Effect of Mechanical Loading on Bone Tissue

Mechanical loading is a well-established stimulus for bone adaptation and remodeling, with increases in the strain stimulus giving rise to increases in the strength characteristics of bone. When a force is appliced to a bone, a "stress" is created and measured in units of pressure (force per cross sectional area). Force application causes a deformation in the structure of the bone tissue, this is measured as a "strain", which is the ratio of the change in length to the original length. Due to the stiffness of bone material, strains are usually presented in microstrain units (με) as little deformation occurs (Burr and Allen, 2013). The action of stress-strain stimuli can determine the level of subsequent bone remodeling (Turner et al, 2009).

2.3.1 Wolff's Law

Wolff (1892) stated that;

"Every change in the form and function of bone or of their function alone is

followed by certain definite changes in their internal architecture and equally definite alteration in their external conformation, in accordance with mathematical laws."

The law essentially highlights that architectural bone orientation is dependent upon the mechanical loading stresses that are experienced and that mechanical bone remodelling occurs in response to changes in bone function to redistribute and realign trabeculae for optimal strength. The law also suggests that mechanical disuse can lead to a loss in BMD, which has been examined further by Frost (1987 & 2003).

2.3.2 Frost's Mechanostat

Frost (1987 & 2003) advanced the "Mechanostat Theory", which states that bone adaptation is threshold dependent in the sense that in order for net bone formation to occur, a minimum mechanical strain threshold must be achieved. Similarly, for net bone resorption to occur, a low level strain threshold must not be surpassed. Frost outlined that there were minimum effective strains for three stages of bone adaptation; remodelling, modelling and microdamage. Habitual strains of 50-100 με are experienced during disuse and lead to net BMU resorption, 1000-1500 με are determined as the minimal effective strain for net BMU modelling and lead to bone formation, 3000 με is suggested to cause bone microtrauma and if bone

is exposed to repeated strains above this threshold value it can cause a range of non-traumatic fractures such as stress fractures (Frost, 2003). Microcracks that occur due to habitual loading are not substantial enough to alter the mechanical strength of the bone and are considered necessary for adaptation purposes (Robling et al, 2006).

Strains of 1000-1500 με can be easily attained with a variety of forms of physical activity, which highlights the importance of exercise and mechanical loading in promoting positive BMU modelling and the formation of sufficiently healthy bones (Fig. 2.3a.) (Frost, 1994 & 2003). Therefore, to optimize bone health throughout the lifespan, it is necessary to engage in correct physical activity with appropriate levels of musculoskeletal loading patterns. Levels of human tibial strain have been reported to be as high as ~ 2000 με during vigorous exercise, which would support the "mechanostat" theory (Burr et al, 1996; Frost, 1987 & 2003).

The level of strain required to fracture a normal bone is approximately 25,000 με (~120 MPa), which is equivalent to a tension that stretches the bone by 2.5%. To create this level of strain stimulus would require a stress of around 110,353,801 $N.m^{-2}$, which is highly unusual in daily living and exercise activities (Frost, 1994). Whilst the mechanostat theory provides the basis for the mechanical action on bone characteristics, there have since been numerous advances in our understanding of the mechanisms underlying bone morphology. It is now

accepted that bone adaptation is both site and strain specific, where the net changes in bone mechanical properties are determined by more factors than just strain magnitude (Robling et al, 2001; Srinivasan et al, 2002; Pivonka et al, 2018). Further contributing mechanical stimuli to bone adaptation are discussed throughout chapter 2.

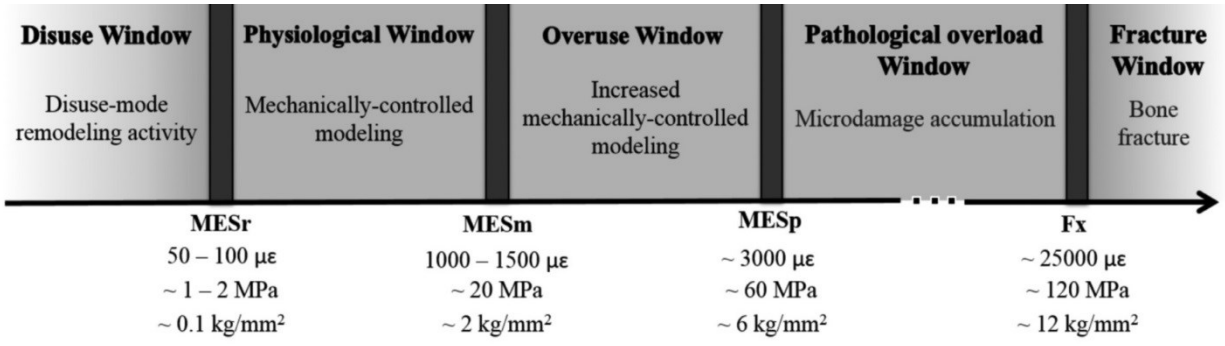

Fig. 2.3.2 Frost's "mechanostat" adapted table of mechanical strains (Rosa et al, 2015).

2.3.3 Three Rules for Bone Adaptation to Mechanical Stimuli

Since the early works regarding mechanical stimuli and bone adaptation more recent theories have been developed as a result of numerous experimental studies.

Turner (1998) outlined three rules that bone adaptation is driven by, these are:

1. Bone adaptation is driven by dynamic, rather than static, loading.

2. Only a short duration of mechanical loading is necessary to initiate an adaptive

response. Extending the loading duration has a diminishing effect on further bone adaptation.

3. Bone cells accommodate to a customary mechanical loading environment, making them less responsive to routine loading signals.

Dynamic loading has been shown to give superior bone adaptation to static loading implying that bone tissue is sensitive to rapid strain reversals as opposed to purely absolute load magnitudes (Hert et al, 1971; Liskova and Hert, 1971). High applications of strain or an increase in the strain rate is also necessary to commence the bone modelling process (Turner et al, 1995). Strain rate is also closely related to the frequency of the loading stimulus, both of which are reflected in one another as higher strain rates allow for higher loading frequencies and vice versa. Higher frequency loading is furthermore linked with greater bone adaptations than lower frequency loading (Turner et al, 1994a). Higher magnitude loads have been shown to initiate a greater bone modelling stimulus than lower magnitude loads, and it is important to note that higher load magnitudes are also closely related to higher strain rates and higher frequency loading, which both show good agreement with bone formation parameters (Rubin and Lanyon, 1985). These factors must be considered in mechanical loading situations and would suggest that higher strains, which are applied at higher strain rates, at higher loading frequencies would better stimulate bone adaptation than the lower level equivalent corresponding conditions. It is widely accepted that strain

magnitude and strain rate / loading frequency determine the level of bone adaptation to a mechanical stimulus (Turner, 1998).

In addition, just a short loading duration and low number of loading cycles can display a similar level of bone adaptation to a much greater duration and higher number of loading cycles. Bone adaptation has been stimulated with as few as four to five loading cycles per day, with many more than 36 loading cycles showing no greater statistical benefit in terms of bone adaptation (Fig. 2.3b.) (Rubin and Lanyon, 1984; Umemura et al, 1997). This indicates that bone is highly sensitive to the initial loading stimulus but then appears to desensitise when many more than 36 loading cycles are applied. The bone stimulus rapidly decreases after a small number of loading cycles where levels of mechanosensitivity give the impression of becoming saturated (Rubin and Lanyon, 1985). Whilst much of the investigation into the loading parameters for optimal bone mineral accretion has been conducted with animals in order to monitor the initial investigations on bone tissue, it is acknowledged that the application of the findings to humans can be limited (Pearce et al, 2007; Wancket, 2015). The findings often provide vital information on the bone response to loading and the subsequent adaptive processes but can only provide an insight into the adaptive responses in human beings, and until the studies are replicated with a human population the findings from animal studies are difficult to extrapolate (Pearce et al, 2007; Bracken, 2008).

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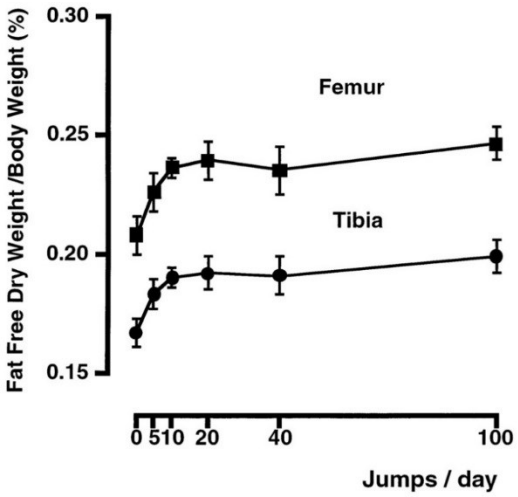


B

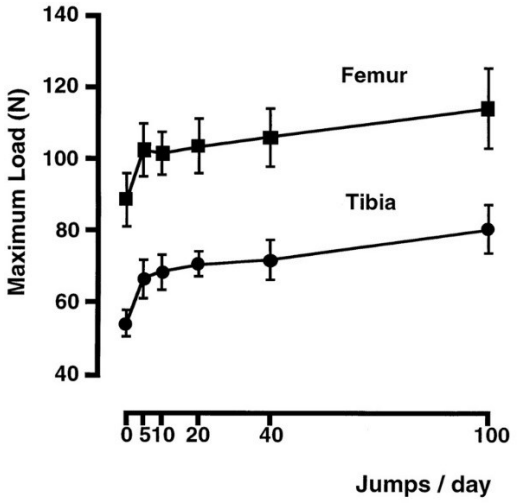


Fig. 2.3.3.

A – Femur and tibia dry weight (as a percentage of bodyweight) in rats that completed differing volumes of jumping exercise per day (Umemura et al, 1997).

B - Femur and tibia maximum strength in rats that completed differing volumes of jumping exercise per day (Umemura et al, 1997).

2.3.4 Young's Modulus

Due to the presence of collagen, bone has a somewhat elastic property and can deform under certain mechanical stresses before recoiling back to the original geometric shape. Leonhard Euler developed the notion of the Young's modulus in 1727, which is the relationship between the stress applied to an object and the subsequent level of deformation, which occurs. Therefore, the higher the Young's modulus, the greater the stress force needed to deform the object. It is a measure of mechanical stiffness. As outlined above, a certain level of strain is required to initiate bone adaptation.

Numerous studies have investigated the mechanical properties of both cortical and trabecular bone, which should be considered as mechanically different when it comes to their material properties (Rho et al, 1993). Cortical bone possesses a much higher compressive strength and also mechanical stiffness than trabecular bone, which has been verified by numerous researchers (Rho et al, 1993; Rho et al, 1998; Hench, 1998; Amaral et al, 2002);

Table 2.3.4 Mechanical properties of cortical and trabecular bone, adapted from Hench et al, 1998 and Amaral et al, 2002

	Cortical bone	Trabecular bone
Compressive strength	100-230 MPa	2-12 MPa
Bending strength	50-150 MPa	NA
Young's Modulus	7-30 GPa	0.05-0.5 GPa

Such is the nature of cortical bone, that it can have over four times higher compressive strength than concrete (Rahal, 2007).

2.4 Mechanical Stimulation of Osteocytes

From mechanical stimulation, bone modelling occurs with a positive BMU balance. However, there is still some debate as to exactly how this mechanical signalling arises from a loading stimulus and then generates an increased osteoblast to osteoclast activity ratio. Bone adaptations to mechanical loading are site specific and are confined to areas that experience high stresses and strains (Hawkins et al, 1999; Kuruvilla et al, 2008; Sugiyama et al, 2010). A number of bone signalling processes have been suggested, which all originate from site

specific mechanical loading.

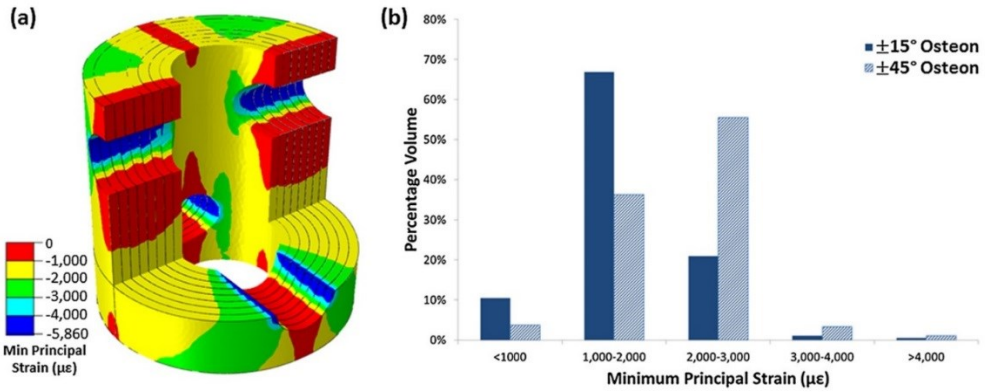
2.4.1 Cell Deformation

When a strain is applied to a bone, a minor deformation occurs in the macrostructure, which heightens the internal stress environment (Vaughan et al, 2013). This type of mechanical strain is purported to cause osteocyte cell deformation where application of a bending force causes the proximal concave surface of the bone to experience compression and the distal convex side of the bone to experience tension (Schaffler et al, 2014).

The presence of Haversian and Volkmann canals can alter the loading stimuli at different locations throughout the bone tissue. This combination of strains can both compress and lengthen osteocytes, which experience strains of up to nine times above bone surface strains and can activate the necessary bone modelling processes (McGarry et al, 2005; Vaughan et al, 2013). Finite element models of osteon strain distribution have shown that osteocytes surrounding these channels in bone tissue, can experience much greater strains than other parts of the bone tissue. For example, when the osteon experiences a habitual longitudinal strain (±15˚ to the longitudinal direction) of 2000 με, it is further amplified if the osteon is aligned at ±45˚ to the longitudinal direction (Fig. 2.4.1.). This process causes osteocyte cells to experience relatively large cell deformations, which are

detected by the dendritic processes and trigger a biochemical reaction that upregulates osteoblast activity and bone modeling (Dallas et al, 2013; Rosa et al, 2015).

A



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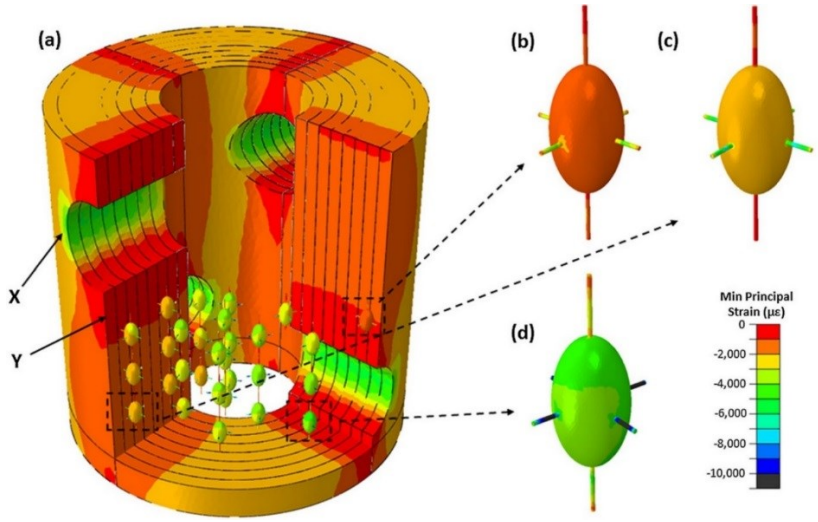


Fig. 2.4.1. Minimum principal strain distribution model of an osteon (A), (a) Distribution of minimum principal strain in a sectioned view of the osteon model and (b) volume average distribution of minimum principal strain in the Osteon model.

(B) Distribution of minimum principal strain in a number of different osteocytes located in different regions (a to d) of the osteon (Vaughan et al, 2013).

2.4.2 Fluid Flow Stress

Another mechanism, which may cause the mechanical stimulation of bone remodelling is fluid flow stress within the internal structure of bone. Internal hydrostatic pressures change dependent upon external loading forces, which subsequently deform the bone macrostructure and increase the internal fluid pressure (Fig. 2.4.2.). Simulations of vigorous activity have shown that high levels of shear stress and fluid velocities are present, which have been closely linked with the mechanosensitivity of the osteocyte (Jacobs et al, 1998; Verbruggen et al, 2014). The generation of in-vitro fluid flow stress has also shown to enhance bone matrix production (Delaine-Smith et al, 2012). Canalicular fluid flow has been shown to respond maximally following the first loading cycle before gradually returning to a homeostatic environment with the application of multiple loading cycles. This provides yet further support to the possible saturation effect that desensitises bone tissue to repeated mechanical loading stimuli (Srinivasan and Gross, 2000).

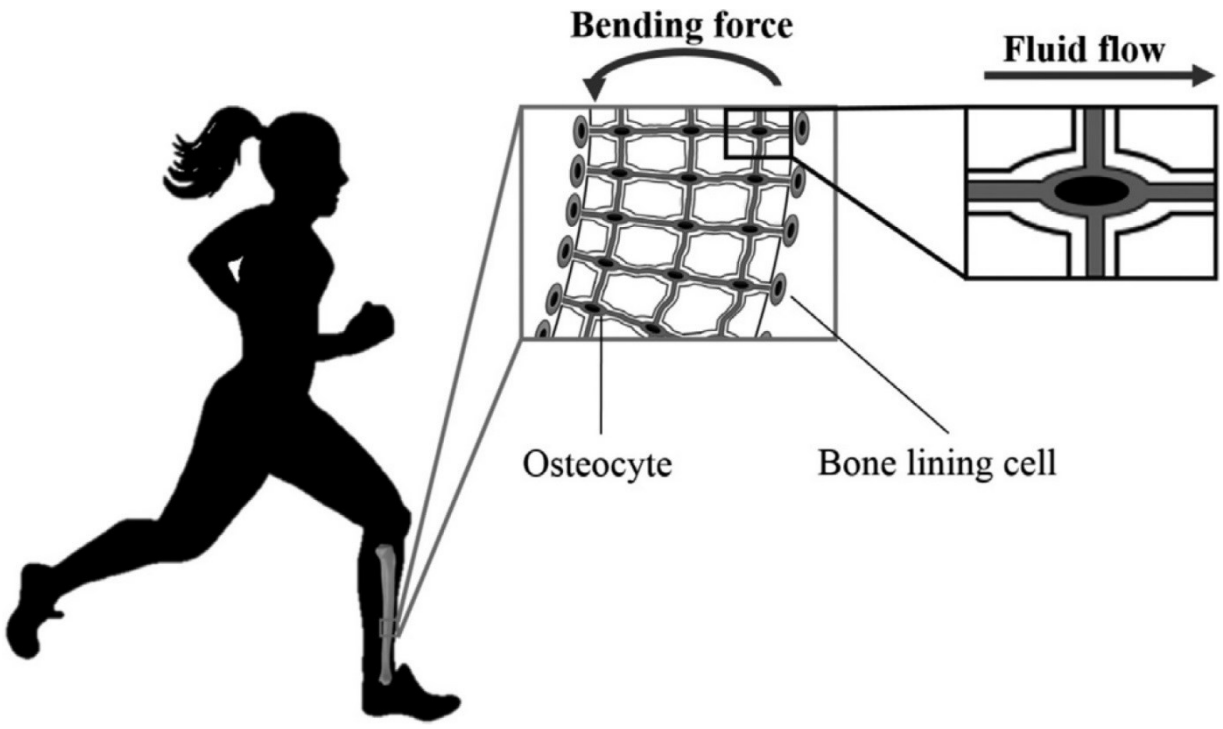


Fig. 2.4.2. Diagram of strain induced bone fluid flow (Rosa et al, 2015).

2.4.3 Streaming Potentials

Electromagnetic fields have been known to stimulate a positive BMU balance when applied in-vivo (Klein-Nulend et al, 2013). Such electromagnetism is reported to arise from negatively charged interstitial fluid flow that alters the flow of ions resulting in a small voltage production. It is this voltage, which is thought to positively affect the BMU balance.

2.4.4 Piezoelectric Effect

Piezoelectric effect is a largely uninvestigated area in which deformations in cortical bone tissue are thought to bring about changes in electrical potential. These changes in electrical potential are presumably detected by osteocytes and increase the net BMU balance to stimulate remodeling, although supporting literature on this process does not currently exist (Ahn and Grodzinsky, 2009). However, in wet bone tissue many authors attribute the change in electrical potential (induced by loading) to streaming potentials. Current thinking states that piezoelectric effect could even contribute to streaming potential.

2.4.5 Microdamage

Microscopic cracks in the bone architecture can occur due to high levels of strain, particularly if the bone is exposed to many repeated strains that exceed ~3000 με (Frost, 1987 & 2003). The presence of microdamage has been linked with a positive BMU balance in the affected area, which suggests damage-driven targeted optimization of the internal bone architecture (Burr et al, 1993; Robling et al, 2006).

Whilst there may be a number of factors that trigger the bone adaptation process, the majority of the current evidence lies with cell deformation and fluid flow stress pathways (Rosa et al, 2015). Further investigation will inform future

practice in this area.

2.5 The Effect of Endocrine Signalling on Bone Tissue

Endocrine signaling as a result of mechanical loading has shown to contribute to the bone adaptation process (Gardinier et al, 2015). This section details some of the endocrine effects that act on bone as a result of mechanical loading.

2.5.1 Parathyroid Hormone

Parathyroid hormone (PTH) is a powerful regulator of both bone formation and also bone resorption. The net effect of each depends upon how PTH is released from the parathyroid glands. PTH is one of the upregulating factors for RANKL expression, RANKL binds to RANK, which then controls the BMU action and balance through facilitating or inhibiting osteoclast activation (Silva and Bilezikian, 2015). PTH can generate a negative BMU balance when stimulated with high levels in a continuous manner but can also generate a positive BMU balance when stimulated with low levels in an intermittent manner (Bellido, 2014). As such, the intermittent administration of PTH has shown to be an osteoanabolic form of therapy for osteoporosis sufferers (Greenspan et al, 2007).

Exercise studies have shown that running can stimulate increases in PTH

concentrations (Bouassida et al, 2006). Continuous exposure can decrease the BMU balance whereas intermittent exposure can increase the BMU balance. The PTH secretion can change dependent upon the type of exercise activity and it is therefore possible that intermittent exercise could provide a greater anabolic stimulus for bone formation than continuous exercise that is completed over long durations (Bouassida et al, 2003; Bouassida et al, 2006).

Intermittent PTH infusion can play an important role in the mechanosensitivity of bone tissue and has demonstrated a reduction in the level of mechanical strain required to elicit an osteogenic response. The effects of mechanical loading and intermittent doses of PTH on bone tissue have been reported as synergistic (Sugiyama et al, 2008; Moustafa et al, 2009).

2.5.2 Oestrogen

Oestrogen levels have been known to affect the bone response to mechanical loading, with lower levels of serum oestrogen contributing to a decline in bone mass (Price et al, 2011). This is thought to be due to the BMU balance that is offset in favour of higher levels of bone resorption (Burr and Allen, 2013). Oestrogen has been proposed to directly block the activity of osteoclasts through inducing apoptosis and upregulate the activity of osteoblasts and osteocytes, which stimulates an increase or maintenance of bone metabolism (Khosla et al,

2012). When linked to the effect of mechanical loading, oestrogen deficiency can therefore potentially blunt the positive action of loading on bone adaptation (Cauley, 2015; Carson and Manolagas, 2015).

2.5.3 Vitamin D

25-hydroxy vitamin D (25 (OH) D) is a steroid hormone that facilitates the absorption of calcium in the gut, that is formed from the ingestion of vitamin D in certain foods or from subcutaneous synthesis as a result of sunlight exposure (Cianferrotti et al, 2015). Deficiency in 25-hydroxy vitamin D (25 (OH) D) can induce secondary hyperparathyroidism, which can lead to high rates of bone loss (Arabi et al, 2012). Higher vitamin D intakes are associated with improved muscle and bone health in postmenopausal women (Rizzoli et al, 2014). Deficiency can alter the osteogenic effect of mechanical loading and exercise on bone tissue by reducing the osteoblast activity that produces osteocalcin and osteopontin for the process of bone mineralisation (Morris et al, 2010; Lanske et al, 2014).

2.6 Osteoporosis

Osteoporosis is a degenerative disease, which is characterised by very low bone density levels at the femoral neck that are 2.5 standard deviations below the

average value for a population of young adults and is assessed by dual-energy X-ray absorptiometry (DXA). These values differ for men and women respectively and are used by practitioners for diagnosis purposes (Kanis et al, 2008a).

The World Health Organization defines osteoporosis as;

"a BMD that lies 2.5 standard deviations or more below the average value for young healthy women (a T-score of <-2.5 SD)" (World Health Organization, 2007).

In the EU and the UK, 46 – 50% of women and 20 – 22% of men over the age of 50 will experience an osteoporotic fracture (van Staa et al, 2001; Hernlund et al, 2013). This high number of fractures generate a medical treatment cost of £3,496 million per year, which is funded by the NHS, this figure is expected to increase an extra 25% by 2025 due to the ever-rising cost of the ageing population (Svedbom et al, 2013; United Nations Department of Economic and Social Affairs, 2017). The most traumatic of these cases is undoubtedly a hip fracture, which causes a 20 - 24% mortality rate within one year of fracture incidence, whether directly or indirectly through the debilitative consequences that they can cause (Cooper et al, 1993; Leibson et al, 2002; Klop et al, 2014).

The most common forms of osteoporosis are caused by a loss of bone mineral density (BMD) during the ageing process, which leaves populations at a higher risk of experiencing fractures (Berard et al, 1997; Shipman et al, 1999; Lems,

2007). Low BMD and the development of osteoporosis are strong predictors of increased fracture risk although other factors can also contribute such as advanced age (Fig. 2.6a.) (Hui et al, 1988; Kanis et al, 2001; Pesonen et al, 2005; Kanis et al, 2009).

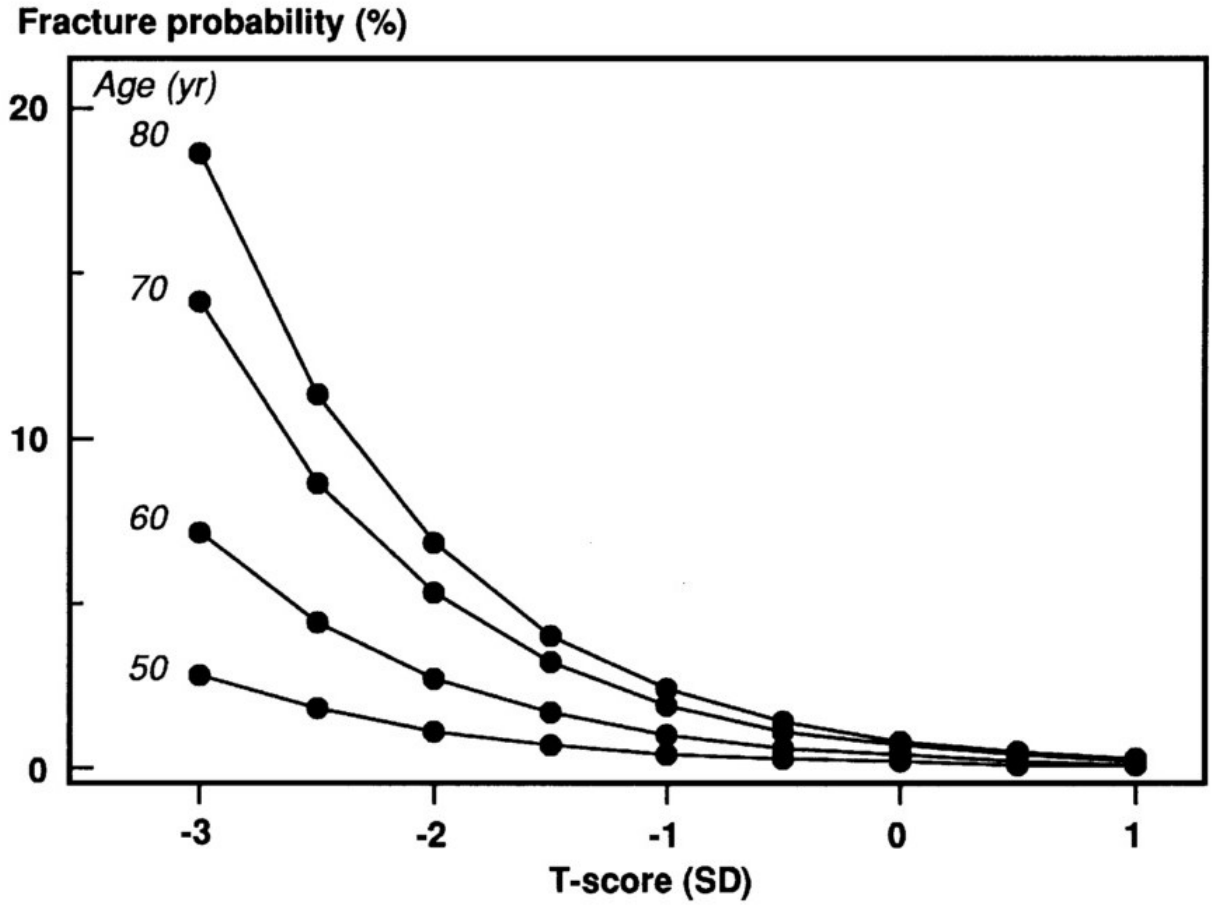


Fig. 2.6a. The relationship between T-score, age and fracture probability (Kanis et al, 2001)

Postmenopausal osteoporosis occurs in women within 15 to 20 years of the menopause. This form of osteoporosis is where women experience rapid declines

in BMD early after the menopause, which leaves them susceptible to reduced bone strength (Raisz, 2007; Burr and Allen, 2013) (Fig. 2.6b.).

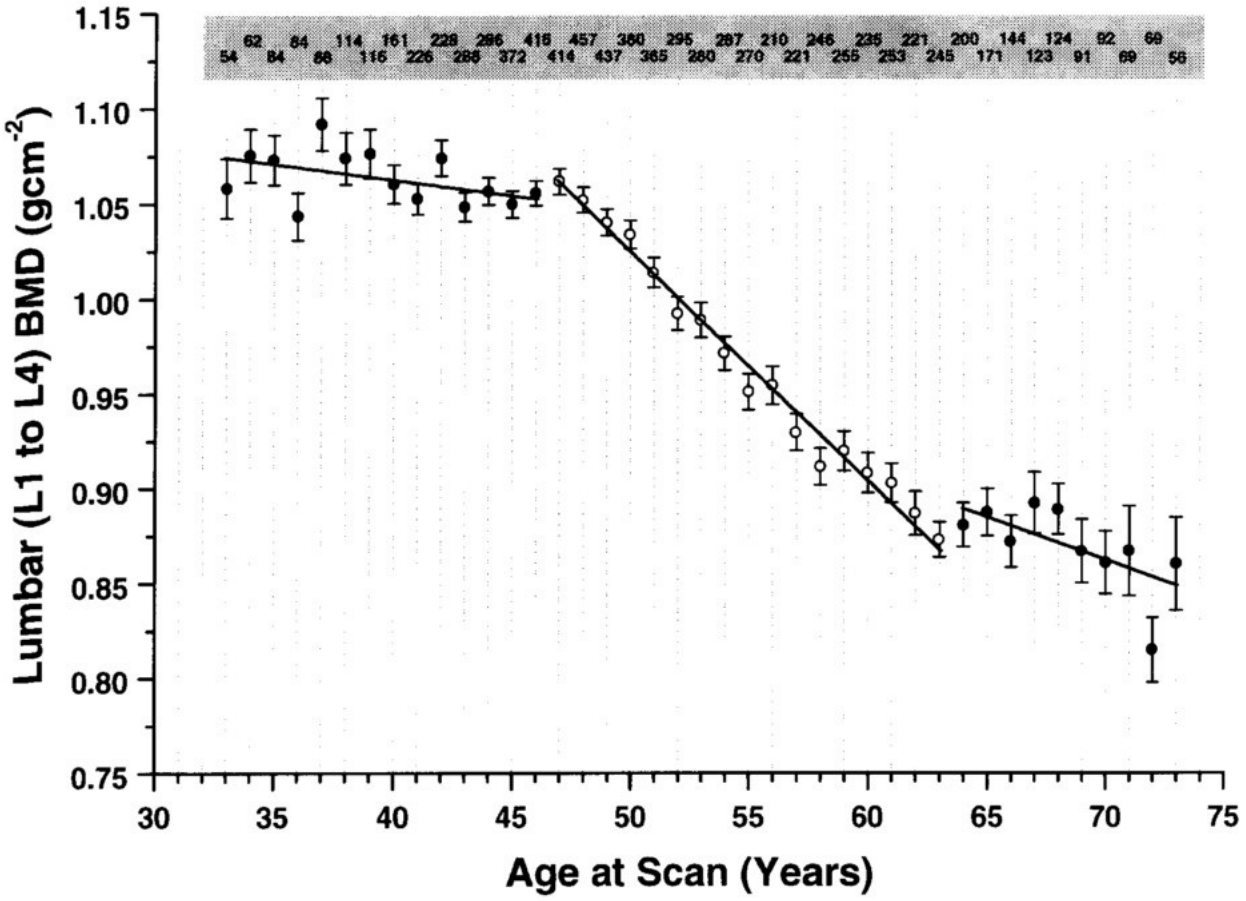


Fig. 2.6b. Cross sectional analysis of lumbar BMD of 8783 Caucasian women (Shipman et al, 1999)

A normal BMD loss in the early years post menopause can easily equate to between 1 – 2% per year (Shipman et al, 1999; Finkelstein et al, 2008). This phenomenon relates to the loss of oestrogen, which has a marked effect on the reduction of trabecular and cortical bone. Trabeculae become disconnected and

thin whereas cortical bone becomes more porous, both of these processes weaken the mechanical structure of the bone. The most devastating factor for bone strength is the actual loss of trabeculae as opposed to trabecular thinning (Van Der Linden et al, 2001). When the trabeculae structures become disconnected the bone is subjected to a much greater level of fragility than if the trabeculae become simply thinner structures. Similar amounts of cortical and trabecular bone are lost during the first 10 years after the menopause despite a more rapid loss of trabecular bone. This is due to the overall larger proportion of cortical bone, which, whilst it is lost at a slower rate, experiences a comparable absolute bone loss (Seeman, 2013). The loss of oestrogen after the menopause contributes to an increase in PTH sensitivity, which leads to elevated levels of calcium resorption. Decreased vitamin D production reduces the uptake of calcium ions, which then causes bone demineralisation and compromises structural integrity. This degenerative process increases the risk of fractures (Cauley, 2015; Klein-Nulend et al, 2015).

Age related osteoporosis typically affects both men and women over the age of 70 years and is caused by lower calcium absorption rates, complications with vitamin D metabolism and diminished stem cell numbers, which decreases the number of osteoblasts (Burr and Allen, 2013). This triggers bone loss through a negative bone turnover balance.

Secondary Osteoporosis can be caused by a variety of diseases, drug treatments

or nutritional deficiencies (Baim et al, 2015). This thesis will focus primarily on the prevention of postmenopausal and age related osteoporosis.

Osteopenia is defined by bone density levels at the femoral neck that are 1 to 2.5 standard deviations below the average value for a population of young adults and is also assessed by DXA scans (World Health Organization, 2007).

2.7 Dual-Energy X-Ray Absorptiometry

Whilst a number of bone density measuring techniques are available including radiography (X-ray), quantitative ultrasound (QUS), dual photon absorptiometry (DPA), magnetic resonance imaging (MRI), computed tomography (CT) and peripheral quantitative computed tomography (pQCT), it is DXA scans that are the gold standard method of BMD assessment. The femoral neck site is advocated by the International Osteoporosis Foundation as the gold standard measure for the diagnosis of osteoporosis due to the extensive validation of this and output of graded fracture risk (Kanis et al, 2013). The two most common sites for DXA BMD assessment are the lumbar spine L1 – L4 and the hip, which encompasses the femoral neck, wards triangle, trochanter, shaft and total hip BMD measures. Both sites, in practice, can show discordance in their T-score classification and the difference can be exacerbated with the ageing process due to the highly variable rates of bone loss at different measurement sites (Arlot et al, 1997;

O'Gradaigh et al, 2003). Although femoral neck BMD is the gold standard measure, analysis of multiple sites prone to fracture is recommended (Lee et al, 2015).

DXA scans return areal bone density scores from a two dimensional (2D) plane of view in which the units given are grams per centimetre squared (g/cm^2). The BMD value obtained is used as a suitable proxy for bone strength ($r^2 = 0.59$ to 0.88), as the actual strength of a bone cannot be evaluated in a living human and therefore must be estimated with non-invasive scanning methods (Cheng et al, 1997). BMD measurement, whilst not totally representative of mechanical strength, is highly correlated with compressive yield stress ($r = 0.64$; $P < 0.005$), and Young's modulus ($r = 0.69$; $P < 0.005$), and therefore serves as a useful measure to indicate bone strength and stiffness (Wachter et al, 2002; Gibson, 2005).

The density value is based upon the levels of X-ray absorption, as measured by X-rays that are taken from above the measurement space and travel through the body where they are detected upon leaving the body. Two different energy X-rays are used, one to detect soft tissue density, another to detect total density. Two energies are used to allow for correction of soft tissue mass and determine the BMD in each pixel of the scan.. BMD values are obtained by dividing the bone mineral content (BMC) by the 2D area of the site measured (Berger, 2002). The greater the density of the object measured, the greater the proportion of the X-ray

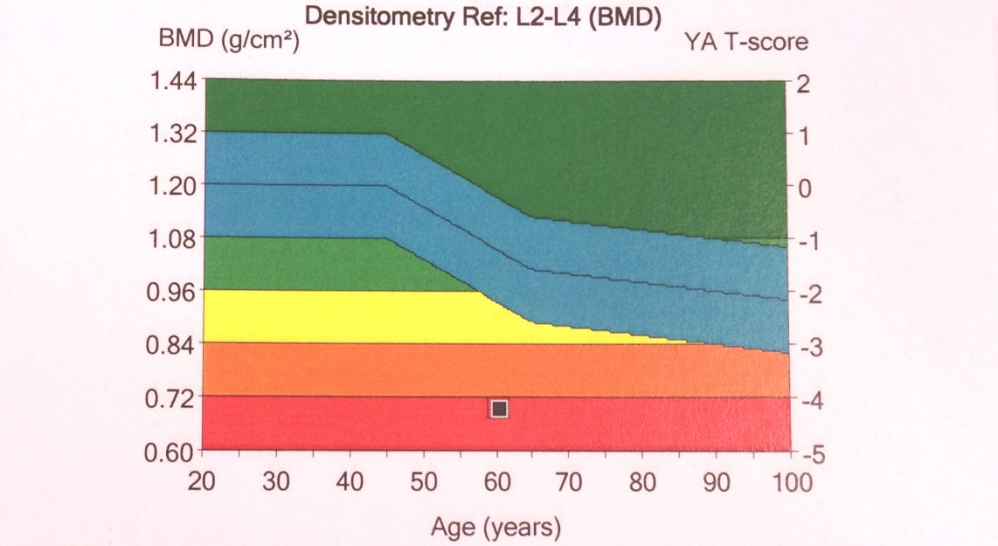
signals that are absorbed whilst passing from one side of the scanner to the other. DXA scans provide a very low dose of ionizing radiation (~3 μSv) as do pQCT scans (<10 μSv), which are seen as preferable to other techniques that give much higher levels of ionizing radiation (1.5 to 2.5 mSv) through multiple scans that construct a 3D image such as a lumbar spine or proximal femur QCT scan (Adams, 2009; Krug et al, 2010). One limitation of this technique is that the third dimension (3D) is omitted from the analysis and the depth component of the bone is ignored, because the scans can only calculate BMD in 2D, this doesn't account for the bone geometry in 3D, which greatly contributes to the mechanical properties of the bone (Felsenberg and Boonen, 2005). In addition to this, DXA results cannot identify the proportions of cortical and trabecular bone, which are present or the geometric properties of the sites involved, they merely give an estimation of the density properties. MRI and CT scans can determine the proportion of cortical and trabecular bone along with identifying the 3D geometry of the bone, which gives a more holistic and accurate estimation of bone strength (Krug et al, 2010; Baum et al, 2013; Johnston et al, 2014; Bandirali et al, 2015).

The reference range for femoral neck bone density measurements in Caucasian women (20-29 years of age) is the National Health and Nutrition Examination Survey III reference database (Looker et al, 1998). This reference range allows clinicians and researchers to establish a comparison between their patient/participant's BMD and the BMD of a normative healthy population. The

comparison is usually given in the form of a T-score.

2.8 T-Scores

DXA outputs are given as BMC, BMD, bone area, T-scores and Z-scores. T-scores are used for the diagnosis of osteoporosis and present the BMD measure as the number of standard deviations above or below the average BMD value of a young and healthy reference population (Looker et al, 1998; Kanis et al, 2008a). As such, a T-score of 0 indicates that the BMD measure is the same as an average young healthy population, whereas a T-score of -1 to -2.5 indicates osteopenia, and a T-score of less than -2.5 indicates osteoporosis (Fig. 2.8.).



Region	1 BMD (g/cm²)	2 Young-Adult T-score	3 Age-Matched Z-score
L1	0.651	-4.0	-2.8
L2	0.676	-4.4	-3.1
L3	0.700	-4.2	-2.9
L4	0.709	-4.1	-2.9
L1-L4	0.685	-4.1	-2.9
L2-L4	0.695	-4.2	-3.0

Fig. 2.8. Bone Density Report (anonymous participant BMD record, Montgomery, 2016)

T-scores are usually calculated for the lumbar spine and femoral neck regions. Lumbar spine measures are either given as L2 – L4 as is sometimes used by clinicians or preferably L1 – L4 as supported by the International Society for Clinical Densitometry (ISCD) (Baim et al, 2015). T-scores are preferred for the BMD measurement of postmenopausal women and men aged 50 years and above.

Z-scores present the BMD measure as the number of standard deviations above

or below the healthy reference age-matched average BMD value. As this is an age matched comparison, Z-scores are preferred for the BMD measurement of premenopausal women and men under the age of 50 years (Baim et al, 2015).

2.9 Hip Structural Analysis

Hip structural analysis (HSA) is an advanced technique that can be obtained from DXA imagery. Whilst direct estimations of the geometric properties of the hip cannot be obtained through 2D DXA scans, they can be estimated using predictive algorithms, which assume that the sites of interest possess a cylindrical shape. This technique can be useful as a change in the geometric properties of a bone can either increase or decrease the mechanical strength of the bone and alter the subsequent fracture risk. A larger cross sectional area for a given level of BMD would possess greater bending and axial strength. Geometric changes of these kind are not reflected in traditional BMD measurements, as it is possible to show a change in geometry without any change in density (Felsenberg and Boonen, 2005). A number of variables can be calculated such as:

Hip axis length, femoral neck area, femoral neck cross sectional area, femoral neck buckling ratio, femoral neck cross sectional moment of inertia, femoral neck section modulus, femoral neck strength index, femoral neck cortical width, femoral neck cortical ratio, femoral neck shaft angle, femoral neck minimum

width.

Hip axis length is the distance between the inner pelvic brim to the greater trochanter. Femoral neck area is the 2D area of the femoral neck region. Femoral neck cross sectional area is the cross sectional area of the femoral neck. Femoral neck buckling ratio is the ratio of the femoral neck radius to the cortical thickness. If the ratio exceeds a value of 10, it is indicative of local instability and potential structural failure. Femoral neck cross sectional moment of inertia is the moment of inertia of the cross section of the femoral neck. Femoral neck section modulus is the femoral neck cross sectional moment of inertia divided by distance from the centroidal axis to the edge of the section. It is inversely related to the maximum bending stress in that section. Femoral neck strength index is an estimate of fracture risk calculated from cross sectional moment of inertia, cross sectional area, BMD, height and weight. Femoral neck cortical width is the estimated thickness of cortical bone of the femoral neck. Femoral neck cortical ratio is the ratio of cortical and trabecular bone of the femoral neck. Femoral neck shaft angle is the angle between the line of the shaft of the femur and the line of the femoral neck that travels through the femur centre. Femoral neck minimum width is the minimum width of the femoral neck (Khoo et al, 2005; Broy et al, 2015; Beck and Broy, 2015).

Hip axis length is the only variable to show an association with fracture risk and is the only variable supported by the ISCD whereas for the majority of other

variables, whilst they might be of use in some instances, they display much poorer precision than DXA BMD values due to complications with participant positioning consistency (Khoo et al, 2005) and have yet to show a convincing relationship to actual fracture risk (Broy et al, 2015).

2.10 Clinical Risk Factors for Osteoporosis

Whilst the assessment of BMD is an important factor to consider in the assessment of mechanical bone strength, a number of other clinical risk factors exist that can have a large influence on future fracture risk (Kanis et al, 2008b). The fracture risk assessment tool (FRAX^{TM}) was developed by the WHO to assess the likelihood of hip, spine, radius and humeral fractures over a 10-year period, this can be used in addition to current femoral neck BMD results or as a stand alone predictor of fracture risk (Kanis et al, 2008b). The eight clinical risk factors used in FRAX^{TM} are; history of fragility fracture, parental hip fracture, smoking, the use of glucocorticoids, high alcohol intake (>3 units per day), body mass index (BMI), rheumatoid arthritis and causes of secondary osteoporosis (Kanis et al, 2008b). Secondary causes of osteoporosis include; untreated hypogonadism, inflammatory bowel disease, immobility, organ transplantation, type 1 diabetes and thyroid disorders (Kanis et al, 2008b). FRAX^{TM} has shown to estimate comparable fracture risk gradients per SD (1.4 to 2.1) to soley using BMD values (1.2 to 3.7), which highlights the usefulness for using FRAX^{TM} when the

measurement of BMD is unavailable, in order to identify patients at high risk of fracture (Kanis et al, 2015). Despite this, FRAX^{TM} does not account for the dose response nature of factors like smoking, alcohol consumption or glucocorticoid use for which a greater consumption would more negatively affect bone tissue (Kanis et al, 2009). In addition to this, the FRAX^{TM} has been criticised for the poor sensitivity for fracture prediction as measured by the area under the receiver operating characteristic (AU-ROC) curve (0.63 [95% CI; 0.56 to 0.69]), which when combined with extra risk factors has provided extra benefit for fracture detection when combined with BMD measurements (0.69 [95% CI; 0.63 to 0.72]), but did not improve fracture prediction ($P = 0.16$), when compared to hip BMD alone (0.66 [95% CI; 0.60 to 0.73]) (Tremollieres et al, 2010). Only three factors; fracture history, having ≥ three pregnancies or current HRT, have shown to predict a major osteoporotic fracture independent of BMD and age, two of which are not included in the current FRAX^{TM} (Tremollieres et al, 2010). To the contrary, the developers of the FRAX^{TM}, have found that fracture risk sensitivity is actually improved when combined with femoral neck BMD when compared to either FRAX^{TM} or femoral neck BMD alone (Johansson et al, 2009). Future extraskeletal risk factors to consider in fracture risk assessment might include fall incidence and type 2 diabetes to potentially predict fractures with greater accuracy (McCloskey et al, 2016). Whilst clinical risk factors can potentially improve the detection of fracture risk, it appears that the assessment of hip BMD is still a valid and reliable indicator of future fracture risk as a stand-alone

measurement (McCloskey et al, 2016).

2.11 Continuous and Intermittent Mechanical Stimulation in Animals

The bone saturation stimulus has been mentioned previously, and dictates that bone cells become accustomed to repeated mechanical loading cycles, and that more than ~40 cycles provides little extra bone adaptation. This research has given rise to a number of projects which have evaluated the effects of rest-inserted mechanical loading (Robling et al, 2001; Robling et al, 2002a; Robling et al, 2002b; Srivivasan et al, 2007; Srinivasan et al, 2015). It is important to determine whether the saturation effect can be altered with either; multiple bouts of loading that are separated by considerable rests or inter-cycle rest periods where each single load application in a multiple loading bout is separated by a brief rest interval.

Early research in this area identified that the rat tibia can express the same level of osteogenic response to multiple loading bouts, providing 24 hours of rest separated each successive bout (Chow et al, 1993; Forwood et al, 1994). Further research established that recovery periods of two to three hours are sufficient to return the bone tissue to the original level of mechanosensitivity when undertaking multiple loading bouts. Exercise that has been divided into four and six bouts have shown to provide a 65 to 94% greater osteogenic response than a

single load matched bout of mechanical stimulation (Robling et al, 2000). This highlights that a saturation effect that can occur in bone tissue, but also that mechanosensitivity can be restored by appropriate rest intervals. Following on from this discovery, a number of other projects ensued with the next one providing a comprehensive overview of how mechanosensitivity is restored with both inter-loading cycle rests and inter-loading bout rest intervals. Robling et al, (2001) found that in delivering 36 loading cycles of a 54 N bending stimulus at a frequency of 2 Hz to a rat tibia, the stimulus was much more potent and osteogenic if loading cycles were separated by a 14 second rest interval as opposed to cycles that were applied every 0.5, 3.5 or 7 seconds (Fig. 2.11a & Fig. 2.11b.).

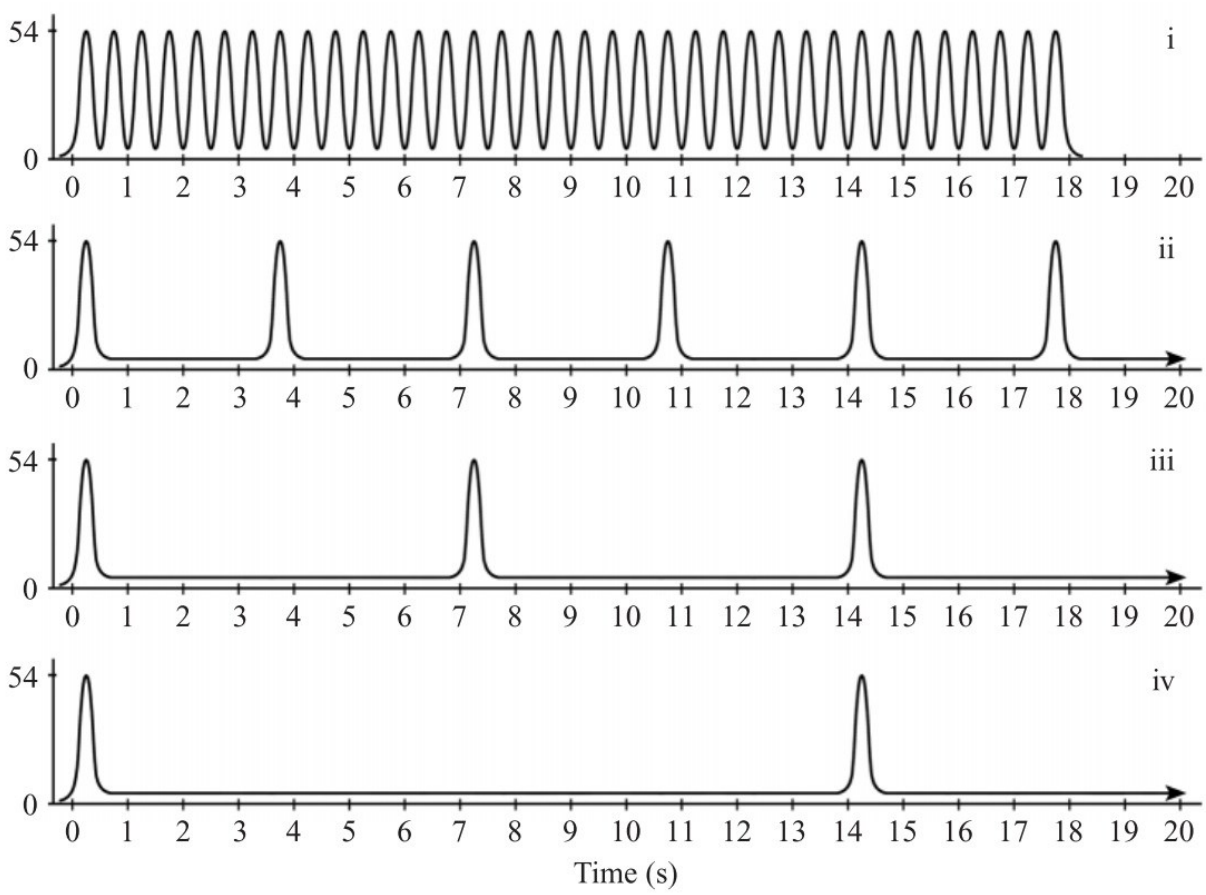


Fig. 2.11a. Application of loading cycles with inter-cycle rests. Rats were administered 36 load cycles per day with one of the following recovery periods introduced between cycles: 0.5 s (back-to-back cycles, i), 3.5 s (ii), 7 s (iii) or 14 s (iv). The 0.5 s group was loaded for 18 s (36 cycles at 2 Hz), the 3.5s group was loaded for 2 min, the 7 s group was loaded for 4 min and 14 s group was loaded for 8 min (the total duration of the loading session is not shown on the figure for the 3.5, 7 and 14 s groups) (Robling et al, 2001).

Interestingly, the loading was designed to deliver peak strains of 2400 με to the lateral periosteal surface and 1300 με to the lateral endocortical surface, which

would stimulate a positive net BMU balance according to the "mechanostat" theory. This current investigation shows supporting evidence for the theory with the increased levels of bone formation in the loading groups (Frost, 1987 & 2003).

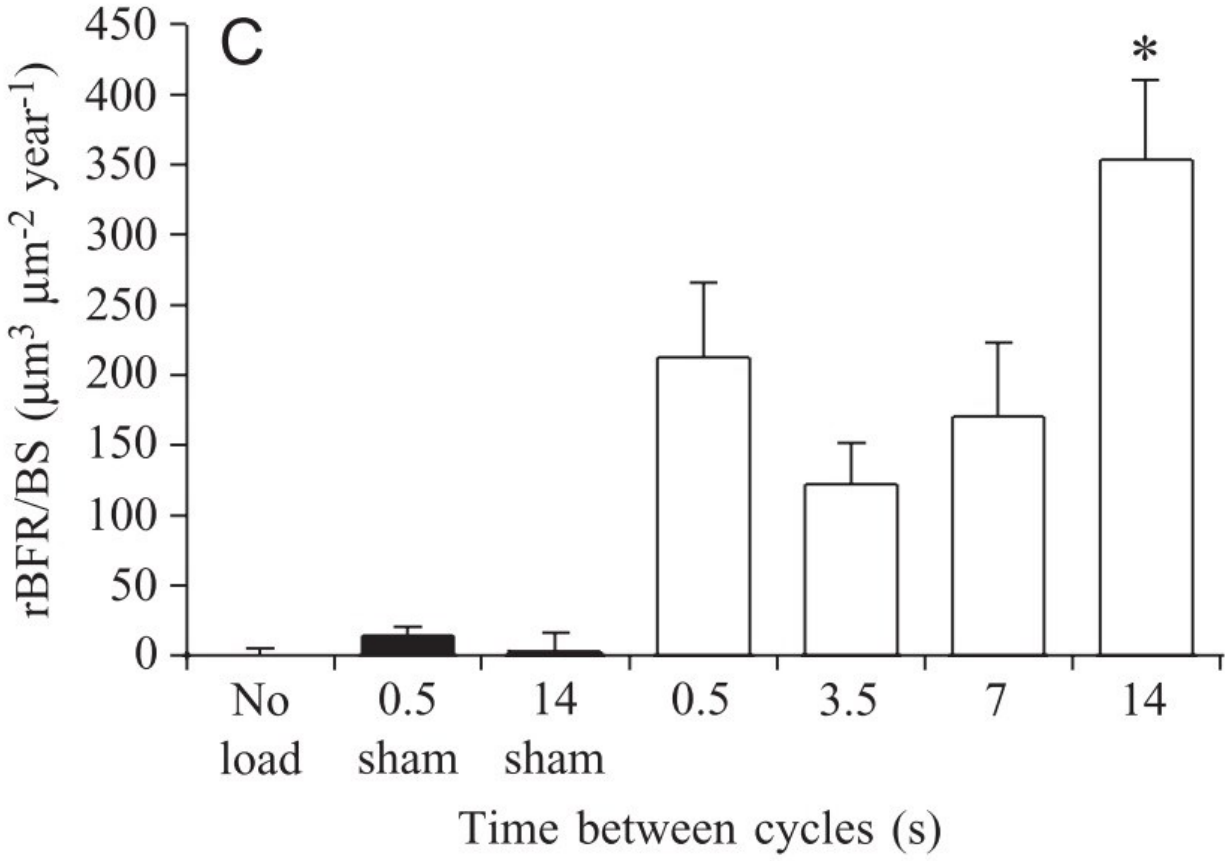


Fig. 2.11b. Relative bone formation rates for sham operated groups and loading cycle groups with 0.5, 3.5, 7 or 14 seconds of inter-cycle rest, * denotes a statistically significant difference from the 0.5, 3.5 and 7 s groups (Robling et al, 2001).

It is important to note that whilst all loading groups displayed a positive response

in terms of bone formation parameters, the more continuous loading groups (with less inter-cycle rest) were superseded by the more intermittent loading group (with 14 s inter-cycle rest). It is therefore possible that the brief rest interval could create a more potent anabolic stimulus for bone formation through mechanically resensitising the bone to the repetitive loading cycles. It is unknown if a longer rest interval would yield greater increases in the endocortical surface of the tibial shaft properties as the rest intervals were limited to < 14 s. As only, the endocortical surface site was measured for mineralization properties, the potential for applying these findings to other sites of interest remains to be quantified. How these results might relate to humans is unknown at the present moment and requires investigation.

The same experiment examined the effect of delivering loading cycles as either a single bout or multiple bouts with differing amounts of inter-bout rest intervals. When comparing 360 of the same loading cycles given in a 24 hour period, it was found that the group that experienced four bouts of 90 cycles with an eight hour inter-bout recovery period, displayed the greatest relative bone formation rate when compared to the volume matched equivalent groups with inter-bout rest intervals of 0 (all 360 cycles delivered in one single bout), 0.5, 1, 2 or 4 hours. A statistically significant difference was present between the eight hour group and the 0.5 hour rest interval group. It was consequently concluded that in order to restore full bone mechanosensitivity, an eight hour rest interval is required. It is

unknown how results might change if the rest interval exceeded eight hours and if greater potential for adaptation would be generated. Similar results were found for a 16 week study, which compared continuous cyclic loading to multiple bouts of the same volume equated stimulus but also added the extent to which aBMD and BMC were increased (Fig. 2.11c.). Separating the stimulus into multiple bouts lead to an 87% greater mechanical strength compared to 64% for the single bout group and a 165% greater fracture energy compared to 94% in the single bout group (Robling et al, 2002a; Robling et al, 2002b).

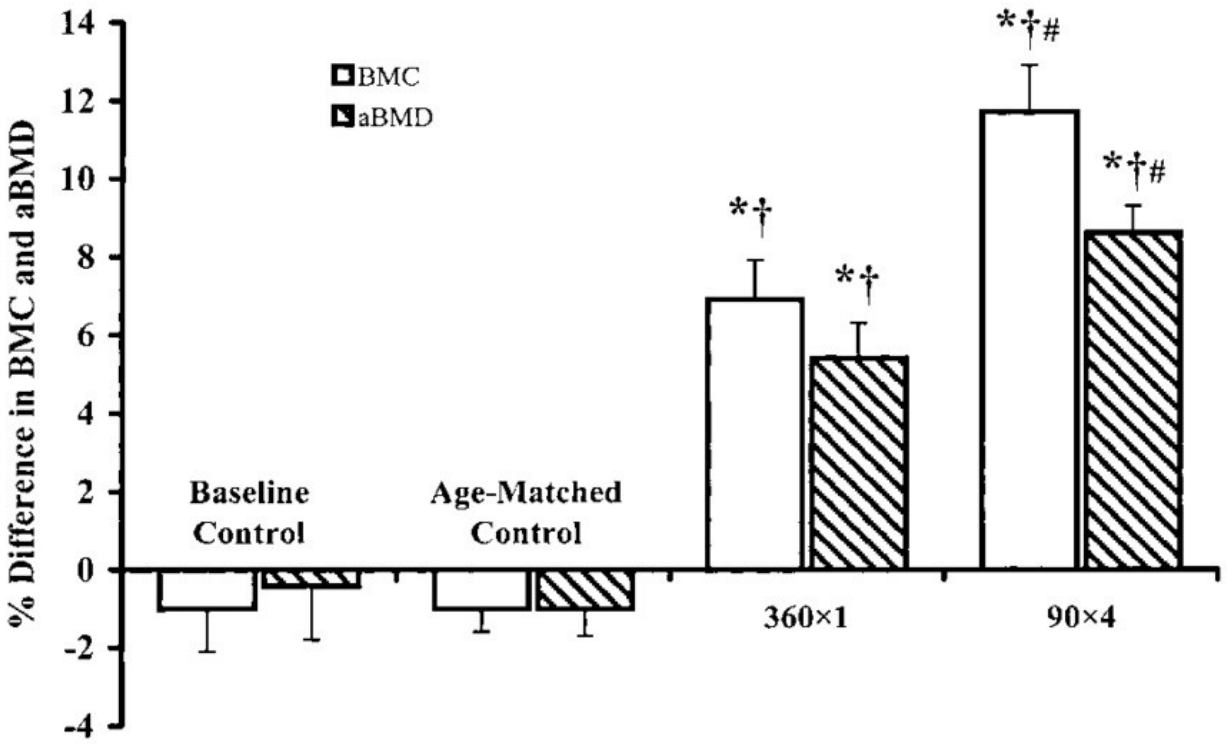


Fig. 2.11c. Areal bone mineral density (aBMD) and bone mineral content (BMC) adaptations to either a single loading bout (360 x 1) or a multiple loading bout (90 x 4) 16 week programme. * denotes statistically significantly different from the baseline control group, † denotes statistically significantly different from the age-matched control group, ♯ denotes statistically significantly different from the 360 x 1 group (Robling et al, 2002b).

The mechanisms for these results are unclear but have been hypothesized to relate to either ion recovery, recovery of the matrix fluid distribution (Srinivasan and Gross, 2000) or desensitisation of G-protein coupled receptors (Robling et al,

2001).

This phenomenon, for which intermittent loading cycles have provided a greater osteogenic response to continuous loading cycles has become a common theme in subsequent literature. It has even been shown at a cellular level where intermittently stimulated cells displayed a greater calcium signaling response in-vitro (Batra et al, 2005). A similar in-vivo study favoured intermittent mechanical loading over cyclic mechanical loading using mice and a 10 second rest interval instead. This project also demonstrated the importance of load magnitude as it highlighted that increasing load magnitudes returned increasing levels of bone adaptation (Srinivasan et al, 2007). Rest-inserted loading studies have also shown that significantly less frequent loading bouts are required to elicit an osteogenic response when compared to cyclic loading bouts (Srinivasan et al, 2015). Low magnitude loading cycles have not changed bone formation rates when applied in a cyclic manner whereas the same loading stimulus has significantly increased bone formation rates when loading has been applied intermittently (10 second inter-cycle rest). This would suggest that the addition of a rest interval can enhance a low magnitude stimulus to the point of creating an osteogenic response from a previously non-osteogenic loading regime (Srinivasan et al, 2002). Although, unsurprisingly, very low strain magnitude vibration loading (300 με at 70Hz) has not altered cortical bone formation levels above baseline in mice whereas higher strain intermittent loading cycles (1000 με, 10 second rest

intervals) in comparison have shown significant increases in bone formation parameters (Kotiya et al, 2011).

Importantly, rest-inserted loading has generated an osteogenic response from aged mice with relatively low magnitude loading stimuli (peak strains of 1200 με) whereas in comparison, the cyclic equivalent loading regime did not enhance bone formation. Interestingly, low magnitude intermittent loading created an equally potent osteogenic stimulus to both doubling the strain magnitude (2400 με) and also increasing the cycle number by fivefold (Srinivasan et al, 2003).

The superiority of intermittent loading over cyclic loading has been advocated on numerous other occasions in more recent years (Kotiya et al, 2011; Srinivasan et al, 2014). The intermittent loading phenomenon has been displayed with dynamic muscle stimulation in addition to the typical external loading equipment that is more frequently used. Dynamic muscle contractions stimulated at 50 Hz have managed to reduce the negative effects of trabecular bone loss through a four week period of disuse (Lam et al, 2011). The optimal contraction to rest ratio for a preservation effect on bone tissue was 2:8, which was in the middle of the range tested with more continuous and more intermittent contraction to rest ratios giving a lesser response. This study not only implies that there is an optimal range for mechanosensitivity but also demonstrated the importance of muscle action upon bone tissue during habitual loading and the positive effects that it can have for the preservation of bone tissue (Lam et al, 2011).

Whilst a great proportion of the literature in this area has been conducted with animal models, it is important to note that the Young's modulus of rat femoral bone and human femoral bone is similar with the main difference being size (Robling et al, 2006). It is therefore possible that there may be a similar mechanism that occurs in human bone tissue as a result of intermittent mechanical loading or intermittent forms of high loading exercise. This would need to be investigated further in well-controlled conditions using human participants.

2.12 Exercise and Bone Density in Humans

2.12.1 Exercise Therapy as an Alternative to Pharmaceutical Treatment

Mechanical loading of the human musculoskeletal system is often generated with exercise participation. In more recent years, exercise has served as an appropriate preventative therapy or treatment for individuals with low bone density (Schwab and Klein, 2008), whereas pharmaceutical treatments such as bisphosphonate therapy and HRT have been used extensively to reduce fracture risk in patients with low bone density (McClung et al, 2013). Whilst bisphosphonate therapy has demonstrated clear beneficial effects upon the reduction of fracture risk through blocking the action of osteoclasts to increase the net bone balance (Levis and Theodore, 2012), bisphosphonate therapy has also been associated with a number

of side effects (MacLean et al, 2008). Commonly reported bisphosphonate side effects have ranged from; osteonecrosis of the jaw, atypical femur fractures, atrial fibrillation, thromboembolic events and esophageal cancer although causal evidence for the direct action of bisphosphonate therapy on these conditions is currently lacking (McClung et al, 2013). In addition, the continuation of oral pharmaceutical treatments has been reported to be around 43% in patients, which highlights an issue with the treatment method for the majority of those receiving it (Netelenbos et al, 2011). Overall, the benefits of bisphosphonate therapy seem to far outweigh the potential risks of experiencing adverse side effects (McClung et al, 2013). For HRT the opposite seems to be the case, in the sense that there appear to be greater risks than the benefit of reduced fracture incidences (Manson et al, 2013). The risk of endometrial cancer and breast cancer were elevated with certain HRT therapies along with elevations in stroke and venous thrombosis risks (Manson et al, 2013). It is clear that HRT in addition to exercise treatment with postmenopausal women can further improve BMD when compared to exercise alone, which is likely due to the upregulation of oestrogen receptor-α activity and shows definite positive actions on the bone architecture (Zhao et al, 2015).

However, despite the strength of evidence advocating the use of some pharmaceutical treatments, the high cost and the potential for side effects have meant that exercise therapy is emerging as an increasingly popular alternative for

the improvement in bone strength and can effectively reduce overall fracture risk (Kemmler et al, 2015; Yuan et al, 2016). There is evidence from a meta-analysis of 754 exercise participants and 670 control group participants to suggest that exercise interventions can decrease overall fracture risk when compared to a control group (relative risk 0.49 [95% CI; 0.31 to 0.76). However, the authors highlighted a potential publication bias with fractures reported as a secondary endpoint and that most exercise trials were underpowered whereas the pharmacological trials are not due to larger budgets (Kemmler et al, 2013). This evidence is comparable for the reduction in fracture risk that is generated by HRT (relative risk 0.48 [95% CI; 0.26 to 0.88]) (Bagger et al, 2004) and also bisphosphonate therapies (relative risk ranging from 0.53 to 0.73) (Cranney et al, 2002), but due to the limitations in the current evidence, it highlights the need for adequately powered randomized control trials of exercise interventions with fractures as a main outcome. Only then could exercise interventions be appropriately investigated as an alternative treatment to pharmaceutical therapy (Body et al, 2010). There is currently very strong evidence for the use of pharmacological treatment for the reduction of fracture risk in populations at a high risk of fracture and bisphosphonate therapy remains the first line of evidence-based medical treatment (Compston et al, 2017).

2.12.2 Cross Sectional Studies

Early cross sectional studies have related bone mechanical properties to the habitual exercise and loading environment in which they have been accustomed to. A large number of investigations have shown discrepancies in the bone architecture in the upper extremities of men and women that are frequent participants in typically unilateral sports (Daly et al, 2004). Differing levels of BMD or bone mechanical strength properties have been present in the loaded and relatively unloaded limbs of a number of athletic populations from tennis to baseball (Ducher et al, 2006; Turner et al, 2009). These types of differences in the bone characteristics show that there are potential benefits of site-specific skeletal loading exercise. These differences have been present at a number of stages all throughout the lifecycle and have been most prominent for younger participants undergoing the maturation/ossification process.

The playing arm of female racquet sports players has been shown to possess significantly higher bone mineral content than the non-playing arm, which has suggested that the increased loading has necessitated greater bone adaptation to safely accommodate the increased loading characteristics (Haapasalo et al, 1994). Interestingly, the same research project found a relationship between the bone mineral content in the upper extremities and the age at which tennis training was first undertaken. The relationship found that the earlier the participants had engaged in tennis training, the greater the differences between the bone mineral

content of the loaded and relatively unloaded limbs (Haapasalo et al, 1994). This could indicate that there is an increased mechanoresponsiveness of bone tissue during the early years of life before menarche and that exercise participation during these years could be of greater long-term benefit to the participants (Haapasalo et al, 1994; Kannus et al, 1995). The differences in playing and non-playing arms may not become apparent until around the age of 12 years however, despite an accumulation of loading for many years prior to that age. This shows that previous exercise and loading history is an important governing factor in bone adaptation although the actual differences in bone adaptation may not appear until later in the maturation process (Haapasalo et al, 1998).

Whilst many studies advocate the importance of exercise for skeletal loading and holistic bone development at very early stages in the lifecycle, emphasis has been placed upon the continuation of the exercise throughout the maturation process and then on into later life. This is thought to ensure that optimal bone mineral accrual occurs during the developmental years (where there appears to be a high level of mechanosensitivity) and the desired level of bone mineral density is then maintained during the ageing process (Heinonen et al, 2000). This particular relationship has been reflected in studies involving racquet sports participants across a variety of stages of life (Haapasalo et al, 2000; Kontulainen et al, 2003; Daly et al, 2004; Ducher et al, 2006).

It comes as no surprise that people who frequently participate in exercise

consisting of high mechanical loads and high muscular forces have been shown to possess high levels of bone mineral density at loading specific areas. This has been found in junior weightlifters that have displayed significantly greater lumbar spine and femoral neck BMD levels than a reference population of 20 – 39 year old men (Conroy et al, 1993). The enhanced BMD levels were significantly related to their relatively high strength levels, which yet further advocates the action of gravitational and muscular loading for stimulating increases in BMD (Conroy et al, 1993). A similar study with adolescent female athletes showed that those involved in predominantly weight-bearing activity (running), had superior BMD at weight baring skeletal sites than those that participated in non-weight-bearing sports (swimming and cycling) (Duncan et al, 2002). Similar associations have been shown in young adult elite female sprint kayakers where upper body BMD and lean mass has been significantly higher than that of a control population, again reflecting the site specific loading response to exercise (Flodgren et al, 1999). Other adult athletes, such as; gymnasts, squash players, aerobic dancers and speed skaters, have also shown to possess significantly higher BMD at skeletal loading sites when compared to controls (Heinonen et al, 1995; Mudd et al, 2007). Furthermore, adult female bodybuilders have likewise displayed significantly higher BMC than swimmers, runners and controls at loading sites, which has been hypothesized to have been due to the greater level of musculoskeletal loading stress that is experienced by this population (Heinrich et al, 1990).

Skeletal loading stress has been reported to be the result of gravitational impact forces and high level muscular forces, which cause compressive, tensile and shear stresses on bone tissue (Judex et al, 2009; Robling et al, 2009). In support of this stress-adaptation concept, some studies have attributed enhanced bone parameters at non-weight-bearing sites to larger estimated joint moments, which highlights the action of high muscular forces on bone tissue adaptation (Nikander et al, 2006). During the maturation process (age ~12-14 years) the peak lean body mass accrual precedes the peak BMD accrual by less than one year. This is also suggestive of muscular actions determining bone adaptations through the maturation process (Rauch et al, 2004). This has been further supported by exercise studies examining the muscle size and bone strength relationship. Significant relationships ($R^2 = 0.73$ to 0.86; $P < 0.001$) have been found between the muscle cross sectional area and the size of the bone in elite level junior tennis players and further emphasizes the influence of muscular contraction on bone tissue. It is important to note that muscle size is not a single predictor of bone size as other factors are involved in the variance of bone strength parameters (Ireland et al, 2013). The initiation of a high gravitational force on the skeleton from a countermovement jump for instance, is inherently linked with a large magnitude eccentric muscle action to control the landing (Ireland et al, 2014). Therefore it would seem that high gravitational and/or high eccentric muscular forces deliver a strain stimulus for bone adaptation.

In a population of young adults the practiced mode of exercise has been found to relate to femoral neck BMD and strength parameters with high impact and odd impact exercise relating to high femoral neck BMD and strength parameters whereas nonimpact exercise such as cycling and swimming did not show any relationship to femoral neck BMD (Nikander et al, 2005). The exercises that showed a beneficial effect upon femoral neck BMD were volleyball, hurdling, squash, speed skating, football, aerobics, weightlifting, orienteering and cross country skiing. This study would also suggest that the high mechanical loading and high eccentric muscular forces are necessary for bone adaptation. The finding that young athletes that compete in high-impact sports have higher levels of BMD than athletes that compete in low-impact sports has been supported by a number of studies over the years with a range of different populations (Fehling et al, 1995; Bennell et al, 1997; Heinonen et al, 2001; Barkai et al, 2007; Gomez-Bruton et al, 2016).

Strong associations between BMD and high-impact team based sports such as football and basketball have also been established in adult populations particularly at the femoral neck, which has been significantly higher (10 to 20%) in a variety of athletes when compared to control populations. This further supports the view that skeletal adaptation can occur with continued adult exercise participation (Lee et al, 1995; Nichols et al, 1995; Duppe et al, 1997).

In a study with older masters athletes a number of different duration running event

athletes were analyzed and it was determined that sprinters had the highest tibial bone mass and strength when compared to long and middle distance runners, race walkers and control participants (Ireland et al, 2011). The velocity of the event was linked with the bone properties with the greater bone mass and geometry being found in the faster events. In addition this mimicked the relationship with the ground reaction forces that are generated suggesting that again, the loading magnitude is related to the bone adaptations. In vivo tibial strain has also been shown to increase with running velocity (Burr et al, 1996). Fascinatingly, as the number of loading cycles of the event increased, the bone parameters decreased, particularly with regards to tibial cortical density, which supports the bone desensitizing and saturation effect (Wilks et al, 2009). Similarly, mechanical bone properties have been linked with the level of ground reaction forces experienced during jumping and sprinting based activities with master athletes.

Although the associations that have been found between populations of individuals that regularly participate in exercise involving high-impacts and/or high muscular forces and desirable levels of bone strength characteristics, they do not imply causality. It is necessary to further investigate the exercise and bone relationship with properly designed randomised control trials.

2.12.3 Intervention Studies

Many exercise training intervention studies have been found to have a positive effect on overall bone health in humans throughout the lifecycle. As previously mentioned, bone mineral accrual is particularly important during the early stages of life, which is why a number of exercise intervention studies have targeted young populations in an attempt to optimize bone architecture (Khan et al, 2000; MacKelvie et al, 2002). Many of the successful exercise programmes have involved some combination of high muscular forces and high gravitational loading (Burrows, 2007; Umemura, 2016). Macdonald et al, (2007) have found that a daily high-impact jumping based programme (along with a mixture of other exercises including skipping, dancing and light resistance exercise) has positively affected pQCT derived bone strength parameters at the tibia in a population of 10 year old boys when compared to aged matched controls. Moreover, in a step-aerobic exercise intervention involving additional countermovement jumps, premenarcheal girls displayed significantly greater BMC changes in the lumbar spine (8.6% compared to 5.3%; $P = 0.012$) and femoral neck regions (9.3% compared to 5.3%; $P = 0.014$) than non-exercising control participants. Differences between intervention and control groups for postmenarcheal girls were negligible however (Heinonen et al, 2000). Bradney et al, 1998 found positive BMD adaptations at the lumbar spine (0.61 ± 0.11% vs. 0.26 ± 0.09% /month; $P < 0.05$) and femoral neck regions (vBMD; 1.14 ± 0.33% /month; $P <$

0.05) in prepubertal boys when compared to a control group, as a result of an eight month weight-bearing exercise programme, which included a wide range of activities from, gymnastics, resistance exercise, aerobic-type exercises, basketball, volleyball, dancing and football. A similar study found that exercise in seven to nine year old girls had a positive effect on the lumbar spine BMD (2.8%; $P < 0.001$), when compared to control participants over the duration of a year. However, this study did not control for the exercise load as the intervention was a daily 40 minute physical education that incorporated a range of running, ball games, and jumping activities. Whilst this is useful supporting research, it gives no further insight into the exact osteogenic exercises to use with young populations (Valdimarsson et al, 2006).

These studies continue to support the efficacy of targeted exercise during the developmental years in order to maximize bone mineral accrual. The effect of weight-bearing exercise during puberty has been widely reported to increase levels of bone mineral density beyond that of non-weight-bearing exercise control populations (MacKelvie et al, 2004; Courteix et al, 2005; Nikander et al, 2010). There is however, a call for more controlled exercise programmes in the future to determine the loading parameters that may contribute to an optimal exercise programme for improving BMD status during the formative years (Hind and Burrows, 2007). Whilst the activities above are undoubtedly weight-bearing in nature, the exact effect upon the bone tissue is unclear insomuch as there may be

many different stresses that contribute to the bone adaptation but as the exercise programme was uncontrolled it is difficult to determine exactly which forms of exercise were having an effect.

Exercise interventions have also shown to benefit the BMD status of adult populations. Although BMD remained unchanged, recreational football training has been shown to increase leg bone mass (41 ± 8 g; $P < 0.05$) in untrained men aged 20 to 43 years with just 12 weeks of training (Krustrup et al, 2009). In a comparable study which lasted 16 months, ~two weekly sessions of recreational football also caused an increase in whole body BMD (2.3%; $P < 0.05$), whilst the equivalent running programme showed an increase in leg BMD (2.4%; $P < 0.05$) in a population of premenopausal women (Krustrup et al, 2010). Progressive high impact exercises in the form of aerobic jumping or stepping exercises have also shown to increase femoral neck (0.012 g/cm^2 [95% CI; 0.003 to 0.020]; $P = 0.006$) and lumbar spine BMD (0.015 g/cm^2 [95% CI; 0.005 to 0.025]; $P = 0.002$) in adult premenopausal women over an 18 month intervention when compared to a control group. Impacts were reported to be between 2.1 to 5.6 g although the exercise prescription was fairly vague in relation to exactly which exercises were used and how they were performed (Heinonen et al, 1996). A combined jumping and lower body resistance training programme (nine sets of 10 to 12 repetitions for each) demonstrated positive effects on BMD in premenopausal women (Winters and Snow, 2000). The exercises were separated by a loosely controlled

rest interval window. The results showed a statistically significant within-group increase in whole body (1.0 ± 1.3%; $P < 0.05$), femoral neck (1.2 ± 3.2%; $P < 0.05$) and trochanter sites (2.7 ± 2.5%; $P < 0.05$) for the exercise group although only the change in trochanter BMD was statistically higher than that of the control group (2.7 ± 2.5% vs 0.8 ± 0.8%; $P < 0.05$). The study showed a trend for the femoral neck BMD change to be greater than the control population but this was not statistically significant (Winters and Snow, 2000). A variety of high impact intervention protocols using a range of exercises from jumping through to football have shown to stimulate increases in lumbar spine and femoral neck BMD in premenopausal women (Vainionpaa et al, 2005; Krustrup et al, 2010; Babatunde et al, 2012; Tucker et al, 2015). One particular study compared the effects of a high-impact exercise and a resistance exercise intervention for a year and concluded that both were an effective means of improving lumbar spine and femoral neck BMD in adult premenopausal women although there was a statistically significantly greater effect at the femoral neck favouring resistance exercise (5.0% vs 2.7%; $P < 0.001$) (McDermott et al, 2001).The inclusion of resistance training to high impact protocols has since been further advocated for creating a larger osteogenic stimulus than high impact exercise alone (Young et al, 2007; Martyn-St James and Carroll, 2010; Kemmler et al, 2015). More research in young to middle aged adults is needed to establish the optimal exercise programmes for achieving optimal levels BMD. A minimum effective threshold for bone adaptation is also yet to be established in these populations.

Postmenopausal populations have demonstrated the capability to respond positively to exercise involving high muscular forces and high gravitational loading with either improved or maintained levels of vBMD or bone strength parameters at the tibia (0.87% [95%CI; 0.37 to 1.37]; $P = 0.0006$), despite the apparent lack of oestrogen (Polidoulis et al, 2012). Just a 30 minute aerobics programme performed three times per week has initiated a protective effect on bone mass (as observed by calcium bone index) in postmenopausal women (50 to 62 years) (0.066 ± 0.036), when compared to control participants that experienced a loss of bone mass (-0.011 ± 0.037; $P = 0.005$) (Chow et al, 1987). Walking, jogging and stair climbing for 50 to 60 minutes, three times per week for nine months has also stimulated an increase in bone mineral content (5.2% [95% CI; 2.0 to 8.4]) in postmenopausal women, when compared to a control group (-1.4% [95% CI; -0.6 to -2.2]; $P < 0.01$) (Dalsky et al, 1988). Another investigation revealed that in a population of postmenopausal women (~62 to 76 years of age), eight months of resistance training improved BMD at the trochanter (2.9%; $P = 0.005$), and total hip regions (1.5%; $P = 0.034$), when compared to an aerobic exercise group and control group that presented no change in BMD (-0.01%; $P > 0.05$). Whilst experiencing a significant within-group decrease in fat mass, the resistance training group significantly increased lean mass when compared to the aerobic and control groups (Marques et al, 2011a). Eight months of moderate impact activity and moderate weight baring exercises have been shown to increase femoral neck BMD both within-group and when compared to

controls in a population of older postmenopausal women (0.018 ± 0.028 g/cm^2 vs -0.007 ± 0.015 g/cm^2; P = 0.008) (Marques et al, 2011b). Studies like this highlight the beneficial effects of different types of exercise regimes but are typically not well controlled for the exercise training volume, meaning that one group could experience a greater BMD adaptation from simply performing a greater amount of exercise. This particular study did not include a control group or randomise the participants to either one of the interventions leaving it open to criticism for selection bias and influence from external factors other than the exercise programme. A systematic review and meta-analysis concluded that progressive resistance training is effective in modestly increasing BMD levels (+0.98%) at the lumbar spine but not the femoral neck in premenopausal women (Martyn-St James and Carroll, 2006a). What's more, a separate systematic review and meta-analysis of controlled trials lasting six months to two years with premenopausal women, reported positive effects on lumbar spine (0.009 g/cm^2 [95% CI; 0.002 to 0.015]; P = 0.011), and femoral neck BMD (0.007 g/cm^2 [95% CI; 0.001 to 0.013]; P = 0.017), from the combination of resistance and also high impact exercise. The effect of only high impact exercise was confined to only benefitting the femoral neck region (0.024 g/cm^2 [95% CI; 0.002 to 0.027]; P < 0.00001) (Martyn-St James and Carroll, 2010). A more recent intervention has shown agreement in that very heavy resistance exercise 80 – 85% 1RM and drop landings have been an effective means of reducing postmenopausal bone loss at the femoral neck (0.3 ± 0.5% vs −2.5 ± 0.8%; P = 0.016), and lumbar spine (1.6

± 0.9% vs −1.7 ± 0.6%; P = 0.005), for osteopenic and also osteoporotic participants in as little as eight months when compared to controls (Watson et al, 2015). A similar study showed that in a 16 year follow up, the participation in a mixed loading exercise programme reduced postmenopausal bone loss and also risk ratio for low-trauma fractures (0.51 [95%CI 0.23 to 0.97], P = 0.046) (Kemmler et al, 2015).

It is widely accepted that pre and postmenopausal women have different BMD responses to weight baring exercise programmes in the sense that the response can be blunted in postmenopausal women (Bassey et al, 1998). When considering this difference, it is however possible for postmenopausal women to improve BMD (as shown in the studies above) or at least maintain BMD levels with correct mechanical loading and exercise involving high muscular forces. Bearing in mind the high level of postmenopausal bone loss that can occur in the early years post-menopause, a maintenance of BMD levels during this stage would be considered beneficial as a greater level of BMD could be maintained later on into life. Many exercise based studies have sought to maintain BMD in populations of early postmenopausal women at important skeletal sites that can be affected by increased rates of BMD loss. To clarify, a maintenance of BMD during this stage of life would be positive when compared to a non-exercising control group that experiences a significant loss in BMD levels. A systematic review and meta-analysis in postmenopausal women showed that again, resistance exercise

modestly but statistically significantly increases absolute lumbar spine BMD (0.006 g/cm^2 [95% CI; 0.002 to 0.011]; $P = 0.006$) but has no statistical effect at the femoral neck region (0.010 g/cm^2 [95% CI; −0.002 to 0.021]; $P = 0.11$) (Martyn-St James and Carroll, 2006b). The adaptations are likely site-specific and are more than likely targeted at the greatest areas of stress as tibial cortical volumetric density has shown no changes in response to a 12 month resistance training programme in 65 to 75 year old postmenopausal women, using a more sensitive pQCT testing procedure (Ashe et al, 2013). Lumbar spine and also femoral neck BMD have been maintained with resistance training in a separate and more recent study with postmenopausal women. Resistance training was reported to be more effective than hormone replacement therapy (HRT) for preserving lumbar spine BMD (0.4 ± 4.3% vs -0.7 ± 3.2%), and equally as effective as HRT for maintaining femoral neck BMD (-1.2 ± 4.3% vs -1.2 ± 3.3%) (Maddalozzo et al, 2007). Yet another systematic review and meta-analysis focussed on impact exercise when combined with resistance exercise in the form of a mixed loading programme for postmenopausal women and demonstrated a reduction in postmenopausal bone loss in the intervention groups for lumbar spine (0.016 g/cm^2 [95% CI; 0.005 to 0.027]; $P = 0.02$), and femoral neck BMD (0.005 g/cm^2 [95% CI; 0.001 to 0.010]; $P = 0.03$), when compared to the controls (Martyn-St James and Carroll, 2009). A different Cochrane systematic review concluded that resistance exercise is the most effective form of activity for maintaining femoral neck BMD and that mixed loading programmes are the most

effective means of preserving lumbar spine BMD. It is important to note that resistance exercise was shown to stimulate significant increases in BMD at the femoral neck (1.03% [95%CI 0.24 to 1.82]) and also lumbar spine (0.86% [95%CI 0.58 to 1.13]) regions when compared to a control group, resistance exercise however, did not improve BMD at the total hip (0.11% [95%CI -0.06 to 0.29]), total body (0.55% [95%CI -0.51 to 1.62]), Ward's triangle (-1.77% [95%CI -3.87 to 0.33]), trochanter (0.40% [95%CI -1.36 to 2.17]). Mixed loading exercise programmes were found to significantly increase BMD at the femoral neck (0.45% [95%CI 0.08 to 0.82]), lumbar spine (3.22% [95%CI 1.80 to 4.64]) and also trochanter regions (1.31% [95%CI 0.69 to 1.92]) when compared to a control group. Mixed loading did not appear to improve BMD of the total hip (-1.07% [95%CI -1.58 to -0.56]), total body (0.14% [95%CI -0.32 to 0.60]), Ward's triangle area (8.38% [95%CI -7.27 to 24.03]) or the arms (0.02% [95%CI -9.43 to 9.47]) (Howe et al, 2011). This opposes the previous review, which showed a positive effect of resistance exercise on lumbar spine BMD (0.006 g/cm^2 [95%CI 0.002 to 0.011]; P = 0.006), but found no effect of resistance exercise on femoral neck BMD, (0.010 g/cm^2 [95%CI -0.002 to 0.021]; *P* = 0.11) or total hip BMD (0.002 g/cm^2 [95%CI -0.001 to 0.005]; *P* = 0.20) (Martyn-St James and Carroll, 2006b). This difference in findings was potentially due to the addition of extra studies that were completed since the first review had taken place or the fact that there were differences in the selection criteria for the separate reviews. In a more recent systematic review and meta-analysis that focussed on both men and

women over the age of 60 years, further support was given in favour of exercise for improving the BMD status during the ageing process. It was established that; mixed loading improved lumbar spine (0.016 g/cm^2 [95%CI 0.002 to 0.030]; P = 0.028) but not femoral neck BMD (0.014 g/cm^2 [95%CI -0.011 to 0.040]; P = 0.268), however, odd impact (team sport/aerobic type) exercise improved both lumbar spine (0.039 g/cm^2 [95%CI 0.002 to 0.075]; P = 0.038) and femoral neck BMD (0.036 g/cm^2 [95%CI 0.012 to 0.061]; P = 0.004). Low impact activity had no effect on lumbar spine (0.009 g/cm^2 [95%CI -0.020 to 0.024]; P = 0.200) or femoral neck BMD (0.002 g/cm^2 [95%CI -0.048 to 0.045]; P = 0.856), neither did only resistance exercise for lumbar spine (-0.002 g/cm^2 [95%CI -0.019 to 0.015]; P = 0.828) or femoral neck BMD (0.023 g/cm^2 [95%CI -0.009 to 0.054]; P = 0.157) (Marques et al, 2012). An overview of systematic reviews in this area has likewise advocated the use of odd/high impact exercise in combination with resistance exercise for the preservation of BMD levels in pre and postmenopausal women (Xu et al, 2016).

The common themes that arise from the current literature are that resistance exercise, high/odd impact exercise or a combination of both can potentially have modest beneficial effects for reducing postmenopausal bone loss at the femoral neck and lumbar spine regions. Having been related to a reduction in fracture risk, these types or exercise have shown to be useful for providing a sufficient mechanical loading stimulus for bone adaptation and could serve to reduce fall

incidence through improving mobility and prevent the event that most frequently causes fractures in the first instance (Gardner et al, 2000; Donath et al, 2016).

Jumping interventions have become common across a whole range of ages and many investigations have found to have stimulated positive bone adaptations throughout different stages of the lifecycle (Fuchs et al, 2001; Mckay et al, 2000; Kohrt et al, 2004; Kato et al, 2006). Jumping is high impact in nature and also requires a high explosive muscular input during the takeoff phase in addition to a high level explosive eccentric muscular action to control the impact upon landing. The high level of muscular force and gravitational loading have been reported as important determining factors for bone adaptation (Zhao et al, 2014). A recent meta-analysis conveyed that jumping and hopping exercises have been reported to improve femoral neck (standardised mean difference = 0.64 [95% CI; 0.38 to 0.90]; P = 0.001), and trochanter BMD (standardised mean difference = 0.36 [95% CI; 0.10 to 0.61]; P = 0.04), in adult premenopausal women but not lumbar spine BMD (standardised mean difference = 0.04 [95% CI; −0.23 to 0.31]; P = 0.79) (Babatunde et al, 2012). All studies that were analysed involved inter-cycle rest intervals as has been previously used in animal studies although not all rest intervals were well defined and none of the more intermittent loading protocols were compared to a continuous equivalent to answer the question of whether a greater osteogenic response can be generated with a brief inter-loading cycle rest intervals.

Bassey and Ramsdale, 1994 also showed positive adaptations in trochanteric BMD (3.4%; $P = 0.01$) in response to a six month jumping based programme with ~30 year old premenopausal women. No differences in femoral neck or lumbar spine were found however. Remarkably, the same intervention was repeated for both pre and postmenopausal women and found significant increases in trochanter BMD after just five months in the premenopausal group (0.024 ± 0.006 g/cm^2; $P < 0.05$) when compared to the control group (0.003 ± 0.008 g/cm^2; $P > 0.05$) (Bassey et al, 1998). No such difference was found between the postmenopausal group and the age matched control after 12 months however. A logical reason for the differences in response between pre and postmenopausal women would be the lack of oestrogen in the postmenopausal group, which would greatly reduce the level of bone adaptation. Nevertheless, it is very important to note that this study included a HRT group, which had bioavailable oestrogen. The HRT groups showed no response to the exercise either when compared to HRT control participants. This study suggests that high impact jumping exercise can be a useful tool for improving BMD status in premenopausal women and that the non-response of postmenopausal women to the same exercise could be due to other factors besides oestrogen deficiency (Bassey et al, 1998). Mechanical strain has been suggested to activate the oestrogen receptor pathway without the need for actual presence of oestrogen in order to upregulate the osteoblast differentiation, which could partly explain the positive effects of exercise on bone tissue that have been highlighted in studies that included postmenopausal women

(Lee and Lanyon, 2004).

With every progressive decrease in T-score in people over the age of 50 years, there is a three to seven fold increase in fracture risk across the forarm, shoulder, spine and hip sites (Kanis et al, 2001). For an average white woman aged 50-59 years, one T-score decrease at the femoral neck is equivalent to a 16% decrease in BMD, which equates to an absolute loss of 0.121 g/cm^2 (Looker et al, 1998). Whilst many of the previous exercise interventions have shown benefits of a much smaller magnitude, it would be logical to expect much smaller reductions in fracture risk from such interventions.

A number of the training/exercise programmes that are outlined above are inherently intermittent in nature in the sense that they contain bursts of activity followed by periods of relatively low activity or even inactivity. For example, team sports contain many breaks in play where participants experience a short rest before recommencing when the ball is back in play. Similarly resistance exercise is broken up into sets of intense activity followed by a rest interval, which is repeated a number of times in the same exercise session. Many of the aerobics programmes involving jumping and impact activities also contain rest intervals but due to the lack of control for exercise to rest ratio, it is impossible to determine whether intermittent exercise could be a more potent osteogenic stimulus than continuous exercise as has been previously shown in animal studies (Robling et al, 2001). In order to answer this question both continuous and

intermittent exercise must be rigorously controlled to be properly evaluated for their effect on BMD.

2.12.4 Measuring Mechanical Loading in Humans

Ground Reaction Force

When measuring the mechanical loading that is experienced by the human body we need to find the external forces, which act upon the body. External ground reaction forces have been closely related to the internal compressive axial forces (Bassey et al, 1997). Dynamic bone strain is difficult to measure in-vivo with living human beings and consequently GRF is measured as practical alternative (Turner and Robling, 2003; Yang et al, 2011). The most common method of identifying loading characteristics is the measurement of GRF with the use of a force platform. This is considered the current gold standard method in establishing GRF. Force platforms are used for measurement of GRF in order to calculate a variety of kinetic variables during walking, running, countermovement jumping and other high-impact exercises (Linthorne, 2001; Riley et al, 2007; Addison and Lieberman, 2015). Peak GRF has been used as a measure of loading intensity when applied to bone with high peak ground reaction force exercises showing beneficial bone adaptations (Bassey and Ramsdale,

1995; Turner and Robling, 2003; Engelke et al, 2006; Allison et al, 2015). When measuring high-impact forces with force platforms, it has been recommended to record force platform GRF data with a sampling frequency that is higher than 1200 Hz in order to correctly determine the peak GRF values (Niu et al, 2014). This is because using a sampling frequency of less than 1200 Hz significantly reduces the measured peak GRF and misinterprets the true signal (aliasing) (Niu et al, 2014). It is important to note that very rapid impact GRF can easily occur in less than $1/100^{th}$ of a second (Lafortune et al, 1995) and that the heel strike during normal walking can create an impact that can occur at frequencies of up to 75 Hz (Simon et al, 1981). Much higher impact activities generate impacts that occur at frequencies >100 Hz, for instance the frequency content of an impact from an accelerometer trace during jumping has shown to be as high as 175 Hz (Vihriala et al, 2000). One limitation of using force platforms is that it requires participants to hit a relatively small area (usually 0.6 x 0.4 m) in order to record the GRF. For some activities this is not a problem, but it can prove overly restrictive for movements that require greater freedom. GRF can be used as an input for musculoskeletal models to estimate bone strain, however this approach requires the prior knowledge of subject specific segmental moment of inertia parameters along with a detailed 3D reconstruction of the movements in question. This usually requires full body MRI data along with a 3D movement analysis for use with a finite element model to obtain acceptable bone strain magnitudes as determined from prior strain gauge research (Al Nazer et al, 2011). These

approaches, whilst incredibly detailed, require expensive imaging techniques in order to obtain acceptable subject specific anatomical parameters and also subject specific strength parameters, which can be time consuming if applied to a multiple participant trial (Schellenberg et al, 2015). Very often, individual differences are overlooked in musculoskeletal models and generic equations are applied to the individual in question, muscle force and recruitment is also often an approximation, both of which can greatly affect the subsequent results (Deng et al, 2017).

Accelerometry

Skin mounted accelerometers provide an alternative to GRF measurement that allows for unrestricted movements. Accelerometers are recommended as a surrogate measure of bone strain during physical activity when the direct measurement of bone strain is often comprimised (Edwards et al, 2009). These can be placed over sites of interest to establish the level of acceleration and how that is transferred throughout the body. Using Newton's second law:

Force (N) = Mass (kg) x Acceleration ($m \cdot s^{-2}$),

(Newton et al, 1726)

the magnitude of the external force is dependent upon the mass of the body when

using accelerometry. Common placings are the lumbar spine, sacrum area, the hip and the tibia, which are all related to site specific bone assessments (Sinclair et al, 2013a; Rispens et al, 2014). Peak acceleration is highly correlated with that of peak GRF and it is important to note that accelerometers have a tendency to overestimate relative loading magnitudes (Lafortune et al, 1995; Vainionpaa et al, 2006; Elvin et al, 2007; Tran et al, 2010). Despite this, as a measurement device to distinguish differences between different loading conditions, accelerometers have shown to be highly reliable (Jamsa et al, 2006). The intensity of the loading stimulus has been linked with BMD adaptations with loading magnitudes greater than 4.9 g being associated with improvements in proximal femur BMD (Vainionpaa et al, 2006), which would support the same finding in a number of animal based studies (Rubin and Lanyon, 1985; Turner et al, 1995). As high loading magnitudes are closely related to loading rates, it is logical that the rates of loading that are greater than 1000 $m \cdot s^{-3}$ have also been associated with BMD adaptations at the hip (Heikkinen et al, 2007). The effect of loading rate has also been previously advocated for positive bone adaptation in animals (Rubin and Lanyon, 1985; Turner et al, 1995, Turner et al, 1998). Peak accelerations have been used to develop the osteogenic index, which combines the number and magnitude of loading cycles to establish a measure of loading volume, which has been closely related to hip and femoral BMD adaptations (Ahola et al, 2010). It is apparent that in more recent years, the governing factors for bone adaptation that have been shown in animals have also emerged to have

similar effects in humans as determined by GRF or accelerometry.

Acceleration traces are subject to the inclusion of skin movement, which could potentially contribute to the overestimations in loading magnitudes when compared to GRF. Attempts have been made to quantify the extent to which skin movement alters the acceleration signal although these have analysed this factor either during a nudge test or when walking and not for the purpose of accurately recording peak impacts (Smeathers, 1989; Morgado Ramirez et al, 2013). Many studies recording peak acceleration as a substitute for peak GRF therefore accept this limitation as part of the procedure as it is incredibly problematic in attempting to eradicate rapid skin movement and retain the true impact signal at the same time (Vihriala et al, 2000; Vainionpaa et al, 2007).

Many accelerometer based investigations are commonly under sampled for the purpose of adequately identifying peak accelerations, with sampling frequencies ranging from 50 – 200 Hz being common (Kelley et al, 2014; Auvinet, 2002; Mansfield and Lyons, 2003). Whilst these frequencies would be adequate for assessing habitual gait events during walking and running, when it comes to accurately measuring the impacts these frequencies are insufficient (Niu et al, 2014). Sampling frequencies greater than 1000 Hz are consequently preferable for the accurate determination of peak acceleration (Lafortune and Henning, 1992; Sinclair et al, 2013a).

Much of previously reported accelerometer data are potentially over filtered. The

peak GRF of a heel strike during walking has shown to occur at frequencies of up to 75 Hz (Simon et al, 1981). Tibial acceleration and GRF data upon ground impact during running trials have been shown to contain frequencies >100 Hz (Lafortune et al, 1995). Therefore, with higher impact activities, surely higher filtering is required to include the high frequency portions of the signal that are created very rapidly. However, many studies opt for relatively low cut-off filters, which serve to eradicate the very components of the impact of which they wish to measure. Common low-pass cut-off frequencies have ranged from 2 Hz (Mansfield and Lyons, 2003), 6 Hz (Kelley et al, 2014), 50 Hz (Auvinet et al, 2002). Filtering at 95% of the signal power, which is identified after a fast Fourier transform provides a more targeted approach to this issue and would retain much more of the higher frequency components of the impact (Lafortune and Henning, 1992; Sinclair et al, 2013a). These studies used low-pass filters of 60 Hz, which was identified from their Fourier analysis, as they analysed walking 1.5 $m \cdot s^{-1}$ and running 4.0 $m \cdot s^{-1}$, which produce impacts of ~2 to 3 g and ~8 g respectively (Lafortune and Henning, 1992; Montgomery et al, 2016), it is logical to expect higher low-pass cut-off frequencies when recording more rapid and higher impact activities, which produce greater high frequency (~175 Hz) acceleration signals (Vihriala et al, 2000).

Recent developments in higher specification accelerometers and their data acquisition technology have allowed for a more detailed and in-depth detection

of high frequency and high magnitude impacts (Jamsa et al, 2011). Advancements in this field will greatly contribute to the assessment of physical activity and inform future studies that require the reporting of loading parameters for the purpose of investigating bone adaptation.

Electromyography

Electromyography (EMG) is the gold standard for the measurement of muscle activation (Webster, 1998), and has shown very good reliability in determining the onset of muscle activation (Di Fabio, 1987). EMG amplitude has not been previously related to bone adaptation but it has been linked with high internal compressive forces on the skeletal system (Bassey et al, 1997). Whilst EMG amplitude provides no direct measure of force or bone characteristics, it provides a reliable means of quantifying the level of muscular activation and has also been highly correlated with muscle force output under static ($R^2 = 0.945$ to 0.950) and dynamic conditions ($R^2 = 0.882$) (Park et al, 2008; Andrade and Andrade, 2012). As muscles provide a key stimulus for bone adaptation, EMG offers a valuable insight into the internal mechanical environment during different loading activities (Robling, 2009). For the purpose of this thesis, EMG is used as a secondary measure to both GRF and acceleration as it does not directly quantify the level of skeletal mechanical loading and is only related to muscle forces. EMG is also limited in determining motor unit activation during rapid eccentric

contractions; as despite the higher force magnitudes, EMG is usually lower during eccentric contractions than concentric contractions due to the reduced neural requirements (Grabiner and Owings, 2002). In addition, the EMG signal can be affected by electrical noise that can be minimised with the use of high quality equipment, electromagnetic radiation can also amplify the EMG signal, motion artefact can also affect the signal, which can derive from the electrode and skin interface or from cable movement, the motor unit firing rate can affect the EMG signal, most of which is eliminated by low-pass filters of around 10 - 20 Hz (Reaz et al, 2006). In addition to the noise accumulation there are a number of factors that affect the EMG signal. Causative factors can be either intrinsic or extrinsic. Intrinsic factors can be number of motor units that are activated, proportions of fast and slow twitch muscle fibres, the volume of circulating blood flow, muscle fibre diameter, the location of active motor units and also the amount and type of surface tissue between the muscle and the electrode (Reaz et al, 2006). Extrinsic factors are mainly due to electrode placements, for instance the area of the electrode surface, the distance between the electrode surface detection sites, the proximity of the electrode placing to the motor units, the proximity of the electrode to the main muscle belly and also the electrode orientation with respect to the muscle fibre pennation angle (Reaz et al, 2006).

2.13 Aims and Hypotheses

The overall aim of this thesis was to examine the effects of continuous and intermittent exercise on BMD in a human population. In order to conduct this research project, firstly it was necessary to establish the most effective modes of exercise from a selection of commonly used exercises for both stimulating improvements in BMD in young adults and also for reducing postmenopausal BMD loss. The most effective exercise, which displayed consistent mechanical loading between continuous and intermittent conditions was used in a 12 month intervention to evaluate the effects of continuous and intermittent exercise on postmenopausal BMD. It was hypothesised that countermovement jumps and box drops would provide a greater mechanical stimulus than heel drops and stamps for bone adaptation, in addition it was hypothesised that intermittent exercise would reduce postmenopausal bone loss to a greater extent than continuous exercise.

3. The effects of continuous and intermittent exercise upon changes in bone mineral density in humans: a systematic review

3.1 Introduction

Bone mineral density is lost during the ageing process, which leaves elderly populations at a higher risk of experiencing fractures (Lems, 2007; Berard et al, 1997; Shipman et al, 1999). Low BMD and the development of osteoporosis can potentially result in fractures, immobility and can increase the risk of general morbidity (Pesonen et al, 2005). It is therefore important to preserve BMD levels during the ageing process. Peak BMD occurs around the ages of 20 to 45 years (Mein et al, 2004; Pedrazzoni et al, 2003). BMD status during these years is a strong predictor of BMD levels throughout the life cycle (Heaney et al, 2000), which highlights the importance of optimising BMD levels in younger populations to ensure that greater BMD levels are maintained throughout the ageing process.

Exercise has been shown to improve BMD status in young populations (Nilsson et al, 2012) and also older populations where exercise has demonstrated a protective effect on the preservation of BMD (Engelke et al, 2006; Kelley et al, 2012). Preservation of healthy levels of BMD is associated with a reduction in the risk of fracture (Hui et al, 1988; Kanis et al, 2009). Osteoporotic and fragility

fractures are common with the risk of fracture exceeding 50% for women over the age of 50 years and exceeding 20% for men over the age of 50 years in the UK (van Staa et al, 2001).

High mechanical loading is necessary to stimulate bone adaptations, whether this is for an increase in BMD or maintenance of BMD in younger or older populations respectively (Frost, 2003). High impact exercise and resistance training have been known to stimulate improvements in BMD status as a result of high mechanical loading (Martyn-St James and Carroll, 2006a; Martyn-St James and Carroll, 2006b; Martyn-St James and Carroll, 2009; Martyn-St James and Carroll, 2010). Bone tissue adapts to a small number of loading cycles with continuous repetitive loading providing minimal further benefit once an optimum number of around 36 - 40 loading cycles has been reached with studies using animal populations (Unemura et al, 1997; Rubin and Lanyon, 1984). However, adding a short rest interval of 10 - 30 seconds in between loading cycles increases the osteogenic response above that of repetitive continuous loading cycles in animals (Robling et al, 2001, Umemura et al, 2002, LaMothe and Zernicke, 2004; Srinivasan et al, 2007). The rest interval and intermittent nature of the loading cycles although not fully understood at present is believed to “re-sensitise” the bone tissue to the mechanical loading cycles and create a more potent anabolic osteogenic stimulus (Srinivasan et al, 2002; De Souza et al, 2005; Lam et al, 2011; Srinivasan et al, 2015). The majority of research involving inter loading cycle rest

intervals has been conducted with animals, few studies have sought to determine whether inserting a set rest interval between high mechanical loading cycles would increase the bone adaptation response above repetitive continuous mechanical loading cycles in humans. Intermittent mechanical loading therefore has the potential to supersede continuous mechanical loading cycles in humans for enhancing bone adaptations; however, research in this area is yet to provide a definitive answer on this topic. The purpose of this systematic review is to summarise and evaluate the evidence on the effect of continuous and intermittent mechanical loading on DXA, pQCT or computerised tomography (CT) derived BMD in humans. This would clarify the current research findings and help to inform further research in this area.

3.2 Methods

3.2.1 Search Strategy for Identification of Studies

The literature search was performed using the ensuing databases: PubMed (1950 to 1st November 2016), Web of Science (1970 to 1st November 2016) and the Cochrane Library (updated daily to 1st November 2016). The search terms that were used included “exercise”, “bone mineral density” and “dual energy x ray absorptiometry”. The full and comprehensive list of search terms that were used

for each database are provided in Appendix A. A PICO system was used to develop the search strategy. Participants: all study participants were eligible for inclusion, during the review process studies were excluded if they included patients (with the exception of osteoporotic patients, which were included in the review). Intervention: Controlled dynamic continuous or intermittent exercise (as defined above). Comparison: Measures of bone mineral density pre and post intervention. Outcome: DXA, pQCT or CT derived bone mineral density in g/cm^2 or g/cm^3. For the studies that were included during the search, study titles and abstracts were screened independently by the author against the inclusion/exclusion criteria (Table 3.2.1). Once the selected studies were determined, full manuscripts were obtained and analysed using the inclusion / exclusion criteria.

Inclusion / exclusion criteria

A systematic search of all studies including the effect of specifically controlled continuous and intermittent exercise on bone mineral density was undertaken. Continuous exercise was defined as "controlled dynamic exercise with defined loading cycles performed at greater than or equal to 1 Hz or a work to rest ratio, which is greater than to 1:1 (with work being greater than the rest interval)". Intermittent exercise was defined as "controlled dynamic exercise with defined loading cycles performed at less than 1 Hz or a work to rest ratio, which is less

than or equal to 1:1 (with work being less than the rest interval e.g. 1:2, 1:4 etc)". Bone mineral density measures were determined in areal bone density in g/cm^2 or volumetric bone density in g/cm^3. These measures were evaluated if they derived from DXA, pQCT or CT scans. Only randomised control trials with human participants (>20 years) were included in the review process with solely articles that were published in peer reviewed journals being selected for inclusion. Articles were excluded from the review process if they examined patient populations (due to medical complications) with the exception of osteoporotic patients. Whole body vibration training was omitted due to insufficient evidence for generating increased BMD (Oliveira et al., 2016; Xu et al., 2016) and the non-dynamic nature of the exercise. All trials in which the exercises were not well defined in terms of exercise and rest intervals, were excluded. All studies that were not written in English, and did not have a translated version of the article were also excluded in accordance with the full inclusion/exclusion criteria (Table 3.2.1).

Table 3.2.1. Inclusion and exclusion criteria

Include	Controlled Dynamic Continuous Exercise/Mechanical Loading (Defined Loading and rest periods >1:1 or ≥1Hz) Controlled Dynamic Intermittent Exercise/Mechanical Loading (Defined Loading and rest periods ≤1:1 or <1Hz) Human Adults, Males and Females, > 20 years of age Randomised Control Trials Healthy participants and osteoporotic patients Intervention Studies Studies where Bone Mineral Density is the dependant or outcome variable Studies which measure Bone Mineral Density using DXA, Quantitative Ultrasound, pQCT, Micro CT or Dual Photon Absorptiometry
Exclude	Humans, < 20 years of age All research designs that are not randomised control trials Static loading protocols Vibration Training interventions (Non-Dynamic) All patient populations except osteoporotic patients All studies where the training load is not properly defined in terms of; duration of load and rest periods

Searching Other Resources

In addition to the database search terms, a manual search of original research articles and review articles including exercise interventions and bone adaptations was completed by the author.

Data Collection and Analysis

For the initial screening process, the author reviewed the titles and abstracts of the database search to assess study eligibility for inclusion. Articles were then either accepted or rejected once evaluated against the review criteria. In the

instance where insufficient information was given for a decision to be made for inclusion or exclusion, a full length article was obtained to further inform the authors. Once the initial screening process was completed, full text articles were retrieved and were screened for inclusion eligibility by the author. The included studies were compared and discussed to generate a final list of included studies for the systematic review and data extraction process.

Data Extraction and Management

From the studies selected for review, the lead author extracted the relevant data including; study population, country of research project, participant number in intervention and control groups, participant drop-out number, adherence of the intervention group, whether the intervention was supervised or not, frequency of the intervention, frequency of follow up testing, study sponsors and primary outcomes at baseline and follow up time points. The primary bone density outcome measures were either recorded or calculated (dependent on whether information was presented) and were displayed as; absolute change, mean difference and 95% confidence intervals (within or preferably between groups where possible), percentage change and also significant change (Table 3.3.2, Table 3.3.3, Table 3.3.5a, Table 3.3.5b).

3.2.2 Risk of Bias

Selected studies were assessed for risk of bias using the Cochrane risk of bias tool Table 8.5a of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011) (Table 3.3.9a, Table 3.3.9b). The Cochrane risk of bias tool is a series of distinct criteria that can be used to assess the quality of the methods and reporting processes of studies for the risk of bias. It is used to detect the likelihood of overinflated study findings due to the inherent bias in the research design or report. As allocation concealment was impossible for an exercise intervention study design this section was ignored leaving seven criteria for the evaluation of selected studies. Study bias was determined as either "high risk", "low risk" or "unclear risk" using the Cochrane risk of bias tool.

3.3 Results

3.3.1 Results of the Search

The initial search returned 2115 articles, which was reduced to 1741 articles once 374 duplicates were removed. Study titles and abstracts were then reviewed leaving 142 potential articles. Full manuscript texts were gathered for the remaining potential articles and were reviewed in detail. This found 16 articles to

match the inclusion criteria. A hand search of the literature identified a further 3 articles that matched the criteria. 19 articles were included in the final analysis, 10 articles were categorised as continuous exercise, 9 articles were categorised as intermittent exercise. Fig. 3.3.1 shows the study selection process using a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Moher et al, 2009). These were later combined into seven continuous studies and eight intermittent studies as some of the articles were part of the same investigation.

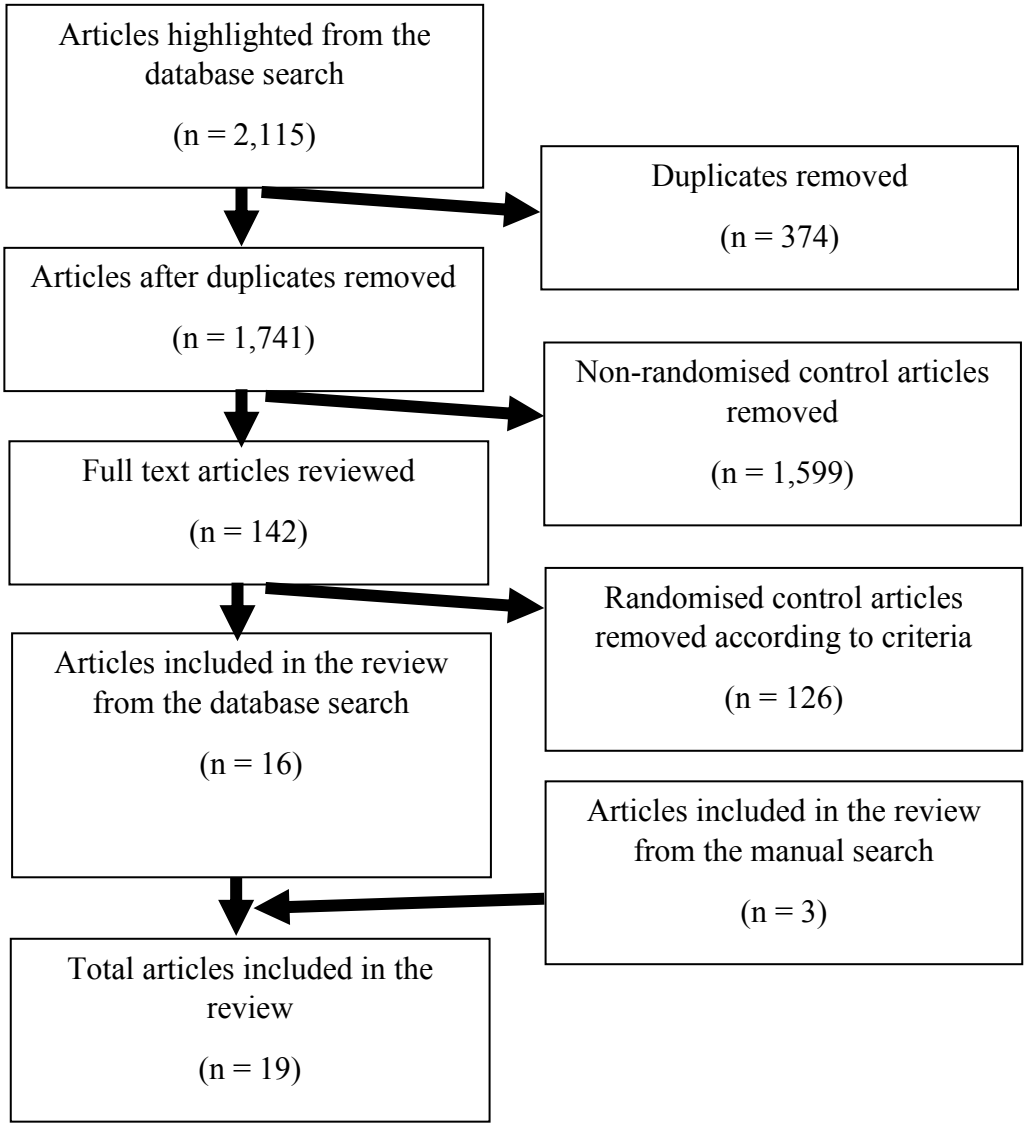


Fig. 3.3.1. PRISMA flow diagram (Moher et al, 2009)

Meta-analyses were not performed due to the heterogeneity of the participants in the selected studies and the heterogeneity of the statistics presented.

3.3.2 Description of Included Studies – Continuous Exercise

Three of the seven continuous exercise studies reported positive effects of the intervention on BMD at various sites (Brooke-Wavell et al, 1997; Hosny et al, 2012; Krustrup et al, 2010), these interventions that had a positive effect included two brisk walking interventions and one running intervention. Four of the seven continuous exercise studies comprised of a brisk walking intervention (Huuskonen et al, 2001; Huuskonen et al, 2002; Remes et al, 2003; Remes et al, 2004; Hosny et al, 2012; Ebrahim et al, 1997; Brooke-Wavell et al, 1997), two studies involved running at 82% maximum heart rate and one study involved a variety of exercise modes performed on an indoor track and also various ergometers (Krustrup et al, 2009; Krustrup et al, 2010; Evans et al, 2007). Of the four studies that did not have a positive effect upon BMD, one involved a continuous running intervention (Krustrup et al, 2009), two involved a continuous walking intervention (Ebrahim et al, 1997, Huuskonen et al, 2001; Huuskonen et al, 2002; Remes et al, 2003; Remes et al, 2004) and one involved a multiple ergometer intervention (Evans et al, 2007). Three studies involved postmenopausal women ranging from 50 to >70 years of age (Ebrahim et al, 1997; Brooke-Wavell et al, 1997, Evans et al, 2007), one study involved young

men (Krustrup et al ,2009), one study involved middle aged men (Huuskonen et al, 2001; Huuskonen et al, 2002; Remes et al, 2003; Remes et al, 2004). One study involved healthy premenopausal women (Krustrup et al, 2010) and one study involved obese premenopausal women (Hosny et al, 2012) (Table 3.3.2).

Table 3.3.2. Continuous studies descriptive information

Study	Population (Age)	Country	Number of Participants Completed	Dropouts	Adherence	Supervised / Unsupervised Intervention	Intervention	Frequency	Follow-up	Sponsor
Brooke-Wavell et al, 1997	Postmenopausal women (60 to 70 years) I = 64.2 ± 3.1 C = 64.9 ± 3.0	UK	Total = 78 I = 38 C = 40	6 I = 5 C = 1	NA	Unsupervised	120 to 280 min brisk walking per week (first 3 months) 280 min brisk walking per week (4 to 12 months) Each walk had to be continuous and 20-50 min long.	NA	12 months	Arthritis and Rheumatism Council grant 50048
Ebrahim et al, 1997	Postmenopausal women (upper arm fracture in the past 2 years) I = 66.4 ± 7.8 C = 68.1 ± 7.8	UK	Total = 97 I = 49 C = 48	68 I = 32 C = 36	NA	Unsupervised	40 mins brisk walking	3 times per week	2 years	The Wolfson Family Trust
Evans et al, 2007	Women at least 2 years post menopause (50 to 65 years) 62.0 ± 5.0	USA	Total = 43 I = 21 C = 22	18 I = 12 C = 6	NA	Supervised	45 min exercise training at 75% to 80% VO2peak. Exercise modes were indoor track, treadmills, rowing ergometers, and stair-climbing ergo-meters.	3 times per week	9 months	The Solae Company (St. Louis, MO). Institutional National Research Service Award (NRSA) AG-00078 and Individual NRSA AG-05874 Individual NRSA HL-10249 Washington University Claude D. Pepper Older American Independence Center, grant AG-13629, General Clinical

										Research Center grant RR-00036, and Diabetes Research and Training Center grant DK-20579. Markers of bone metabolism were supported by the University of Kentucky General Clinical Research Center, grant M01-RR-02602.
Hosny et al, 2012	Premenopausal obese women (30 to 40 years) I = 35.9 ± 2.4 C = 35.2 ± 2.9	Egypt	Total = 40 I = 20 C = 20	0	NA	Supervised	30 min brisk walking at 70% HRmax	3 times per week	3 months	NA
Huuskonen et al, 2001 & 2002 & Remes et al, 2003 & 2004	Middle aged men (50 to 60 years) I = 58.1 ± 2.9 C = 58.2 ± 2.9	Finland	Total = 132 I = 66 C = 66	8	NA	Unsupervised	30–45 min brisk walking at 40–60% VO2max, 3 times per week (first 3 months) 60 min brisk walking at 40–60% VO2max, 5 times per week (3 months onward)	5 times per week	4 years	Ministry of Education in Finland, City of Kuopio, Juho Vainio Foundation
Krustrup et al, 2009	Untrained men (20–43 years) I = 31.0 ± 2.0 C = 30.0 ± 2.0	Denmark	Total = 20 I = 10 C = 10	3 I = 2 C = 1	NA	NA	60 min running at 82% HRmax (split into 3-4 segments over first 6 weeks only)	2.5 times per week	12 weeks	Danish Ministry of Culture and the Danish Football Association
Krustrup et al, 2010	Untrained females (19–47 years) I = 40.0 ± 2.0 C = 38.0 ± 4.0	Denmark	Total = 15 I = 8 C = 7	4 I = 2 C = 2	NA	NA	60 min running at 81% and 82% HRmax during the first 4 and last 12 months, respectively	1.9 times per week (0 to 4 months) 1.7 times per	16 months	FIFA – Medical Assessment and Research Centre (F-MARC), the Danish Football Association (Dansk Boldspil-Union) and by 3F (United Federation of

								week (5 to 12 months)		Danish Workers).

I represents “intervention group”, C represents “control group”, NA represents “not available”.

3.3.3 Description of Included Studies – Intermittent Exercise

Five of the eight intermittent exercise studies reported positive effects of the intervention on BMD (Allison et al, 2013 & 2015; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006; Tucker et al, 2015), these interventions that had a positive effect included two multidirectional hopping interventions, two countermovement jumping interventions and one wrist loading intervention. However, for one of these studies, significance was only found once the confidence level had been adjusted to 90% and the results were adjusted for multiple covariates (Tucker et al, 2015). Of the three studies that did not have a positive effect upon BMD, two of them included resistance training interventions and one included a wrist loading intervention (Chilibeck et al, 1996; Lester et al, 2009; Troy et al, 2013). Seven studies involved young premenopausal women (Bhatia et al, 2015; Kato et al, 2006; Bailey and Brooke-Wavell, 2010; Chilibeck et al, 1996; Lester et al, 2009; Troy et al, 2013) whilst only one study involved older men (Allison et al, 2013 & 2015) (Table 3.3.3).

Table 3.3.3. Intermittent studies descriptive information

Study	Population (Age)	Country	Number of Participants Completed	Dropouts	Adherence	Supervised / Unsupervised Intervention	Intervention	Frequency	Follow-up	Sponsor
Allison et al, 2013 & 2015	Older men (65 to 80 years) 69.9 ± 4.0	UK	Total = 35	15	90.5 ± 9.1%	Unsupervised	5 sets of 10 multidirectional hops, Each set was interspersed with 15 s rest (gentle on the spot walking).	7 days per week	12 months	Medical Research Council (MRC) Interdisciplinary Bridging Award.
Bailey and Brooke-Wavell, 2010	Premenopausal women I Ex7 34.6 ± 7.9 I Ex4 32.2 ± 10.0 I Ex2 30.7 ± 7.4 C 32.9 ± 9.4	UK	Total = 64 I Ex7 = 16 I Ex4 = 13 I Ex2 = 16 C = 19	21 I Ex7 = 6 I Ex4 = 9 I Ex2 = 5 C = 1	Ex7 = 86% Ex4 = 90% Ex2 = 84%	Unsupervised	5 sets of 10 multidirectional hops, Each set was interspersed with 15 s rest (gentle on the spot walking).	7, 4 or 2 days per week	6 months	NA
Bhatia et al, 2015	Premenopausal women (21-35 years)	USA	Total = 35 I = 23 C = 12	0	82 ± 17%	Unsupervised	Wrist loading, 50 cycles per Day, 2 second rest in between cycles	3 days per week	14 weeks	NIAMS of the National Institutes of Health
Chilibeck et al, 1996	Premenopausal women (~20 years) I = 20.3 ± 1.0 C = 20.2 ± 4.0	Canada	Total = 30 I = 20 C = 10	0	96%	Supervised	Upper and lower body resistance training with 2min rest between sets.	4 days per week	22 weeks	Natural Sciences and Engineering Research Council of Canada
Kato et al, 2006	Premenopausal women (~21 years) I = 20.5 ± 0.6 C = 20.9 ± 0.8	Japan	Total = 36 I = 18 C = 18	6 I = 3 C = 3	82%	Unsupervised	10 maximal countermovement jumps. The interval of each jump was 8–12 s	3 days per week	6 months	NA
Lester et al, 2009	Premenopausal women (~20 years)	USA	Total = 27 I = 17 C = 10	11 grouping not stated	NA	Supervised	Full body resistance training programme, following a non-linear periodized model.	3 days per week	8 weeks	US Army Medical Research and Materiel Command Bone Health and Military

	20.2 ± 1.7						Rests between sets were 90 to 150 s depending on training cycle.			Medical Readiness Research Program to BCN.
Troy et al, 2013	Premenopausal women (19 to 32 years) I = 22.0 ± 3.0 C = 22.0 ± 2.0	USA	Total = 22 I = 16 C = 6	8 I = 7 C = 1	89%	Unsupervised	Wrist loading, 50 cycles per Day, 2 second rest in between cycles	3 days per week	28 weeks	NA
Tucker et al, 2015	Premenopausal women (25 to 50 years) I20 = 39.8 ± 4.8 I10 = 41.1 ± 4.4 C = 37.7 ± 6.4	USA	Total = 60 I20 = 14 I10 = 23 C = 23	28	>40%	Unsupervised	Either 10 or 20 countermovement jumps (depending upon group allocation) separated by 30 s inter-jump rest, performed twice daily with an interest rest of at least 8 hrs.	6 days per week	16 weeks	NA

I represents “intervention group”, C represents “control group”, I Ex7 represents “7 days per week intervention group”, I Ex4 represents “4 days per week intervention group”, I Ex2 represents “2 days per week intervention group”, I20 represents “20 jump intervention group”, I10 represents “10 jump intervention group” , NA represents “not available”.

3.3.4 Bone Mineral Density Outcomes

The reporting of continuous studies was inconsistent with three studies comparing absolute changes in BMD pre and post intervention (Hosny et al, 2012; Krustrup et al, 2009; Krustrup et al, 2010), whilst three studies compared the absolute changes in BMD between the intervention and control groups (Brooke-Wavell et al, 1997; Ebrahim et al, 1997; Evans et al, 2007) and one study merely stated that there was no difference in BMD changes between the intervention and control groups (Huuskonen et al, 2001; Huuskonen et al, 2002; Remes et al, 2003; Remes et al, 2004).

The reporting of intermittent studies was inconsistent with four studies comparing the percentage changes in BMD in the intervention and control groups (Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Troy et al, 2013; Tucker et al, 2015), three studies comparing absolute within group changes pre and post intervention (Allison et al, 2013 & 2015; Kato et al, 2006, Lester et al, 2009), one study stated that there were no statistical changes in hip BMD, lumbar spine BMD was unmentioned (Chilibeck et al, 1996).

3.3.5 Exercise Intervention Details

Continuous exercise interventions were mainly running/walking based, two of which were uncontrolled for exercise intensity (Brooke-Wavell et al, 1997; Ebrahim et al, 1997), the remaining five determined exercise intensity with a variety of different measures from % of maximum heart rate to % VO2 peak to % VO2 max. A range of different intensities were analysed. Intermittent exercise interventions were mainly loading cycle based in terms of high

impact exercise and resistance exercise. They ranged from relatively brief exercise protocols consisting of 10 to 50 jumps / hops to 50 wrist loading cycles to a full body resistance training programme per exercise session including concentric and eccentric muscle actions (Table 3.3.5a, Table 3.3.5b).

Table 3.3.5a. Continuous exercise data extraction

Study	Outcomes	Group	Baseline (Mean±SD)	Post Intervention (Mean±SD)	Absolute Change (SD)	Mean Diff (Post-Pre) (95% CI)	% Change	Improvement
Brooke-Wavell et al, 1997	DXA BMD (g/cm^2) L2-L4, Femoral Neck and Calcaneus		**L2 - L4**	**L2 - L4**	**L2 - L4** *(±SE)*	**L2 - L4**	**L2 - L4**	**L2 - L4**
		I	1.044 ± 0.175	1.050 (NA)	0.006 ± 0.004	0.006 (NA)	0.57	NS (intervention vs control)
		C	1.035 ± 0.199	1.030 (NA)	-0.005 ± 0.004	-0.005 (NA)	-0.48	
			Femoral Neck	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**
		I	0.843 ± 0.109	0.859 (NA)	0.016 ± 0.006	0.016 (NA)	1.90	NS (intervention vs control)
		C	0.840 ± 0.112	0.851 (NA)	0.011 ± 0.007	0.011 (NA)	1.31	P < 0.01 (within group change for intervention)
			Calcaneus	**Calcaneus**	**Calcaneus**	**Calcaneus**	**Calcaneus**	**Calcaneus**
		I	0.499 ± 0.093	0.500 (NA)	0.001 ± 0.004	0.001 (NA)	0.20	P = 0.04 (intervention vs control)
		C	0.528 ± 0.108	0.518 (NA)	-0.010 ± 0.004	-0.010 (NA)	-1.89	P < 0.01 (within group change for control)
Ebrahim et al, 1997	DXA BMD (g/cm^2) Lumbar Spine and Femoral Neck		**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**
		I	0.997 ± 0.194	1.014 (NA)	0.017 ± 0.051	0.017 (NA)	1.71	NS (intervention vs control)
		C	0.938 ± 0.168	0.955 (NA)	0.017 ± 0.054	0.017 (NA)	1.81	
			Femoral Neck	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**
		I	0.806 ± 0.122	0.804 (NA)	-0.002 ± 0.042	-0.002 (NA)	-0.25	NS (intervention vs control)
		C	0.765 ± 0.145	0.744 (NA)	-0.021 ± 0.065	-0.021 (NA)	-2.75	
Evans et al, 2007	DXA BMD (g/cm^2) Lumbar Spine and Femoral Neck		**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**	**Lumbar Spine**
		I	Milk 0.850 ± 0.154 Soy 0.961 ± 0.228	NA due to split grouping	−0.015 ± 0.022 (interventions grouped)	-0.015 (NA)	NA	NS (intervention vs control)
		C	Milk 0.939 ± 0.150 Soy 0.915 ± 0.146	NA due to split grouping	−0.009 ± 0.028 (interventions grouped)	-0.009 (NA)	NA	
			Femoral Neck	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**
		I	Milk 0.710 ± 0.116 Soy 0.722 ± 0.129	NA due to split grouping	−0.004 ± 0.026 (interventions grouped)	-0.004 (NA)	NA	NS (intervention vs control)

		C	Milk 0.738 ± 0.090 Soy 0.673 ± 0.111	NA due to split grouping	0.000 ± 0.022 (interventions grouped)	0.000 (NA)	NA	
Hosny et al, 2012	DXA BMD (g/cm^2) L2-L4 and Total Hip	**I** **C** **I** **C**	**L2 – L4** 1.16 ± 0.08 1.15 ± 0.07 **Total Hip** 0.96 ± 0.07 0.96 ± 0.08	**L2 – L4** 1.24 ± 0.13 1.08 ± 0.09 **Total Hip** 1.02 ± 0.09 0.93 ± 0.06	**L2 – L4** 0.08 -0.07 **Total Hip** 0.06 -0.03	**L2 – L4** 0.080 (0.013 to 0.147) -0.070 (-0.120 to -0.020) **Total Hip** 0.060 (0.010 to 0.110) -0.030 (-0.074 to 0.014)	**L2 – L4** 6.90 -6.09 **Total Hip** 6.25 -3.13	**L2 – L4** P = 0.007 (within group) P = 0.004 (within group) **Total Hip** P = 0.009 (within group) P = 0.001 (within group)
Huuskonen et al, 2001 & 2002 & Remes et al, 2003 & 2004	DXA BMD (g/cm^2) L2-L4 and Femoral Neck	**I** **C** **I** **C**	**L2 – L4** 1.233 ± 0.178 1.192 ± 0.185 **Femoral Neck** 1.010 ± 0.138 1.024 ± 0.137	**L2 – L4** NA NA **Femoral Neck** NA NA	**L2 – L4** NA NA **Femoral Neck** NA NA	**L2 – L4** NA NA **Femoral Neck** NA NA	**L2 – L4** NA NA **Femoral Neck** NA NA	**L2 – L4** NS (intervention vs control) **Femoral Neck** NS (intervention vs control)
Krustrup et al, 2009	DXA BMD (g/cm^2) Whole body	**I** **C**	**Whole Body** 1.33 ± 0.03 1.28 ± 0.03	**Whole Body** 1.33 ± 0.03 1.27 ± 0.03	**Whole Body** 0.00 (NA) -0.01 (NA)	**Whole Body** 0.00 (-0.026 to 0.026) -0.01 (-0.036 to 0.016)	**Whole Body** 0 -0.78	**Whole Body** NS (within group) NS (within group)
Krustrup et al, 2010	DXA BMD (g/cm^2) Whole body and Legs	**I** **C** **I** **C**	**Whole Body** 1.158 ± 0.026 1.188 ± 0.035 **Legs** 1.229 ± 0.018 1.312 ± 0.044	**Whole Body** 1.161 ± 0.022 1.208 ± 0.035 **Legs** 1.258 ± 0.013 1.330 ± 0.036	**Whole Body** 0.003 0.020 **Legs** 0.029 0.018	**Whole Body** 0.003 (-0.021 to 0.027) 0.020 (-0.017 to 0.057) **Legs** 0.029 (0.014 to 0.044) 0.018 (-0.024 to 0.060)	**Whole Body** 0.26 1.68 **Legs** 2.36 1.37	**Whole Body** NS NS **Legs** P < 0.05 NS

I represents “intervention group”, C represents “control group”, NA represents “not available”, NS represents “not significant”

Two INT studies measured BMD of the lumbar spine and femoral neck (Chilibeck et al, 1996; Kato et al, 2006), whilst three studies measured BMD of the femoral neck only (Allison et al, 2013 & 2015; Bailey et al, 2010; Lester et al, 2009) and one study reported total hip BMD (Tucker et al, 2015). Two studies measured volumetric BMD of the radius using CT scans (Bhatia et al, 2015; Troy et al, 2013). BMD precision information was reported for seven INT studies (Allison et al, 2013; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Chilibeck et al, 1996; Kato et al, 2006, Lester et al, 2009, Troy et al, 2013). (Table 3.3.5b).

Table 3.3.5b. Intermittent exercise data extraction

Study	Outcomes	Group	Baseline (Mean ± SD)	Post Intervention (Mean ± SD)	Absolute Change (SD)	Mean Diff (Post-Pre) (95% CI)	% Change	Improvement
Allison et al, 2013 & 2015	DXA BMD (g/cm^2) L1-L4 and Femoral Neck	**I**	**L1 -L4** 1.258 ± 0.030 (control not possible)	**L1 - L4** 1.270 ± 0.030 (control not possible)	**L1 - L4** 0.012 (NA)	**L1 - L4** 0.012 (-0.002 to 0.026)	**L1 - L4** 0.95 (NA)	**L1 - L4** NS (within group)
		I	**Femoral Neck** 0.948 ± 0.018	**Femoral Neck** 0.954 ± 0.017	**Femoral Neck** 0.006 (NA)	**Femoral Neck** 0.006 (-0.002 to 0.014)	**Femoral Neck** 0.63 (NA)	**Femoral Neck** P = 0.003 (group x time interaction)
		C	0.954 ± 0.018	0.945 ± 0.018	-0.009 (NA)	-0.009 (-0.017 to -0.001)	-0.94 (NA)	
Bailey and Brooke-Wavell, 2010	DXA BMD (g/cm^2) Femoral Neck		**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck** *Mean (95% CI)*	**Femoral Neck**
		I (Ex7)	NA	NA	NA	NA	1.7 (0.7 to 2.7)	P = 0.003 (compared to control)
		I (Ex4)	NA	NA	NA	NA	0.9 (−0.2 to 2.0)	P = 0.015 (compared to Ex2)
		I (Ex2)	NA	NA	NA	NA	0.2 (−0.8 to 1.2)	(BMD change in the exercise leg between groups
		C	NA	NA	NA	NA	−0.3 (−1.2 to 0.5)	(with change in the control leg and baseline BMD as covariates)).
Bhatia et al, 2015	CT vBMD (g/cm^3) Distal 12 cm Arm	**I**	**Distal Arm** 0.26 ± 0.05	**Distal Arm** 0.262 (NA)	**Distal Arm** 0.002 (NA)	**Distal Arm** 0.002 (NA)	**Distal Arm** 0.7 ± 2.0	**Distal Arm** P < 0.05 (intervention vs control)
		C	0.25 ± 0.04	0.244 (NA)	-0.006 (NA)	-0.006 (NA)	-2.6 ± 2.6	
Chilibeck et al, 1996	DXA BMD (g/cm^2) Lumbar Spine and Femoral Neck	**I**	**Lumbar Spine** 1.06 ± 0.10	**Lumbar Spine** NA	**Lumbar Spine** NA	**Lumbar Spine** NA	**Lumbar Spine** NA	**Lumbar Spine** NS
		C	1.06 ± 0.09	NA	NA	NA	NA	NS

			Femoral Neck					
		I	0.94 ± 0.14	NA	NA	NA	NA	NS
		C	0.94 ± 0.12	NA	NA	NA	NA	NS
Kato et al, 2006	DXA BMD (g/cm^2) L2-L4 and Femoral Neck		**L2 - L4**	**L2-L4**	**L2-L4**	**L2-L4**	**L2-L4**	**L2-L4**
		I	0.991 ± 0.115	1.015 ± 0.113	0.024 (NA)	0.024 (-0.050 to 0.098)	2.42 (NA)	P < 0.01 (within group)
		C	1.007 ± 0.113	1.013 ± 0.110	0.006 (NA)	0.006 (-0.067 to 0.079)	0.60 (NA)	NS
			Femoral Neck	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**
		I	0.984 ± 0.081	1.010 ± 0.080	0.026	0.026 (-0.027 to 0.079)	2.64 (NA)	P < 0.01 (within group)
		C	0.985 ± 0.143	0.974 ± 0.134	-0.011	-0.011 (-0.102 to 0.080)	-1.12 (NA)	NS
Lester et al, 2009	DXA BMD (g/cm^2) Femoral Neck pQCT BMD (g/cm^3)		**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**	**Femoral Neck**
		I	1.12 ± 0.15	1.12 ± 0.14	0.000	0.000 (-0.098 το 0.098)	0	NS
		C	1.11 ± 0.14	1.10 ± 0.14	-0.010	-0.010 (-0.133 το 0.113)	-0.9	NS
			Tibia 4%	**Tibia 4%**	**Tibia 4%**	**Tibia 4%**	**Tibia 4%**	**Tibia 4%**
		I	317.98 ± 45.57	317.27 ± 48.34	-0.71	-0.71 (-33.26 το 31.84)	-0.2	NS
		C	312.14 ± 17.52	310.50 ± 19.51	-1.64	-1.64 (-17.9 το 14.6)	-0.5	NS
Troy et al, 2013	CT vBMD (g/cm^3) Distal 12 cm Arm		**Distal Arm**	**Distal Arm**	**Distal Arm**	**Distal Arm**	**Distal Arm**	**Distal Arm**
		I	0.42 ± 0.05	0.42 (NA)	0.00 (NA)	0.00 (NA)	-0.33 (0.84)	NS (intervention vs control)
		C	0.40 ± 0.03	0.40 (NA)	0.00 (NA)	0.00 (NA)	-1.02 (0.85)	
Tucker et al, 2015	DXA BMD (g/cm^2) Total Hip	**I20**	**Total Hip**	**Total Hip**	**Total Hip**	**Total Hip**	**Total Hip**	**Total Hip**
			0.915 ± 0.079	0.917 (NA)	0.002 (NA)	0.002 (NA)	0.23 (3.02)	NS (intervention vs control)
		I10	0.935 ± 0.081	0.938 (NA)	0.003 (NA)	0.003 (NA)	0.28 (1.62)	NS (intervention vs control)
		C	0.907 ± 0.088	0.899 (NA)	-0.008 (NA)	-0.008 (NA)	-0.85 (1.55)	

I represents “intervention group”, C represents “control group”, I Ex7 represents “7 days per week intervention group”, I Ex4 represents “4 days per week intervention group”, I Ex2 represents “2 days per week intervention group”, I20 represents “20 jump intervention group”, I10 represents “10 jump intervention group” , NA represents “not available”, NS represents “not significant”.

3.3.6 Adherence

No adherence data was presented for any of the continuous studies.

All intermittent studies presented adherence data with two exceptions, one that did not mention adherence (Lester et al, 2009) and one that excluded data for participants whose adherence was less than 40% but did not give adherence data (Tucker et al, 2015). For the six studies that gave adherence information, the adherence was very good and exceeded 80% in all cases.

3.3.7 Length of Intervention

Continuous studies ranged from 12 weeks to 4 years, intermittent studies ranged from 8 weeks to 12 months. The continuous studies that generated positive BMD outcomes were all 3 months duration and above (Brooke-Wavell et al, 1997; Hosny et al, 2012; Krustrup et al, 2010). The intermittent studies that generated positive BMD outcomes were all 22 weeks duration and above (Allison et al, 2013 & 2015; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006).

3.3.8 Study Quality

The Cochrane risk of bias tool highlighted that none of the studies selected were

free from a high risk of bias in at least two main areas with some studies showing a high risk of bias in up to six main areas. Blinding the participants and investigators to the intervention and control group allocation was usually impossible in these types of exercise interventions although the blinding of the DXA operator whilst possible, was only conducted in four out of fifteen studies (Ebrahim et al, 1997; Brooke-Wavell et al, 1997, Allison et al, 2013 & 2015; Kato et al, 2006). Only five out of fifteen studies showed a low risk of bias for incomplete outcome data (Brooke-Wavell et al, 1997; Hosny et al, 2012; Huuskonen et al, 2001 & 2002 and Remes et al, 2003 & 2004; Chilibeck et al, 1996; Kato et al, 2006). Three continuous studies (Brooke-Wavell et al, 1997; Evans et al, 2007; Krustrup et al, 2009) and six intermittent studies (Allison et al, 2013 & 2015; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006; Lester et al, 2009; Troy et al, 2013) showed a low risk of bias from selective reporting. Table 3.3.8a and Table 3.3.8b display the Cochrane risk of bias evaluation for the selected studies.

Table 3.3.8a Continuous exercise – Cochrane risk of bias tool

	Brooke-Wavell et al, 1997	**Ebrahim et al, 1997**	**Evans et al, 2007**	**Hosny et al, 2012**	**Huuskonen et al, 2001 & 2002 and Remes et al, 2003 & 2004**	**Krustrup et al, 2009**	**Krustrup et al, 2010**
Random sequence generation	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Allocation concealment	High risk	High risk	High risk	High risk	High risk	High risk	High risk
Blinding of participants and personnel	High risk	High risk	High risk	High risk	High risk	High risk	High risk
Blinding of outcome assessment	Low risk	Low risk	High risk	High risk	High risk	High risk	High risk
Incomplete outcome data	Low risk	High risk	High risk	Low risk	Low risk	High risk	High risk
Selective reporting	Low risk	Unclear risk	Low risk	High risk	High risk	Low risk	High risk
Other sources of bias	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	High risk

Table 3.3.8b Intermittent exercise – Cochrane risk of bias tool

	Allison et al, 2013 & 2015	**Bailey and Brooke-Wavell, 2010**	**Bhatia et al, 2015**	**Chilibeck et al, 1996**	**Kato et al, 2006**	**Lester et al, 2009**	**Tucker et al, 2015**	**Troy et al, 2013**
Random sequence generation	Low risk	Low risk	High risk	High risk	Low risk	Low risk	Low risk	High risk
Allocation concealment	High risk	High risk	High risk	High risk	High risk	High risk	High risk	High risk
Blinding of participants and personnel	High risk	High risk	High risk	High risk	High risk	High risk	High risk	High risk
Blinding of outcome assessment	Low risk	High risk	High risk	High risk	Low risk	High risk	High risk	High risk
Incomplete outcome data	High risk	High risk	High risk	Low risk	Low risk	High risk	High risk	High risk
Selective reporting	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	High risk	Low risk
Other sources of bias	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	High risk	Low risk

3.4 Discussion

3.4.1 Main Findings

The main findings of this review were that one out of seven studies using continuous exercise had a positive effect on BMD at the calcaneus when compared to a control group (Brooke-Wavell et al, 1997). Three out of eight studies using intermittent exercise found positive effects on BMD at the femoral neck or distal arm when compared to a control group (Allison et al, 2013; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015). From this evidence, it is not possible to determine if continuous or intermittent exercise would be more beneficial for improving BMD. This review also highlights a number of issues regarding the lack of controlled, specific and defined exercise periods and rest intervals in exercise interventions and also the inconsistency of BMD outcomes reporting which make it difficult to compare and summarise the effects of either continuous or intermittent exercise on BMD.

For a direct comparison of the effects of continuous and intermittent exercise upon BMD the same form of exercise must be completed during a continuous and intermittent condition where the only variable that is manipulated is the length of the rest interval. As most of the continuous interventions comprised of walking and running whilst the intermittent interventions comprised of high impact

activity, loading cycles or resistance exercise this comparison becomes complicated. Four intermittent studies found statistically significant improvements in BMD when compared to a control group (Allison et al, 2013; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006). By contrast, only one of the continuous studies showed a statistically significant improvement in BMD when compared to a control group (Brooke-Wavell et al, 1997) (Table 3.3.5a). Whilst a greater number of intermittent studies showed positive effects on BMD than continuous studies, this could merely be due to the use of exercises with a greater osteogenic potential or the use of younger populations in which the adaptations are magnified (Bassey et al, 1998) (Table 3.3.5b). The use of different types of exercise confounds any findings that may have arisen during this review.

3.4.2 Study Number and Design

Once the exclusion criteria had been applied to the full texts there was only a small number of well controlled continuous and intermittent exercises studies left (seven and eight respectively). Many other exercise programmes targeted at improving bone health have been intermittent in nature but have not reported well defined exercise and rest intervals and therefore were omitted from this review. This applies particularly to the large number of resistance training interventions, which are typically split into sets with inter-set rests although only two studies

met the strict criteria in terms of a well-controlled rest interval for this review (Chilibeck et al, 1996; Lester et al, 2009). A substantial number of mixed loading exercise interventions were also omitted due to the multicomponent nature of the exercises, which were less well controlled and involved unrecorded changeover time between stations/exercises.

3.4.3 Specific Findings

Although percentage change in BMD is widely reported, absolute reported values or absolute changes in BMD are preferable (Brown et al, 2002; Baim et al, 2015). Six continuous studies and three intermittent studies conformed with these guidelines (continuous: Brooke-Wavell et al, 1997; Ebrahim et al, 1997; Evans et al, 2007; Hosny et al, 2012; Krustrup et al, 2009, Krustrup et al, 2010; intermittent; Allison et al, 2013; Kato et al, 2006; Lester et al, 2009) whilst five intermittent studies presented percentage changes (Bailey and Brooke-Wavell, 2010, Bhatia et al, 2015, Chilibeck et al, 1996; Troy et al, 2013; Tucker et al, 2015), and one continuous study provided no values (Huuskonen et al, 2001). When determining true changes in lumbar spine and femoral neck BMD, with the use of intervention group and control group comparisons, there were only four studies to show a statistically significant benefit and these were all intermittent. These included different skeletal sites and ranged from the intervention groups displaying an improvement of 0.63%, 1.7 [95%CI: 0.7 o 2.7]%, 0.7 ± 2.0%,

2.64% and the respective control groups showing a loss of -0.94%, -0.3 [95%CI: -1.2 to 0.5]%, -2.6 ± 2.6%, -1.12% (Allison et al, 2013; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006).

3.4.4 Study Quality

Random sequence generation was performed for all continuous exercise studies and five out of eight intermittent studies. Allocation concealment was not used in any of the studies that were reported. Due to the nature of these exercise interventions, it was impossible to blind participants or researchers to whether the participants performed the intervention or were placed in the control group. The exercises must have been monitored and explained thoroughly in order to complete the intervention. However, it is possible to blind the DXA or pQCT operators as to participant group allocation and this was only conducted in two out of seven continuous exercise interventions (Brooke-Wavell et al, 1997; Ebrahim et al, 1997) and also two out of eight intermittent exercise interventions (Allison et al, 2013; Kato et al, 2006), which is surprising. Many of the studies presented incomplete data sets mainly through large drop-out rates, which highlights the difficulties in maintaining participant numbers and intervention adherence rates with human populations.

3.4.5 Strengths and Limitations of the Review

A range of different modes of exercise were found to have a positive effect on BMD including continuous brisk walking and running (Brooke-Wavell et al, 1997; Hosny et al, 2012; Krustrup et al, 2010) and intermittent hopping, jumping and wrist loading (Allison et al, 2013 & 2015; Bhatia et al, 2015; Kato et al, 2006; Bailey and Brooke-Wavell, 2010). This is consistent with previous research, which has concluded that both walking and high impact exercise are beneficial for hip BMD adaptations (Martyn-St James and Carroll, 2008; Martyn-St James and Carroll, 2010; Xu et al, 2016). Interestingly the two intermittent resistance training studies reported no positive response in lumbar spine or femoral neck BMD (Chilibeck et al, 1996; Lester et al, 2009), despite research showing a clear benefit of resistance training upon lumbar spine BMD in postmenopausal women (Martyn-St James and Carroll, 2006a). This is likely due to the length of the interventions as opposed to the method of exercise in these two cases as resistance exercise is a known anabolic stimulus for bone in premenopausal women (Xu et al, 2016). It is possible that these interventions may have increased BMD if they were prolongued over the duration of a year or longer but with durations of 2 months and 5 months, there will have been insufficient time for the detection of a change in BMD using DXA (Lester et al, 2009; Chilibeck et al, 1996) and insufficient time for the detection of a change using pQCT (Lester et al, 2009).

Greater than six months has been recommended to allow sufficient time for DXA

derived BMD changes whilst three months has been recommended for QCT derived bone geometric changes in premenopausal women (Ahola, 2009; Nikander et al, 2010) and durations of over 12 months have been recommended for DXA derived BMD changes in postmenopausal women (Kemmler and Engelke, 2004). Four continuous studies (Brooke-Wavell et al, 1997; Ebrahim et al, 1997; Huuskonen et al, 2001 & 2002; Remes et al, 2003 & 2004; Krustrup et al, 2010) and five intermittent studies (Allison et al, 2013 & 2015; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006; Troy et al, 2013) met these recommendations. Of these studies two continuous studies found positive changes in BMD (Brooke-Wavell et al, 1997; Krustrup et al, 2010) and four intermittent studies found positive changes in BMD (Allison et al, 2013 & 2015; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006). Three continuous studies (Evans et al, 2007; Hosny et al, 2012; Krustrup et al, 2009) and three intermittent studies (Chilibeck et al, 1996; Lester et al, 2009; Tucker et al, 2015) did not last long enough in duration to meet these recommendations. Unsurprisingly two continuous studies (Evans et al, 2007; Krustrup et al, 2009) and all three intermittent studies (Chilibeck et al, 1996; Lester et al, 2009; Tucker et al, 2015) did not find positive changes in BMD, however, remarkably Hosny et al, 2012 found extremely large and statistically significant increases in lumbar spine (+6.9%) and femoral neck BMD (+5.2%) using a three month brisk walking intervention. There were equally large and statistically significant reductions in the control group BMD levels despite only lasting 12 weeks. The walking group

increases appear to be miscalculated based on the information provided as we calculated them as +6.9% and +6.3% at the lumbar spine and total hip respectively. The control group BMD losses were reported as -6.1% and -5.1% at the lumbar spine and total hip respectively whereas we calculated the same values based on the information provided as -6.1% and -3.1%. In addition, the same results were presented as different values when used in a figure, which would question the reliability of these findings. It is important to note that the precision error increases when scanning obese participants, which was the target population used in this study (body mass index: 30 to 35 kg/m^2) (Knapp et al, 2012). This could potentially help explain the large differences in the results.

Recent recommendations state that BMD measurement precision should be reported as root mean square standard deviation in absolute units (g/cm^2) which is used to calculate the least significant change in BMD within a 95% confidence limit (Baim et al 2015; The International Society for Clinical Densitometry, 2015). The reviewed studies reporting of DXA precision ranged from standard error in g/cm^2 (Brooke-Wavell et al, 1997), a single percentage value (Evans et al, 2007), root mean square average of coefficient of variation percentage (Huuskonen et al, 2001; Bhatia et al, 2015), coefficient of variation (Allison et al, 2013; Bailey and Brooke-Wavell, 2010; Chilibeck et al, 1996; Kato et al, 2006; Lester et al, 2009; Troy et al, 2013). pQCT and QCT precision was reported as root mean square average of coefficient of variation percentage (Bhatia et al,

2015) and coefficient of variation (Troy et al, 2013). In addition, changes in BMD were often reported without the precision measurement for continuous studies. Precision measurements were much more regularly reported for intermittent studies (Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015; Kato et al, 2006). Without individual changes being presented, it is not possible to determine whether changes in BMD were large enough to be detected in an individual.

3.5 Conclusions

This review has shown that continuous and intermittent exercise and their effects upon BMD require further study in human populations. We highlighted only seven continuous studies and eight intermittent studies that were sufficiently controlled enough to meet our criteria. The study quality analysis highlighted the large potential for inherent methological bias in some cases. The length of the interventions, consistency in reporting BMD value changes and consistency of reporting the effect of the intervention when compared to the control group were lacking in many cases. Future studies should address these issues, as it is important to establish whether intermittent exercise could provide a more potent osteogenic stimulus for bone adaptations. It is important to optimise exercise interventions in order to maximise bone health in a range of populations, in particular those at risk of developing osteoporosis to reduce the risk of bone fractures (Kanis et al, 2001). Whilst both continuous and intermittent exercise can

be used to stimulate bone adaptation, intermittent exercise has the potential to provide a greater osteogenic stimulus than continuous exercise (Robling et al, 2001), but requires further investigation in humans with well-controlled exercise interventions that include specific rest intervals.

4. Tibial impacts and muscle activation during walking, jogging and running when performed overground, and on motorised and non-motorised treadmills

4.1 Introduction

Walking and running are the most common forms of human locomotion and are usually performed overground. However, walking and running are often performed on treadmills as attractive alternatives and to facilitate studies under controlled conditions.

The motorised treadmill is the most common ergometer and is powered by a motor that keeps the treadmill belt at a constant velocity. The non-motorised treadmill is less common and is characterised by a freely moveable treadmill belt powered by the individual by means of a horizontal tether attached at the waist. This allows the self-propelled belt to rotate according to the speed of the participant. Several studies have compared motorised treadmill vs overground locomotion to examine kinematics (Sinclair et al, 2013b), ground reaction forces (Riley et al, 2007) and muscular activation differences (Murray et al, 1985; Lee and Hidler, 2008; Prosser et al, 2011). Similarly, non-motorised treadmill and overground locomotion have been compared for 5000 m performance time, EMG amplitude, blood lactate, oxygen uptake kinetics, heart rate (Stevens et al, 2015),

maximal sprinting performance (Highton et al, 2012) and 6-minute walk distance (Janaudis-Ferreira et al, 2010) that have all highlighted differences between the conditions, which could affect the mechanical loading environment and also the musculoskeletal adaptations generated by different locomotion conditions.

Walking and running, either overground or on a treadmill are recommended for the health of the general population and are feasible methods of exercise for all ages (Garber et al, 2011; Engelke et al, 2006), with benefits including reduced body fat, lowered resting heart rate and increased maximal oxygen uptake (Hespanhol Junior et al, 2015). Walking and running are also recommended for maintaining bone health during ageing (Brooke-Wavell et al, 1997; Kohrt et al, 2004; Martyn-St James and Carroll, 2008; NHS, 2015). For bone health, it is important to establish the magnitude of mechanical loading and muscle activation generated by walking and running as the intensity of loading encourages skeletal adaptation (Vainionpaa et al, 2007). Muscular activation has been linked with internal compressive forces that increase the mechanical loading on bones (Bassey et al, 1997). In addition, muscles impose a force on the skeletal system, which increases bone remodelling (Robling, 2009). Impact forces and muscle activation patterns are well recognised in the habitual human gait, with accelerometry recommended as an indicator of bone strain and EMG amplitude indicating the internal forces experienced (Mizrahi et al, 2000; Turcot et al, 2008; Edwards et al, 2009, Murley et al, 2010, Andrade and Andrade, 2012). Due to the

biomechanical differences between overground, motorised treadmill and non-motorised treadmill conditions, there is also the potential for the impact forces and EMG to show differences across the locomotion conditions, which would alter the mechanical loading environment. It is therefore important to establish the mechanical loading generated during each condition to determine their osteogenic potential.

Given the popularity of walking and running overground and on treadmills, it is important to understand how the impacts and muscle activity respond under different conditions in these types of locomotion. The NMT presents type of freely moveable treadmill that allows for the undertaking of intermittent exercise protocols (Brown et al, 2007; Highton et al, 2012; Aldous et al, 2014; Tofari et al, 2015). The device also permits the quantification of instantaneous GRF measurement throughout an exercise protocol, which would quantify mechanical loading characteristics and allow for the control of these variables during continuous and intermittent protocols. The NMT loading parameters have not been well established or compared to that of overground or motorosed treadmill conditions previously. It was previously stated in chapter 3 that well controlled exercise interventions are required to examin the effects of continuous and intermittent exercise on BMD. Should loading conditions be found to be sufficient whilst either running, jogging or walking on a NMT, this device could provide an ideal training tool for the manipulation of continuous and intermittent

exercise for the purpose of an exercise intervention. Accordingly, the aim of this study was to compare the ground impacts via accelerometry and muscle activation via surface EMG generated between overground (OG), motorised treadmill (MT) and non-motorised treadmill (NMT) conditions when walking, jogging and running at matched speeds. This was the first study to comprehensively examine impacts and muscle activation during locomotion at different velocities and in different conditions within the same population. We hypothesised that as differences have been highlighted in a number of physiological variables during NMT locomotion compared with overground locomotion, that the impact forces would be reduced and the EMG amplitude would be increased when using a NMT, which could change the mechanical loading stimulus for musculoskeletal adaptations.

4.2 Methods

4.2.1 Participants

Participants were recruited from the University of Hull via email and word of mouth. Participants were excluded if they were outside the age range of 18 to 35 years or if they had any of the following; a past history of cardiovascular, renal, hepatic, thyroid disease, a history of physical disability, a family history of

sudden cardiac death or any existing musculoskeletal injuries that would affect their ability to walk, jog or run. Familiarisation was undertaken at least 48 h before the main testing, and involved walking, jogging and running at a constant speed on a non-motorised treadmill (NMT). Participants were already familiar with overground and motorised treadmill locomotion. The protocol was approved by the institutional ethics committee and informed consent was obtained from all participants prior to testing.

4.2.2 Procedures

Tibial acceleration (ACC) and lower body muscle activation were measured during OG, MT and NMT locomotion whilst walking, jogging and running at matched velocities using a cross sectional repeated measures design. Although GRF would have been a preferable measure, it was not viable due to the lack of force plate technology in the treadmills used. In addition, the NMT could only sample at a maximum of 200 Hz which, when measuring impacts would underestimate the true magnitude of the forces experienced (Lafortune et al, 1995). Following a warm up of walking, jogging, running and dynamic stretching, participants walked, jogged and ran along a 40 m indoor laboratory at a self-selected constant velocity whilst instantaneous velocity was recorded at 100 Hz with a speed meter via a waist harness (Speed Real Time, AP Lab, V3.1 – 2012, Rome, Italy). This speed meter has shown to provide reliable assessment

of instantaneous centre of mass velocity (Morouco et al, 2006). One trial was completed but trials were repeated if necessary to achieve a constant velocity (determined from manual inspection of velocity data). Overground walking (1.56 ± 0.15 $m.s^{-1}$), jogging (2.88 ± 0.35 $m.s^{-1}$) and running (4.28 ± 0.36 $m.s^{-1}$) were individually replicated during 30 s bouts on a MT (Woodway ELG55, Woodway, Weil an Rhein, Germany) and NMT (Woodway Force 2.0. Woodway, Weil an Rhein, Germany) in a randomised order. MT speeds were constant whereas NMT speeds were matched when walking (1.56 ± 0.13 $m.s^{-1}$), jogging (2.88 ± 0.35 $m.s^{-1}$) and running (4.25 ± 0.37 $m.s^{-1}$). Participants were instructed to walk, jog or run "naturally". Trials were separated by 4-5 min rest allowing sufficient recovery and to reduce any effects of fatigue. Umbro 5v5 trainers (Umbro, Cheshire, UK) were worn by all participants in their correct size to standardise footwear.

ACC and EMG data were collected synchronously via Noraxon hardware (sampling rate = 1500 Hz, input impedance > 100 MΩ, CMRR > 100 dB, baseline noise < 1μV RMS, base gain = 200, final gain = 500) and stored on a computer using a 16-bit resolution wireless system (Desktop DTS, Noraxon USA Inc, Arizona, USA). An accelerometer (DTS 3D accelerometer-16 g, Noraxon USA Inc, Arizona, USA) was attached to the midanterior right tibia, parallel with the long axis of the tibia to assess compressive forces (50% of the distance between the tibial tuberosity and medial malleolus) in order to reduce the preserve the fast frequency components of the signal and reduce the effect of skin movement

(LaFortune et al, 1995; Vihriala et al, 2000). Surface EMG electrodes (Ambu Blue Sensor N, Ambu, Cambridgeshire, UK) were placed over the rectus femoris (RF), semitendinosus (ST), tibialis anterior (TA), and soleus (SL) muscles of the participant's right leg in accordance with SENIAM surface electromyography recommendations (Hermens et al, 1999). These specific muscles were chosen due to their biarticulate direct compressive action on the femur across the hip and knee joints or their attachment to the tibia to link with the ACC measurements. Prior to electrode attachment, the skin was shaved, abraded and cleansed with a 70% alcohol swab. ACC and EMG wearable hardware were secured with surgical tape and elasticated bandages to reduce unwanted movement and signal artefacts.

4.2.3 Data Processing

Each gait cycle was identified using ACC data parallel to the long axis of the tibia, beginning at the lowest trough preceding the impact peak of the right tibia (which represented initial ground contact) and ending at the same point preceding the next impact peak of the right tibia (Ben Mansour et al, 2015). Eight cycles were selected for analysis from a section where the participant was moving at a matched constant velocity in each condition. Point of ground contact was established using pilot data where synchronised motion capture, ground reaction force, sacrum and tibia accelerometers were used. The ground reaction force data served as the gold standard measure from which the identification of the point of

ground contact and the following impact peak was determined. Once these instances were identified, it enabled the point of ground contact and following impact peaks to be identified on the accelerometer traces using the time of each ground contact event and the subsequent peak acceleration value.

ACC data were low-pass filtered at cut-offs of 16, 33 and 40 Hz for walking, jogging and running respectively across all conditions based on a cut-off frequency set at 95% of the signal energy from a mean of the trials from the first 10 participants (Sinclair et al, 2013a). Acceleration peak was established as the immediate impact peak following ground contact. Acceleration gradient was calculated as the slope from the point of ground contact to the acceleration peak (Heikkinen et al, 2007) and cycle time was calculated as the duration between right foot ground contacts upon landing. Acceleration peak, acceleration gradient and cycle time were averaged across 8 cycles per trial.

EMG data were band-pass filtered (bi-directional Butterworth, 10 - 500 Hz), full wave rectified and low-pass filtered at 15 Hz to obtain linear envelopes. EMG amplitude was calculated as the area under the curve (trapezium method) for each of the 8 identified cycles. EMG amplitude was taken as the mean across 8 cycles per trial and normalised to the NMT run trial, which produced the highest averaged EMG amplitudes across the RF, ST and SL muscles (Prosser et al, 2011). EMG co-contraction values were calculated, expressing the EMG amplitude of the agonist musculature as a percentage of the antagonistic

musculature. RF values were expressed as a percentage of the ST values whilst TA values were expressed as a percentage of the SL values. A value of 100 indicates equal activation of the agonist and antagonist muscles. Values over 100 indicate greater RF or greater TA muscle activation compared to the ST and SL muscles respectively (Kellis and Kouvelioti, 2009), for the purpose of this experiment, anterior muscles were classed as agonists and posterior muscles were classed as antagonists (Raiteri et al, 2015).

Data were processed using Myoresearch XP software (Myoresearch XP Master Edition 1.08.27, Noraxon USA Inc, Arizona, USA) and a bespoke MATLAB programme (MATLAB R2011a, Mathworks, Cambridge, UK).

4.2.4 Statistical Analysis

Data containing excessive signal interference were removed. Parametric data were statistically analysed using two-way (3 conditions x 3 velocities) repeated measures ANOVAs (Sidak adjustments) with post-hoc pairwise comparisons using SPSS (IBM SPSS Statistics Version 20.0. IBM Corp, NY, USA). Where applicable, non-normally distributed data were log-transformed and analysed using parametric methods. Cohen's *d* effect size is reported and evaluated using the following scale: 0 - 0.19 trivial, 0.2 - 0.59 small, 0.6 - 1.19 moderate, 1.2 - 1.99 large, 2.0 - 3.99 very large. Uncertainty in the population estimates are

expressed as 95% confidence intervals along with the likelihood that the effect is substantially positive, trivial or substantially negative (Hopkins, 2006). For non-parametric data where log-transformation was not possible, Friedman's tests were used to compare main effects of treadmill and velocity conditions. Wilcoxon signed-rank tests with Bonferroni corrections were used for pairwise comparisons and post hoc tests in SPSS, resulting in an alpha level set at $P < 0.017$ due to 3 groupings. Cliff's Delta (δ) effect size was calculated in R (R Foundation for Statistical Computing 3.2.1, Vienna, Austria) effsize package (Torchiano, 2016), and evaluated using the following scale: 0 - 0.146 trivial, 0.147 - 0.32 small, 0.33 - 0.473 moderate, >0.474 large. Uncertainty in the population estimates are expressed as 95% confidence intervals (Cliff, 1996).

4.3 Results

4.3.1 Participant Characteristics

The 15 male participants that completed the testing protocol (mean ± SD: 24.2 ± 3.8 years, 179.5 ± 3.9 cm, 81.0 ± 7.2 kg) were recreationally active.

4.3.2 Accelerometry

There were significant main effects for both condition ($P = 0.014$) and velocity ($P < 0.001$). The running condition resulted in large increases in acceleration peaks and gradients when compared to walking and jogging trials, while jogging trials resulted in large increases in acceleration peaks and gradients when compared to walking trials (Fig. 4.3.2a). The NMT produced large reductions in acceleration peaks when compared to OG and MT conditions across all walking ($\delta = -0.56$ [95%CI: -0.81 to -0.13], $P = 0.004$; , $\delta = -0.58$ [95%CI: -0.83 to -0.15], $P = 0.002$), jogging ($\delta = -0.64$ [95%CI: -0.85 to -0.23], $P = 0.001$; $\delta = -0.78$ [95%CI: -0.92 to -0.45], $P = 0.001$) and running conditions ($\delta = -0.51$ [95%CI: -0.77 to - 0.11], $P = 0.004$; $\delta = -0.51$ [95%CI: -0.78 to -0.01], $P = 0.001$). OG and MT conditions were similar. The treadmill condition had no main effect on acceleration gradients, ($P = 0.759$) although there was a main effect for velocity ($P < 0.001$) (Fig. 4.3.2b).

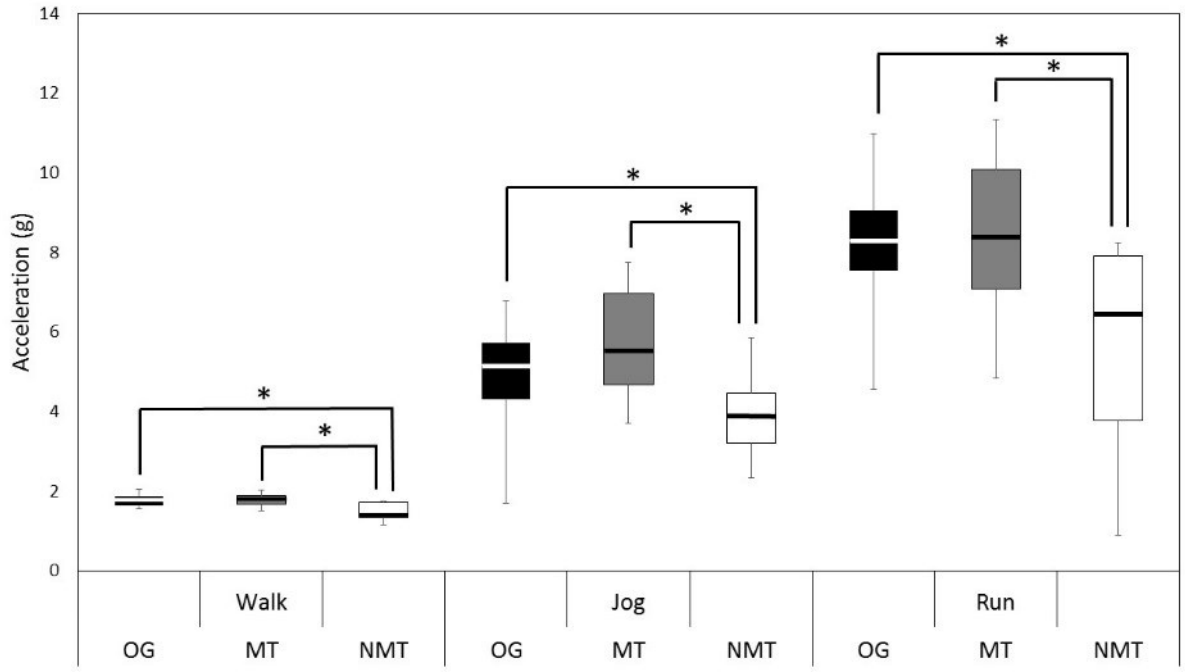


Fig. 4.3.2a. Median (interquartile range, minimum and maximum) acceleration peaks across overground (OG), motorised treadmill (MT) and non-motorised treadmill (NMT) conditions whilst walking, jogging and running. ┌*┐ indicates a significant difference between groups (P<0.05). OG is black box, MT is Grey box, NMT is white box.

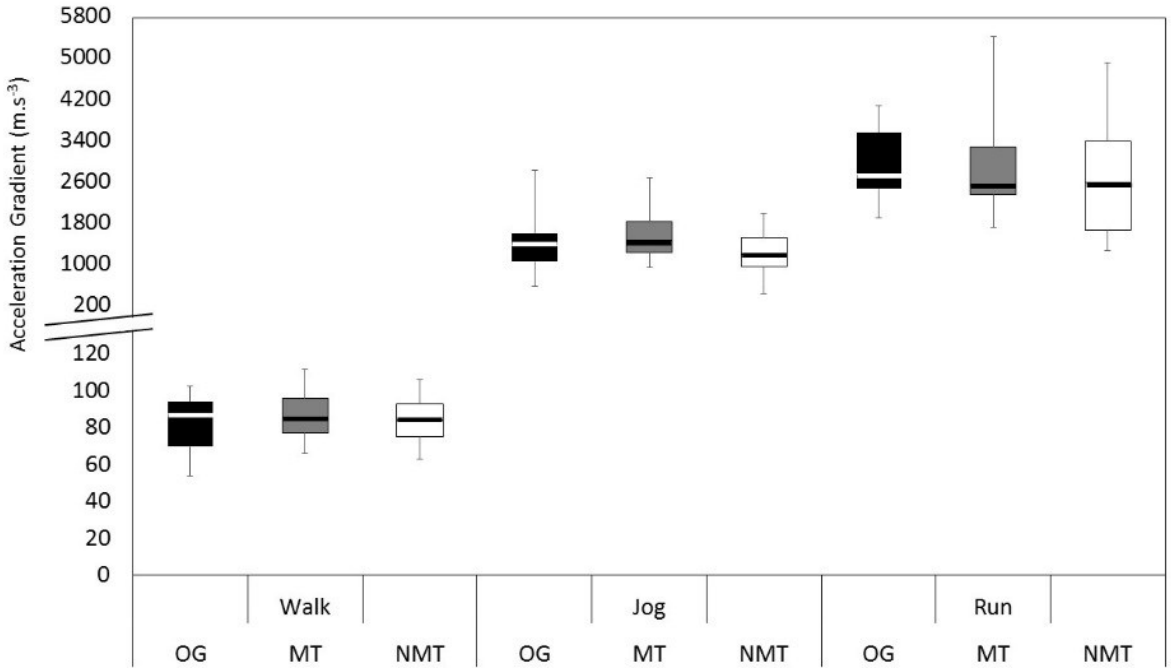


Fig. 4.3.2b. Median (interquartile range, minimum and maximum) acceleration gradient across overground (OG), motorised treadmill (MT) and non-motorised treadmill (NMT) conditions whilst walking, jogging and running. OG is black box, MT is Grey box, NMT is white box.

4.3.3 Electromyography Amplitude

There were significant main effects for condition ($P < 0.001$ to $P = 0.001$) and velocity ($P < 0.001$ to $P = 0.027$) across all four muscles. The interaction was significant for the RF EMG ($P = 0.005$), ST EMG ($P < 0.001$) and TA EMG ($P = 0.019$). There was a small to very large increase in all EMG amplitudes (four muscles) during the running condition when compared to walking and jogging trials, while jogging trials generated small to large increases in all EMG

amplitudes above walking trials (Table 4.3.3). There was a small increase in RF EMG during the NMT condition when compared to the OG condition and a moderate increase when compared to the MT condition across all gait conditions (Table 4.3.3). The NMT generated large increases in ST EMG when compared to the OG condition and very large increases in ST EMG when compared to the MT condition whilst the OG condition gave small increases in ST EMG when compared to the MT condition during walking (Table 4.3.3). ST EMG was similar across all conditions for jogging and running. OG and NMT conditions produced moderate increases in TA EMG when compared to the MT condition during walking, additionally the OG condition produced moderate and small increases in TA EMG when compared to the MT condition for jogging and running respectively (Table 4.3.3). OG and NMT conditions created large increases in SL EMG when compared to the MT condition during jogging, while the NMT created large increases in SL EMG when compared to OG and MT conditions during running (Table 4.3.3).

Table 4.3.3 Electromyography **(**EMG) amplitude (area under the curve) for each of the four muscles across overground, motorised treadmill and non-motorised treadmill conditions whilst walking, jogging and running. EMG amplitude is normalised to the non-motorised treadmill running trial and presented as a percentage

	EMG Amplitude (Mean±SD)			*P* value			Cohen's *d* [95% confidence intervals]		
RF	**OG**	**MT**	**NMT**	**OG -MT**	**OG-NMT**	**MT-NMT**	**OG - MT**	**OG - NMT**	**MT - NMT**
Walking	22 ± 12	22 ± 10	35 ± 17	*P* = 0.997	*P* = 0.015	*P* = 0.015	*d* = 0.06 [-0.15 to 0.28], 90% trivial	*d* = -1.06 [-1.65 to -0.48], 100% negative	*d* = -0.96 [-1.65 to -0.27], 98% negative
Jogging	38 ± 16	40 ± 18	52 ± 17	*P* = 0.628	*P* = 0.012	*P* = 0.012	*d* = -0.14 [-0.42 to 0.15], 67% trivial	*d* = -0.86 [-1.34 to -0.38], 99% negative	*d* = -0.57 [-1.00 to -0.14], 96% negative
Running	70 ± 31	69 ± 20	100 ± 0	*P* = 0.993	*P* = 0.02	*P* < 0.001	*d* = 0.07 [-0.25 to 0.40], 75% trivial	*d* = -0.97 [-1.54 to -0.41], 99% negative	*d* = -0.95 [-1.39 to -0.51], 100% negative
ST	**OG**	**MT**	**NMT**						
Walking	39 ± 10	36 ± 9	58 ± 15	*P* = 0.04	*P* < 0.001	*P* < 0.001	*d* = 0.36 [0.09 to 0.63], 88% positive	*d*= -1.81 [-2.46 to -1.17], 100% negative	*d* = -2.17 [-2.91 to -1.43], 100% negative
Jogging	64 ± 11	65 ± 11	68 ± 9	*P* = 0.965	*P* = 0.34	*P* = 0.605	*d* = -0.07 [-0.40 to 0.27], 74% trivial	*d* = -0.32 [-0.75 to 0.11], 72% negative	*d* = -0.26 [-0.73 to 0.22], 60% negative
Running	96 ± 19	89 ± 19	100 ± 0	*P* = 0.538	*P* = 0.843	*P* = 0.125	*d* = 0.34 [-0.24 to 0.93], 70% positive	*d* = -0.19 [-0.71 to 0.34], 48% negative	*d* = -0.53 [-1.04 to -0.02], 91% negative
TA	**OG**	**MT**	**NMT**						
Walking	83 ± 24	68 ± 18	89 ± 22	*P* = 0.008	*P* = 0.131	*P* = 0.002	*d* = 0.61 [0.25 to 0.96], 99% positive	*d* = -0.24 [-0.47 to -0.01], 64% negative	*d* = -0.86 [-1.26 to -0.43], 100% negative
Jogging	92 ± 21	74 ± 20	81 ± 18	*P* = 0.003	*P* = 0.185	*P* = 0.142	*d* = 0.79 [0.38 to 1.19], 100% positive	*d* = 0.48 [-0.04 to 1.00], 87% positive	*d* = -0.31 [-0.61 to 0.00], 76% negative
Running	111 ± 31	95 ± 28	100 ± 0	*P* = 0.039	*P* = 0.461	*P* = 0.857	*d* = 0.50 [0.12 to 0.88], 94% positive	*d* = 0.34 [-0.18 to 0.86], 71% positive	*d* = -0.16 [-0.63 to 0.31], 51% trivial
SL	**OG**	**MT**	**NMT**	**Bonferroni Correction (*P* < 0.017)**			**Cliff's Delta (δ) [95% confidence intervals]**		
Walking	73 ± 29	61 ± 17	90 ± 30	*P* = 0.041	*P* = 0.002	*P* = 0.012	δ = 0.12 [-0.28 to 0.49]	δ = -0.2 [-0.55 to 0.22]	δ = -0.37 [-0.69 to 0.08]
Jogging	81 ± 25	73 ± 14	91 ± 14	*P* = 0.001	*P* = 0.609	*P* = 0.001	δ = 0.49 [0.04 to 0.78]	δ = -0.11 [-0.50 to 0.32]	δ = -0.65 [-0.87 to -0.21]
Running	91 ± 16	77 ± 11	100 ± 0	*P* = 0.001	*P* = 0.056	*P* = 0.016	δ = 0.33 [-0.06 to 0.64]	δ = -0.57 [-0.85 to -0.04]	δ = -0.71 [-0.92 to -0.19]

* Soleus Data are presented as Medians ± Interquartile Range

Rectus femoris (RF), n=12; semitendinosus (ST), n=15; tibialis anterior (TA), n=15; soleus (SL), n=13;

overground (OG); motorised treadmill (MT); non-motorised treadmill (NMT)

4.3.4 Co-contraction Rectus Femoris / Semitendinosus

There was a significant main effect for condition ($P = 0.004$), velocity ($P = 0.002$) and interaction term ($P = 0.002$). No statistical differences were observed between conditions during walking trials, however the NMT condition generated moderate increases in co-contraction values when compared to OG conditions and small increases when compared to MT conditions during jogging ($d = 0.69$ [95%CI: 0.17 to 1.21], $P = 0.033$; $d = 0.33$ [95%CI: -0.42 to 1.09], $P = 0.006$). The NMT condition generated very large increases in co-contraction values when compared to OG and MT conditions during running ($d = 2.57$ [95%CI: 1.05 to 4.1], $P = 0.024$; $d = 2.2$ [95%CI: 0.93 to 3.47], $P = 0.038$) (Fig. 4.3.4).

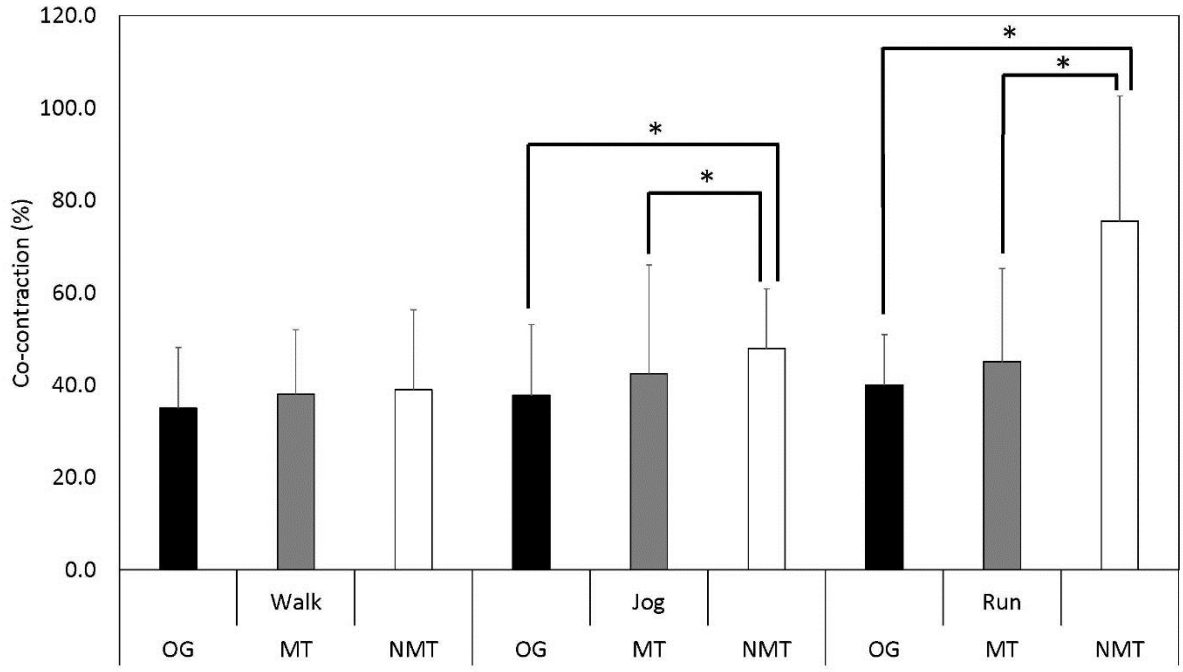


Fig. 4.3.4. Mean (±SD), rectus femoris (RF)/ semitendinosus (ST) co-contraction percentage across overground (OG), motorised treadmill (MT) and non-motorised treadmill (NMT) conditions whilst walking, jogging and running. ┌*┐ indicates a significant difference between groups ($P<0.05$). OG is black box, MT is Grey box, NMT is white box.

4.3.5 Co-contraction Tibialis Anterior / Soleus

There was a significant main effect for condition ($P = 0.002$), but not velocity ($P = 0.13$) and a significant interaction term ($P = 0.006$). No statistical differences were observed between conditions during walking trials, however the NMT

condition displayed small reductions in co-contraction values when compared to OG and MT conditions during jogging (d = -0.4 [95%CI: -0.6 to -0.2], P = 0.009; d = -0.35 [95%CI: -0.61 to -0.1], P = 0.027) and running trials (d = -0.44[95%CI: -0.71 to -0.17], P = 0.023; d = -0.45 [95%CI: -0.75 to -0.14], P = 0.028) (Fig. 4.3.5).

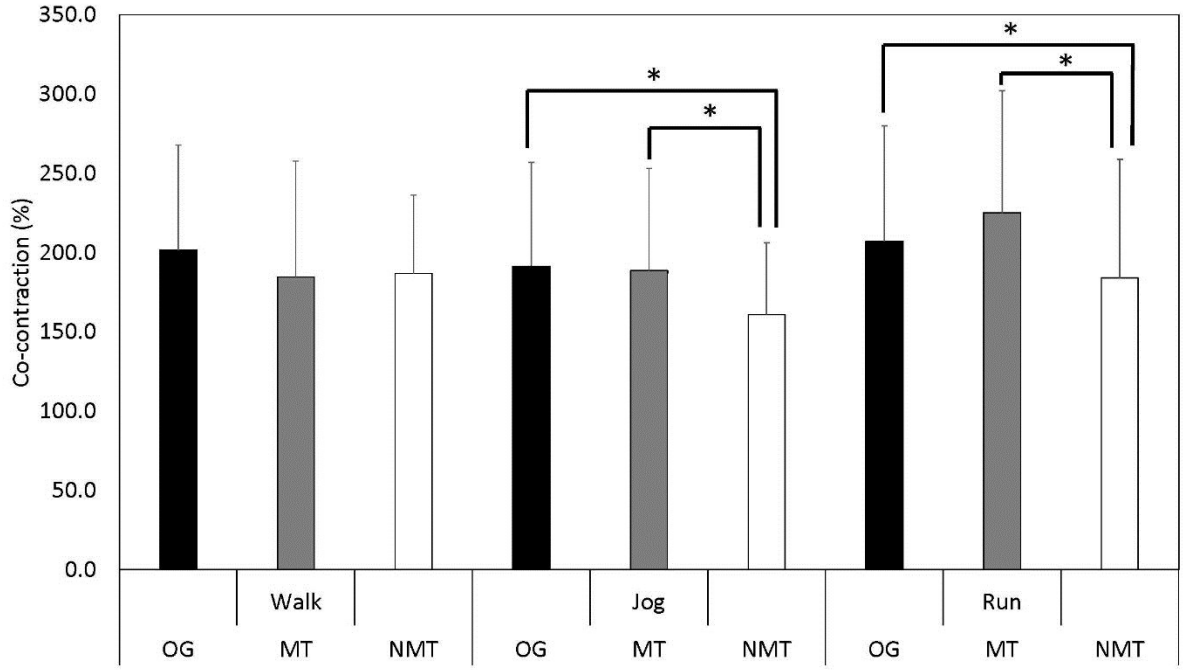


Fig. 4.3.5. Mean (±SD), tibialis anterior (TA) / soleus (SL) co-contraction percentage across overground (OG), motorised treadmill (MT) and non-motorised treadmill (NMT) conditions whilst walking, jogging and running. ┌*┐ indicates a significant difference between groups (P<0.05). OG is black box, MT is Grey box, NMT is white box.

4.3.6 Cycle Time

There was a significant main effect for condition ($P < 0.001$), velocity ($P < 0.001$) and interaction term ($P = 0.012$). The NMT condition generated very large decreases in cycle time when compared to OG and MT conditions during walking (d = -2.24 [95%CI: -2.85 to -1.63], $P < 0.001$; d = -2.04 [95%CI: -2.64 to -1.44], $P < 0.001$), with large and very large decreases during jogging (d = -1.98 [95%CI: -2.5 to -1.46], $P < 0.001$; d = -2.03 [95%CI: -2.56 to -1.51], $P < 0.001$) and very large decreases during running (d = -3.03 [95%CI: -3.73 to -2.33], $P < 0.001$; d = -2.55[95%CI: -3.24 to -1.86], $P < 0.001$). OG and MT conditions produced similar cycle times.

4.4 Discussion

The main findings were that exercising on a NMT resulted in large reductions in peak acceleration on impact across all walking, jogging and running trials when compared to OG and MT conditions. Additionally, the NMT condition generated small and moderate increases in RF EMG (all trials), large and very large increases in ST EMG (walking) and large increases in SL EMG (running) in comparison with OG and MT conditions respectively. Findings indicate that habitual locomotion is altered when using a NMT, which may decrease the level of mechanical loading and potentially the osteogenic nature of the exercise (as

determined by the OI) but could provide useful rehabilitation purposes due to the reduction in impact forces.

Differences in ground reaction forces have been reported between OG and MT locomotion with the OG condition generating a 6% higher bodyweight percentage (Riley et al, 2007), although our data indicates that OG and MT conditions give similar acceleration peaks, which supports more recent research (Kluitenberg et al, 2012). The large reduction in peak acceleration during NMT locomotion could be caused by a pronounced forward lean favouring forefoot striking as opposed to heel/midfoot striking, but this assertion warrants research using motion analysis as this was not specifically measured (Wood and Kip, 2014). Large reductions in acceleration peaks during NMT conditions suggest that it is unsuitable for eliciting an osteogenic response, as previously determined thresholds (>4.9 g accounting for standing being 1 g) required to stimulate an increase in bone remodelling (Vainionpaa et al, 2007) are not consistently met, while OG and MT conditions elicit acceleration peaks above the required threshold when running (Fig. 4.3.1a). Peak acceleration has been shown to have a graded effect on BMD adaptations with higher accelerations eliciting greater bone adaptations (Vainionpaa et al, 2007). In the current study, this would indicate that the NMT could reduce BMD adaptations when compared to OG and MT conditions. The reduced accelerations during NMT locomotion would make the device an unideal treadmill for the purpose of assessing the differences

between continuous and intermittent exercise and their effects on bone adaptation. It is necessary to design a training intervention with adequate peak accelerations to sufficiently generate increases in BMD, as the peak acceleration levels on a NMT do not meet osteogenic thresholds, any intervention using the NMT would be unlikely to show any changes in BMD (Vainionpaa et al, 2007). Peak accelerations showed good agreement with previous MT studies (Mizrahi et al, 2000; Wood and Kipp, 2014) and the large reductions in acceleration peaks during the NMT condition might imply that the NMT is better for rehabilitation when gradually re-introducing impact activity to individuals with lower extremity injuries. Acceleration gradient is also important in stimulating bone adaptation with OG, MT and NMT conditions producing similar results for this variable. Jogging and running consistently surpassed the threshold of 1000 $m.s^{-3}$ indicating that adaptations due to this variable might be similar (Heikkinen et al, 2007). Acceleration gradients remained similar across treadmill conditions whereas acceleration peaks showed a large reduction during the NMT condition, this may have been caused by a shift in NMT kinematics, which preserved the gradient of the acceleration on ground contact but cushioned the magnitude of the acceleration.

The NMT condition produced small and moderate increases in RF EMG when compared to all OG and MT trials, with large and very large increases in ST activation during walking and large increases in SL EMG during running. While

the NMT could enhance training adaptations in these muscle groups it will alter natural OG movement patterns, which highlights that information gathered from NMT locomotion should be interpreted with caution. Increased RF EMG has been linked with high internal compressive forces on the femur (Bassey et al, 1997), in addition EMG amplitude is highly correlated with muscle force output, indicating that higher EMG amplitudes could indicate higher internal loading (Andrade and Andrade, 2012). Muscle forces have also been suggested as the main driver of bone adaptation (Robling, 2009). Despite generating large reductions in peak acceleration, NMT locomotion could potentially initiate skeletal adaptations at the hip, which for osteoporotic patients could provide a means of stimulating bone maintenance without risk of osteoporotic fractures from high impact activity. This warrants further investigation as debate over the main stressor for bone adaptation continues and data from EMG studies are yet to show causal evidence for bone adaptation (Robling, 2009; Judex and Carlson, 2009). No statistical difference was observed between RF EMG in the OG and MT (-1% lower) conditions. This is contrary to previous studies, where up to 130% larger RF EMG values were reported during the MT condition while walking (Murray et al, 1985; Lee and Hidler, 2008; Prosser et al, 2011). These differences are possibly due to walkway limitations (< 8 m), an elliptical walkway or inconsistent footwear.

The moderate and small increases in RF/ST co-contraction data during the NMT

condition while jogging and the very large increase in RF/ST co-contraction data while running (in comparison with OG and MT conditions) indicates that a proportionally higher RF input was present. The small reductions in TA/SL co-contraction data during the NMT condition implied that a higher SL contribution was present throughout jogging and running trials. OG and MT trials were similar but the NMT appeared to induce higher levels of RF and SL activation with the exception of walking trials, which indicates that different jogging and running techniques are required to match OG velocities. This suggests that the NMT creates large to very large reductions in cycle times and therefore infers increases step frequency in order to match OG and MT velocities. This would also question the similarity of NMT locomotion and OG locomotion.

4.4.1 Strengths and Limitations

Whilst this study provided an estimate of tibial acceleration using high specification accelerometry with a high sampling frequency, the direction of force transfer is difficult to ascertain with skin mounted accelerometers due to orientation of the tibia (albeit in line with the long axis of the tibia). Few alternatives however, permit acceleration recording over multiple cycles during overground locomotion (Turcot et al, 2008). Direct bone strain measurement would have been preferred but was not measured due to the invasive nature of the procedure. Instead, bone strain was inferred from peak acceleration values, which

provide an estimate of the internal loading and can be affected by skin movement (Vihriala et al, 2000; Edwards et al, 2009). One familiarisation session on the NMT may be insufficient, two familiarisations could be optimal (Sirotic and Coutts, 2008). However, previous studies did not use only constant velocities, which might negate the need for extra familiarisation, particularly as one familiarisation session has shown good reliability (Tofari et al, 2015) and that participants all sufficiently met target velocities during familiarisation. High inter and intra participant variability for EMG amplitude was present, likely due to individual walking, jogging and running techniques, which was expected, yet unavoidable.

4.4.2 Conclusions

In summary, the NMT generates large reductions in tibial acceleration, large to very large increases in step frequency and small to very large increases in muscular activation when compared to OG and MT locomotion. The reduction in tibial accelerations during NMT locomotion might reduce osteogenic adaptation, although could better suit individuals avoiding high impact exercise due to ongoing rehabilitation for lower limb injuries. The greater EMG amplitude response to NMT locomotion could indicate a higher training stimulus and higher internal compressive forces on the skeletal system, which has been suggested to create a larger osteogenic stimulus, although this would require further

investigation due to insufficient causal evidence for higher EMG amplitude and higher bone remodelling rates.

5. The osteogenic potential of four common exercises used in osteoporosis prevention for postmenopausal women

5.1 Introduction

Postmenopausal women experience the most rapid decline in BMD of any human population, which could expose them to a greater risk of developing osteoporosis during their lifetime (Kroger et al, 1992; Shipman et al, 1999). Exercise interventions in postmenopausal women have become popular in an attempt to reduce BMD loss (Engelke et al, 2006; Kelley et al, 2012). High impact exercise can reduce postmenopausal BMD loss and in some cases improve BMD during the early years post-menopause, therefore reducing the likelihood of developing osteoporosis and experiencing a subsequent bone fracture (Berard and Gauthier, 1997; Wolff et al, 1999; Wallace and Cumming, 2006). However, there is still no consensus on the most osteogenic mode of exercise for post-menopausal women (Xu et al, 2016), so it is important to establish the most effective forms of high-impact exercise in order to optimise future exercise programmes targeted at maintaining bone health throughout the ageing process.

Countermovement jumps (vertical jumping for height) (Zhao et al, 2014), box drops (stepping off a box and landing) (Vainionpaa et al, 2006), heel drops (raising onto the toes before relaxing and dropping back on to the floor) (Hans et

al, 2002) and stamping (unilateral foot stamping) (Young et al, 2007) have all been used during high-impact exercise interventions to stimulate increases in BMD. However, a direct comparison of these osteogenic exercises with regards to mechanical loading and differing stimulus frequencies (number of loading cycles per second) has yet to be undertaken.

A number of parameters have been used to quantify the osteogenic potential of high-impact exercise, including the magnitude of impacts (Jamsa et al, 2006; Vainionpaa et al, 2007) and acceleration gradient (Heikkinen et al, 2007; Jamsa et al, 2011), which are commonly measured with accelerometry. Although direct measures of bone strain are preferable, due to the strong relationship with the magnitude of accelerometer recorded impacts and bone strain, accelerometers are recommended as a surrogate measure of bone strain during physical activity when the direct measurement of bone strain is often comprimised (Edwards et al, 2009). In addition, muscular activation as measured with EMG (Bassey et al, 1997; Robling, 2009), stimulus frequency (Robling et al, 2001), and number of loading cycles (Turner and Robling, 2003) are all associated with the stimulation of bone remodelling. EMG amplitude has been linked with high internal compressive forces on the femur, which would increase the mechanical loading environment (Bassey et al, 1997). Muscular forces are thought to provide the main stressor on the skeletal system during mechanical loading conditions, which drives increases in bone remodelling (Robling, 2009). A combination of ACC and EMG would

consequently provide a good representation of the loading placed on the bones and would indicate the likelihood of site-specific bone remodelling.

Intensity of impacts and number of loading cycles are required for the calculation of the osteogenic index (OI) as designed by Turner and Robling, 2003. The OI serves to quantify the mechanical loading generated by exercise programmes and has been found to relate to longitudinal bone adaptations (Nilsson et al, 2012; von Stengel et al, 2005) and bone turnover markers (Erickson and Vukovich, 2010; Rantalainen et al, 2011) following exercise participation, and also cross-sectionally when relating OI to current BMD status (Rantalainen et al, 2010; Weeks and Beck, 2008). The OI has also been used to determine the most osteogenic forms of activity (Weeks and Beck, 2008). However, loading conditions are often poorly reported in studies targeting bone health and the OI calculation regularly disregards the previously mentioned factors governing bone adaptation (Daly and Bass, 2006; Lester et al, 2009; Nilsson et al, 2012).

Intermittent mechanical loading cycles have been shown to enhance BMD adaptations in rats and mice when compared to continuous mechanical loading cycles. The 10 – 14 second recovery interval is thought to reduce the desensitisation effect on bone that occurs with repetitive continuous loading cycles to create a more potent osteogenic stimulus for bone adaptation (Robling et al, 2001; LaMothe and Zernicke, 2004; Srinivasan et al, 2007). Intermittent mechanical loading therefore has the potential to supplant continuous mechanical

loading for exercise interventions aimed at reducing postmenopausal bone loss. In order to examine whether the addition of recovery periods can increase bone adaptation in a population of postmenopausal women, the osteogenic potential of continuous (CTS) and intermittent (INT) exercise modes must be examined to ensure they meet previously established osteogenic loads (Jamsa et al, 2006; Vainionpaa et al, 2007; Heikkinen et al, 2007). Additionally, the mechanical loading intensity must remain consistent across CTS and INT loading conditions for which a protocol has not been previously developed. This will allow for the future testing of the effects of stimulus frequency upon bone remodelling in humans where it is the only manipulated variable and all other parameters are consistent.

Therefore, the aim of this study was to calculate the OI, accelerations and muscle activation of four common CTS and INT exercises used in osteoporosis prevention in order to inform future exercise interventions targeted at improving BMD in a population of early post-menopausal women. We hypothesise that the osteogenic index, magnitude of acceleration and muscular activation will be greater in counter-movement jumps (CMJ) and box drop (BD) exercises than the heel drop (HD) and stamping (STP) exercises. In addition, we hypothesise that the difference between continuous and intermittent protocols will be trivial with regards to loading parameters and muscle activity.

5.2 Methods

5.2.1 Overview

Healthy early postmenopausal women (1 - 5 years post menopause, defined cessation of menstrual cycle for more than 12 months), were recruited through contacting local companies and university staff via email, posters and word of mouth. The study was estimated to require 14 participants to establish an 80% level of statistical power based on the peak ACC values recorded during pilot testing ($P < 0.05$). This calculation was conducted using G*Power 3.0 software (G*Power 3.0, Düsseldorf, GER), (Faul et al, 2007) and was based on an assumption that the peak ACC values would be similar to that recorded during pilot testing of the first five participants. From this, a Cohen's *d* effect size of $d = 0.95$ was used. The first screening was performed with telephone conversations and email communications to establish eligibility. Fourteen volunteers fit the inclusion criteria and were successfully recruited (Appendix C). Participants completed 10 repetitions each of four exercises both in a CTS (stimulus frequency of 0.25 Hz, one repetition every four seconds / 15bpm) INT condition (stimulus frequency of 0.067 Hz, one repetition every 15 seconds / 4bpm) in accordance with previous stimulus frequencies (Robling et al, 2001), in a randomised order. Exercises were performed barefoot in the laboratory with an accelerometer (Noraxon, DTS 3D accelerometer 16 g, Arizona, USA) attached to the sacrum

(aligned with the vertical axis to assess compressive forces) and four surface electromyography electrodes (Noraxon, DTS Desk Receiver System, Arizona, USA) placed over the rectus femoris (RF), semitendinosus (ST), tibialis anterior (TA) and the lateral head of the gastrocnemius (GL) muscles. These muscles were selected due to the compressive forces that they exert on the femur across the hip and knee joints and the compressive forces that they exert on the tibia. In addition, for this population, larger superficial muscles were selected to ensure that there were less complications associated with EMG signal detection, which can occur with older populations. The accelerometer was attached to the sacrum due to it's close proximity to the lumbar spine, hip region and body centre of mass. Differing exercise techniques can alter and sometimes dampen the transfer of loading throughout the skeletal system (Hamill et al, 1995; Kavanagh and Menz, 2008). The sacrum site allows estimation of the loading transfer to specific bone sites of interest that are at greater risk of osteoporotic fracture (Lee et al, 2015). The protocol was approved by the institutional ethics committee and informed consent was obtained from all participants prior to testing.

5.2.2 Main Testing Session

Following a brief warm up consisting of jogging and dynamic stretching a 30 second static standing EMG trial was recorded to calculate the baseline EMG amplitude.

The exercises consisted of 10 repeated CMJ, BD, HD and unilateral STP at both CTS and INT frequencies in a randomised order with 2 - 3 minutes rest in between trials to reduce the effect of fatigue from multiple trials and keep an adequate number of loading cycles to sufficiently represent the exercise. Frequency was measured by an audible metronome app (Metronome Version 1.3 for iPhone, Marketwall.com) to coincide with previously determined loading frequencies (Robling et al, 2001). For the CMJ, participants were instructed to jump for maximum height, using their arms and land with bent knees on the balls of their feet. BD were initiated from a 0.2 m box (Reebok Step, Reebok International Limited, Canton, MA, USA), participants stepped backwards up onto the box before stepping off the box ensuring a 0.2 m freefall onto the floor, then immediately stepping back up onto the box for the duration of the rest interval (always leading with the right leg). For the HD, participants were instructed to "stand as high as they can on their toes before instantly relaxing the leg muscles and dropping onto their heels to create an impact". For the STP, participants were instructed to stamp with their right leg "as hard as physically possible without generating discomfort". Participants were familiarised with each exercise before data recording was undertaken and trials were repeated if necessary. For all trials vertical ACC and EMG were recorded synchronously at 1500 Hz (EMG: input impedance > 100 MΩ, CMRR > 100 dB, baseline noise < 1μV RMS, base gain = 200, final gain = 500). ACC and EMG were automatically synchronised with the Noraxon hardware (Noraxon, DTS Desk Receiver System, Arizona, USA).

5.2.3 Sensor Placement

Surface EMG electrodes (Ambu Blue Sensor N, Ambu, Cambridgeshire, UK) were placed over the (RF), (ST), (TA), and (GL) muscles of the participant's right leg in accordance with SENIAM surface electromyography recommendations (Hermens et al, 1999). Skin was shaved, abraded and cleansed with a 70% alcohol swab before electrode attachment. The accelerometer was vertically aligned and attached to the participant's sacrum (S1) in order to best represent whole body loading and also identify force transfer close to the hip and lumbar spine, which are at risk of osteoporotic fracture (Kelley et al, 2014; Heilmeier et al, 2016). ACC and EMG wearable hardware were secured with surgical tape and elasticated bandages to reduce unwanted movement and signal artefacts.

5.2.4 Osteogenic Index

OI was calculated from the ACC data using the following formula as outlined by Turner and Robling, 2003:

$$OI = \text{Intensity} \times \ln(\text{Number of loading cycles} + 1) \quad \text{(Eq. 1)}$$

Intensity in this case was measured by peak acceleration (mean of 10 loading cycles) from ACC data to represent whole body loading (Vainionpaa et al, 2007), and ln is the natural log.

5.2.5 Accelerometry

ACC data were filtered optimally using 95% of the signal energy of the data from a base value of 1 g before the impact to the impact peak for each of the 10 highest impact peaks per exercise (Fig. 5.2.5a). ACC data were presented as g while ACC gradients (Grad ACC) from 1 g to the peak, were converted to $m \cdot s^{-3}$. The 10 highest peaks in the ACC data (Peak ACC) were calculated using the peak from each loading cycle across 10 cycles (Fig. 5.2.5b). The 10 Grad ACC from 1 g (9.81 $m \cdot s^{-2}$) leading to the peak for each loading cycle were calculated using the following formula (Heikkinen et al, 2007) (Fig. 5.2.5c):

$$\text{Grad ACC} = (\text{Peak ACC} - 9.81\ m \cdot s^{-2}) / (t\ @\ \text{Peak ACC} - t\ @\ 9.81 m \cdot s^{-1}) \quad \text{(Eq. 2)}$$

Where "t @" denotes the time point at either Peak ACC or at 9.81 $m \cdot s^{-2}$

Peak ACC and Grad ACC were averaged across the 10 loading cycles.

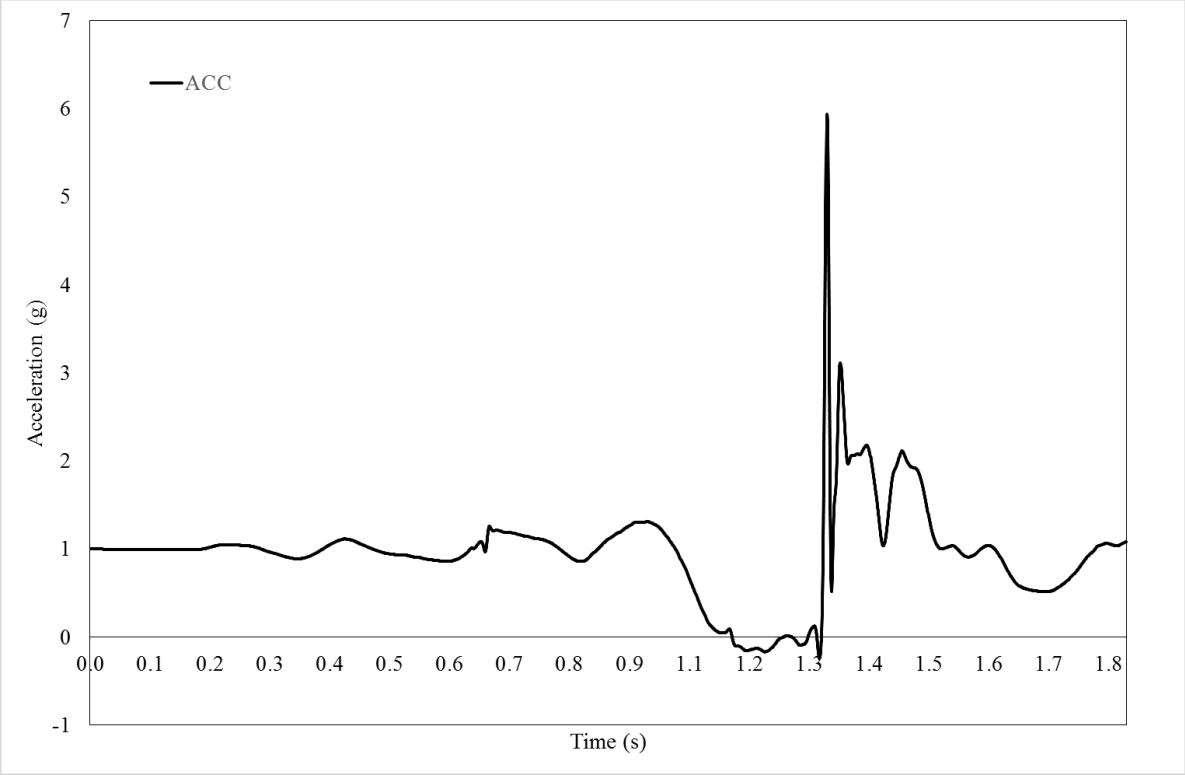


Fig. 5.2.5a Acceleration trace for one loading cycle of the box drop exercise. Solid black line indicates the filtered acceleration trace

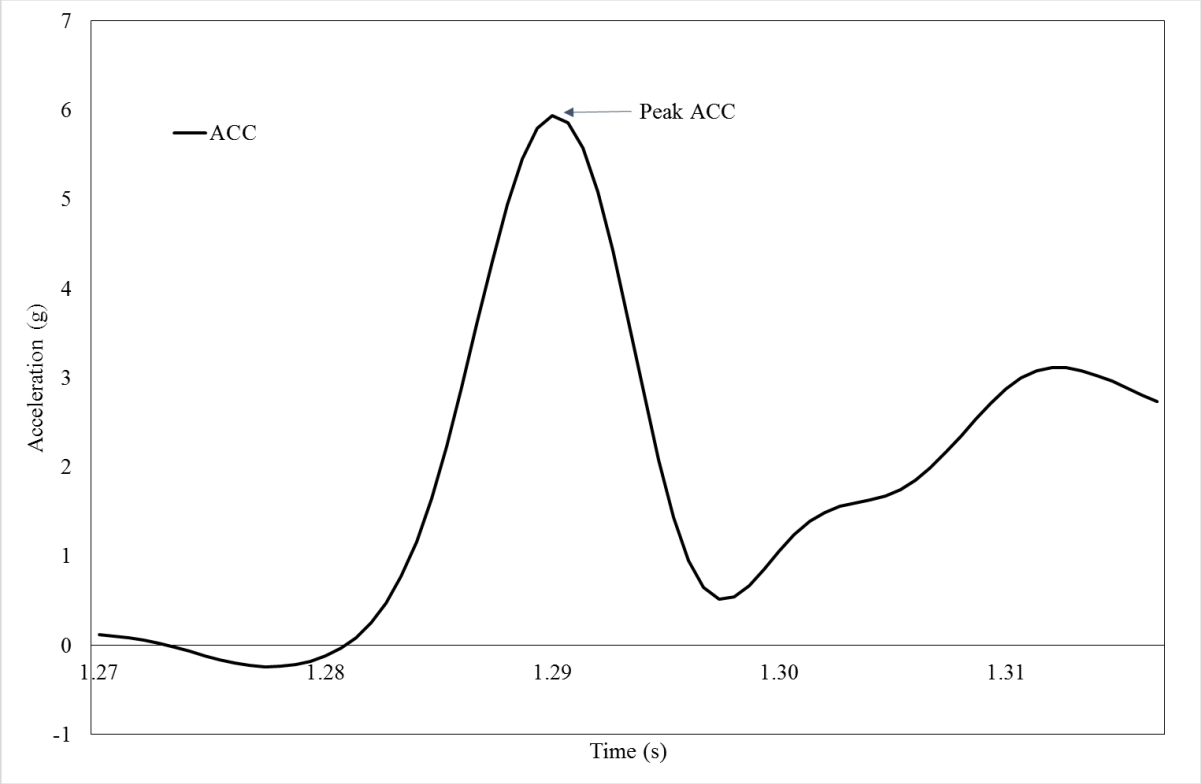


Fig. 5.2.5b Acceleration trace for one loading cycle of the box drop exercise. Solid black line indicates the filtered acceleration trace. Peak ACC indicates the peak acceleration value.

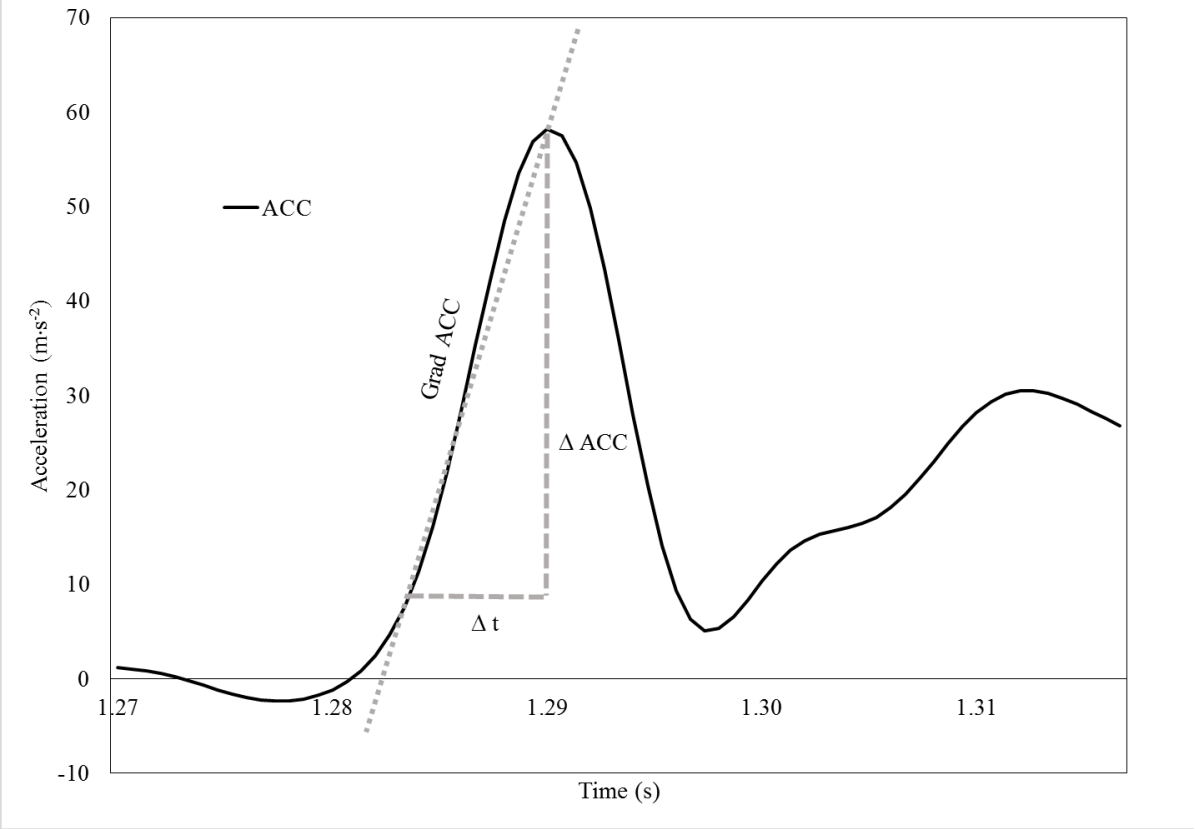


Fig. 5.2.5c Acceleration trace for one loading cycle of the box drop exercise. Solid black line indicates the filtered acceleration trace. Δ ACC indicates the change in the acceleration, Δ t indicates the change in time. Grad ACC indicates the acceleration gradient as highlighted by the dotted grey line.

5.2.6 Electromyography

EMG data were band-pass filtered (bi-directional Butterworth, 10-500 Hz), full wave rectified and low-pass filtered at 8 Hz to obtain linear envelopes (Shaharudin et al, 2014). Baseline EMG amplitude for each muscle was calculated as 3 standard deviations above the mean value from the standing static

trial and was removed from the exercise trials for each muscle to leave the EMG activity due to the exercise only (Prosser et al, 2011) (Fig. 5.2.6). EMG Mean amplitude was calculated using the mean of the EMG activity above the baseline EMG amplitude from 2.5 seconds before the first impact peak to 2.5 seconds after the last impact peak as determined by the ACC data. EMG Mean amplitude was averaged across the 10 loading cycles for each exercise and was normalised to the corresponding CMJ CTS trial, which showed the highest mean values for the RF and ST muscles (Prosser et al, 2011).

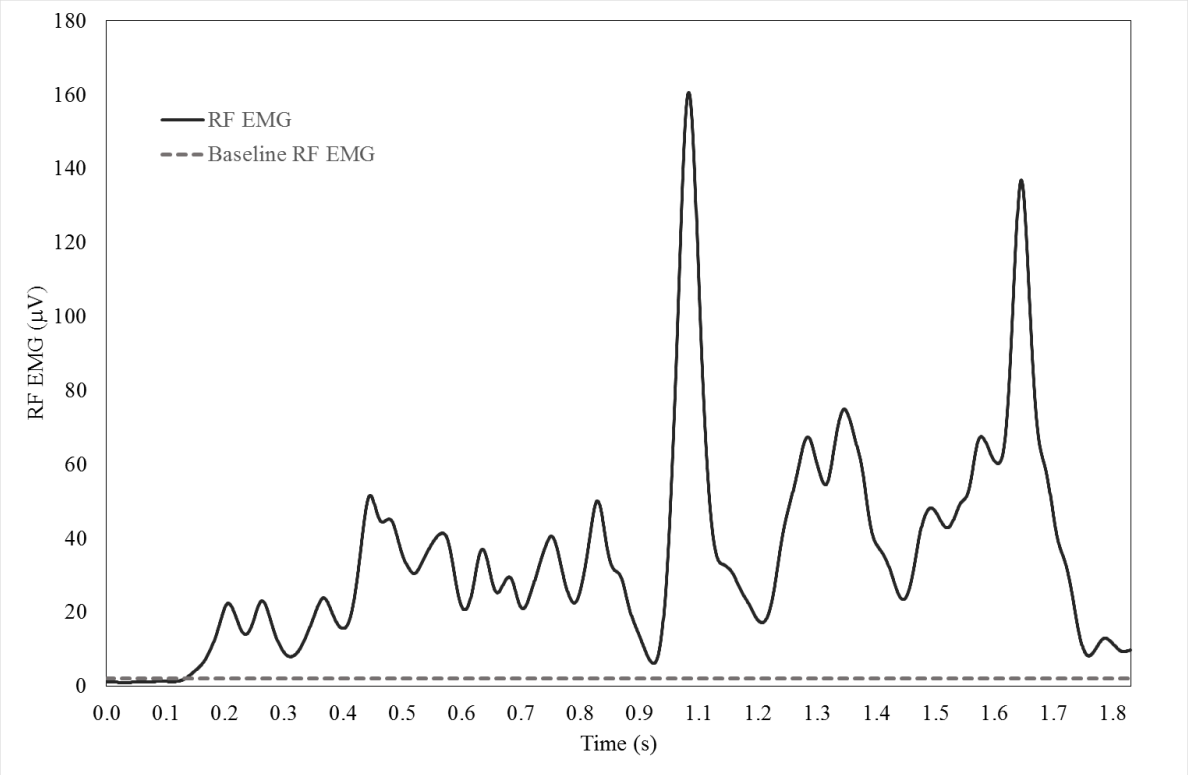


Fig. 5.2.6 Rectus femoris electromyography trace for one loading cycle of the box drop exercise. Solid black line indicates the filtered rectus femoris electromyography trace before normalisation has occurred. Dashed grey line indicates the baseline rectus femoris electromyography amplitude as calculated from a standing static trial.

5.2.7 Statistical Analysis

Data were processed using a bespoke MATLAB programme (MATLAB R2011a, Mathworks, Cambridge, UK). Parametric data were statistically analysed using two-way (4 exercises x 2 stimulus frequencies) repeated measures ANOVAs (Sidak adjustments) with post-hoc pairwise comparisons using SPSS (IBM SPSS

Statistics Version 20.0. IBM Corp, NY, USA). Where applicable, non-normally distributed data were log-transformed and analysed using parametric methods. Using a bespoke template (Hopkins, 2006), Cohen's *d* effect size is reported and evaluated using the following scale: 0 - 0.19 trivial, 0.2 - 0.59 small, 0.6 - 1.19 moderate, 1.2 - 1.99 large, 2.0 - 3.99 very large. Uncertainty in the population estimates are expressed as 95% confidence intervals along with the likelihood that the effect is substantially positive, trivial or substantially negative. For non-parametric data when log-transformation was not possible, Friedman's tests were used to compare main effects of exercises and stimulus frequency conditions. Wilcoxon signed-rank tests with Bonferroni corrections were used post-hoc pairwise comparisons SPSS. Cliff's Delta (δ) effect size was calculated in R effsize package (Torchiano, 2016) and evaluated using the following scale: 0 - 0.146 trivial, 0.147 - 0.32 small, 0.33 - 0.473 moderate, >0.474 large. Uncertainty in the population estimates are expressed as 95% confidence intervals (Cliff, 1996). Effect sizes were presented for statistically significant results.

5.3 Results

5.3.1 Participant Characteristics

The fourteen healthy early postmenopausal women that completed the testing

protocol were recreationally active (mean ± SD: 55.7 ± 3.8 years, 163.0 ± 4.3 cm, 65.8 ± 11.9 kg, 1 - 5 years post menopause, defined cessation of menstrual cycle for more than 12 months).

5.3.2 Osteogenic Index

There was a main effect for exercise ($P < 0.001$) but not for stimulus frequency ($P = 0.433$). For CTS conditions, CMJ produced a moderate and very large increase in OI than HD and STP ($d = 0.83$ [95%CI: 0.27 to 1.4], $P = 0.043$; $d = 2.38$ [95%CI: 1.36 to 3.39], $P = 0.001$). BD and HD produced a very large and large increase in OI when compared with STP ($d = 2.17$ [95%CI: 1.13 to 3.2], $P = 0.003$; $d = 1.54$ [95%CI: 0.71 to 2.37], $P = 0.009$). For INT conditions, CMJ and BD generated very large increases in OI when compared to STP while HD generated large increases in OI when compared to STP ($d = 2.21$ [95%CI: 1.15 to 3.26], $P = 0.004$; $d = 2.15$ [95%CI: 1.2 to 3.1], $P = 0.002$; $d = 1.88$ [95%CI: 0.98 to 2.79], $P = 0.004$). HD INT showed small increases in OI compared to HD CTS ($d = 0.33$ [95%CI: 0.00 to 0.65], $P = 0.047$). (Fig. 5.3.2).

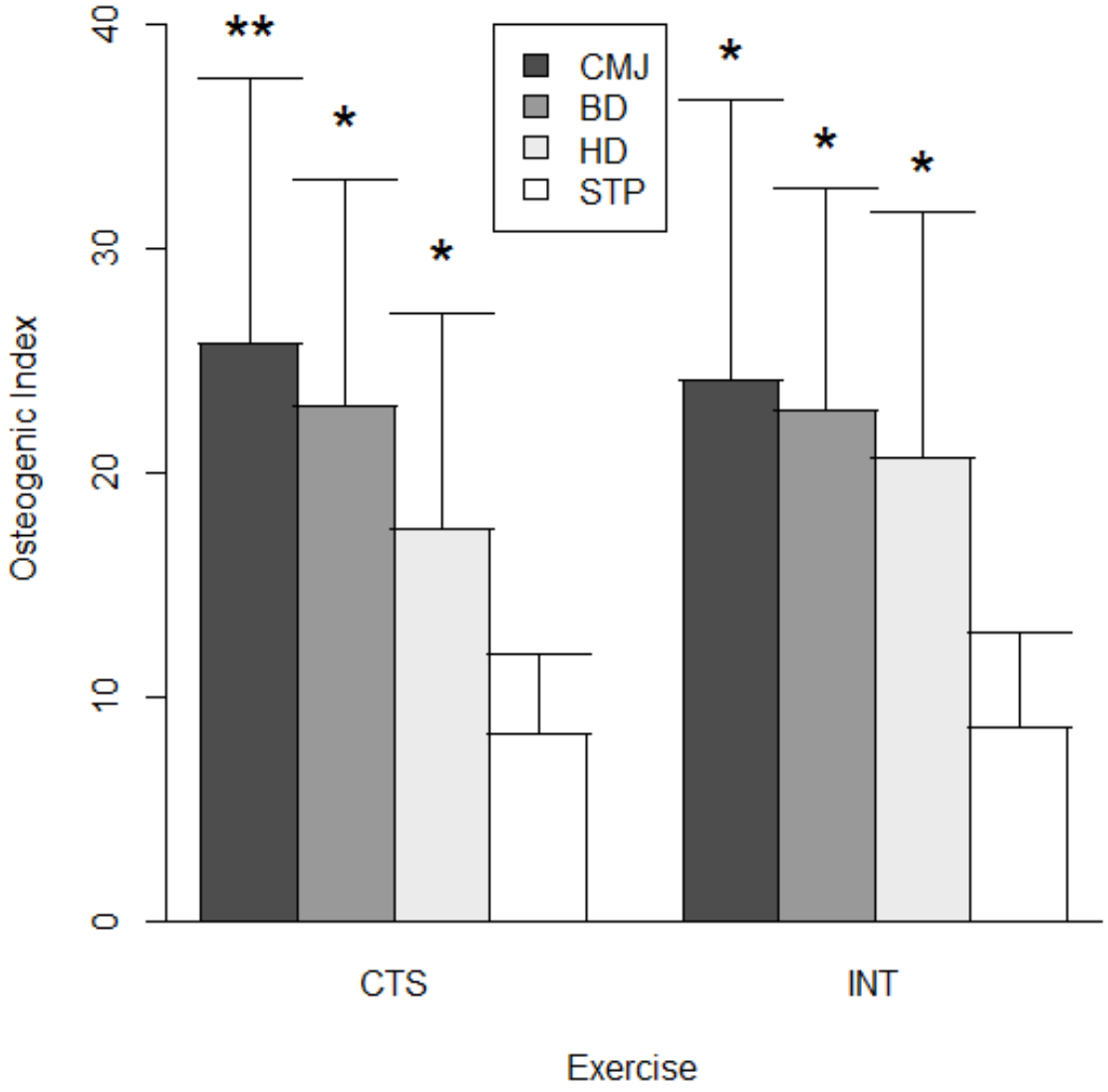


Fig. 5.3.2 Mean (±SD) osteogenic index of countermovement jumps (CMJ), box drops (BD), heel drops (HD) and stamps (STP) when performed both continuously and intermittently. Bars are means, error bars are SD. ** indicates condition is statistically significantly higher than the corresponding HD and STP condition, * indicates condition is statistically significantly higher than the corresponding STP condition. n = 14

5.3.3 Peak Acceleration

For CTS exercises Peak ACC was 10.7 ± 4.8 g for CMJ, 9.6 ± 4.1 g for BD, 7.3 ± 3.8 g for HD and 3.5 ± 1.4 g for STP. For INT exercises Peak ACC was 10.0 ± 5.0 g for CMJ, 9.5 ± 4.0 g for BD, 8.6 ± 4.4 g for HD and 3.6 ± 1.7 g for STP. Peak ACC pattern and statistics were proportional to OI statistics due to the nature of equation 1.

5.3.4 Acceleration Gradient

There was a main effect for exercise ($P < 0.001$) but not for stimulus frequency ($P = 0.097$). For CTS conditions, CMJ and BD produced large increases in Grad ACC when compared to STP ($d = 1.89$ [95%CI: 1.03 to 2.75], $P = 0.002$; $d = 1.74$ [95%CI: 0.88 to 2.61], $P = 0.005$). For INT conditions, CMJ, BD and HD produced large increases in Grad ACC when compared to STP ($d = 1.77$ [95%CI: 0.89 to 2.66], $P = 0.005$; $d = 1.78$ [95%CI: 1.02 to 2.54], $P = 0.001$; $d = 1.57$ [95%CI: 0.7 to 2.44], $P = 0.011$) (Fig. 5.3.4).

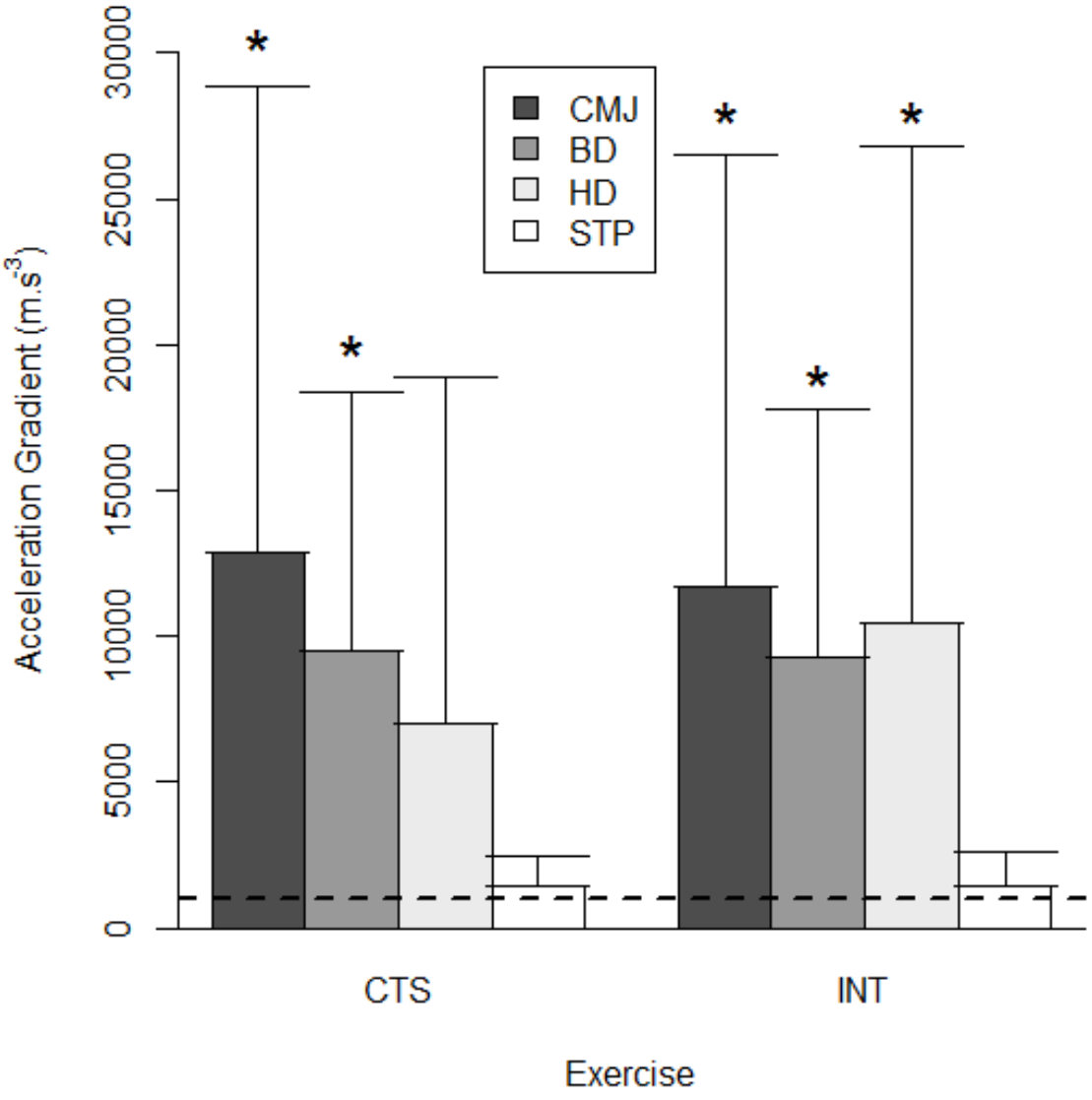


Fig. 5.3.4 Mean (±SD) acceleration gradient of countermovement jumps (CMJ), box drops (BD), heel drops (HD) and stamps (STP) when performed both continuously and intermittently. * indicates condition is statistically significantly higher than the corresponding STP condition. Dashed line indicates osteogenic threshold of 1000 $m \cdot s^{-3}$ (Heikkinen et al, 2007). n = 14

5.3.5 Rectus Femoris Electromyography

There was a main effect for exercise ($P < 0.001$) and stimulus frequency ($P < 0.001$). CTS conditions were statistically significantly higher than INT conditions for CMJ, BD and STP ($\delta = 0.57$ [95%CI: 0.27 to 0.77], $P = 0.009$; $\delta = 0.85$ [95%CI: 0.48 to 0.96], $P = 0.001$; $\delta = 0.41$ [95%CI: -0.04 to 0.72], $P = 0.008$). For CTS conditions, CMJ was significantly higher than HD and STP ($\delta = 0.86$ [95%CI: 0.64 to 0.95], $P = 0.001$; $\delta = 0.93$ [95%CI 0.75 to 1.00], $P = 0.001$). BD were significantly higher than HD and STP ($\delta = 0.86$ [95%CI: 0.36 to 0.98], $P = 0.001$; $\delta = 0.82$ [95%CI: 0.48 to 0.94], $P = 0.002$). For INT conditions, CMJ was significantly higher than BD, HD and STP ($\delta = 0.69$ [95%CI: 0.31 to 0.88], $P = 0.001$; $\delta = 0.92$ [95%CI 0.48 to 1.00], $P = 0.001$; $\delta = 0.89$ [95%CI: 0.64 to 0.97], $P = 0.001$). BD were significantly higher than HD and STP ($\delta = 0.83$ [95%CI: 0.50 to 0.95], $P = 0.001$; $\delta = 0.54$ [95%CI: 0.08 to 0.81], $P = 0.002$) (Fig. 5.3.5).

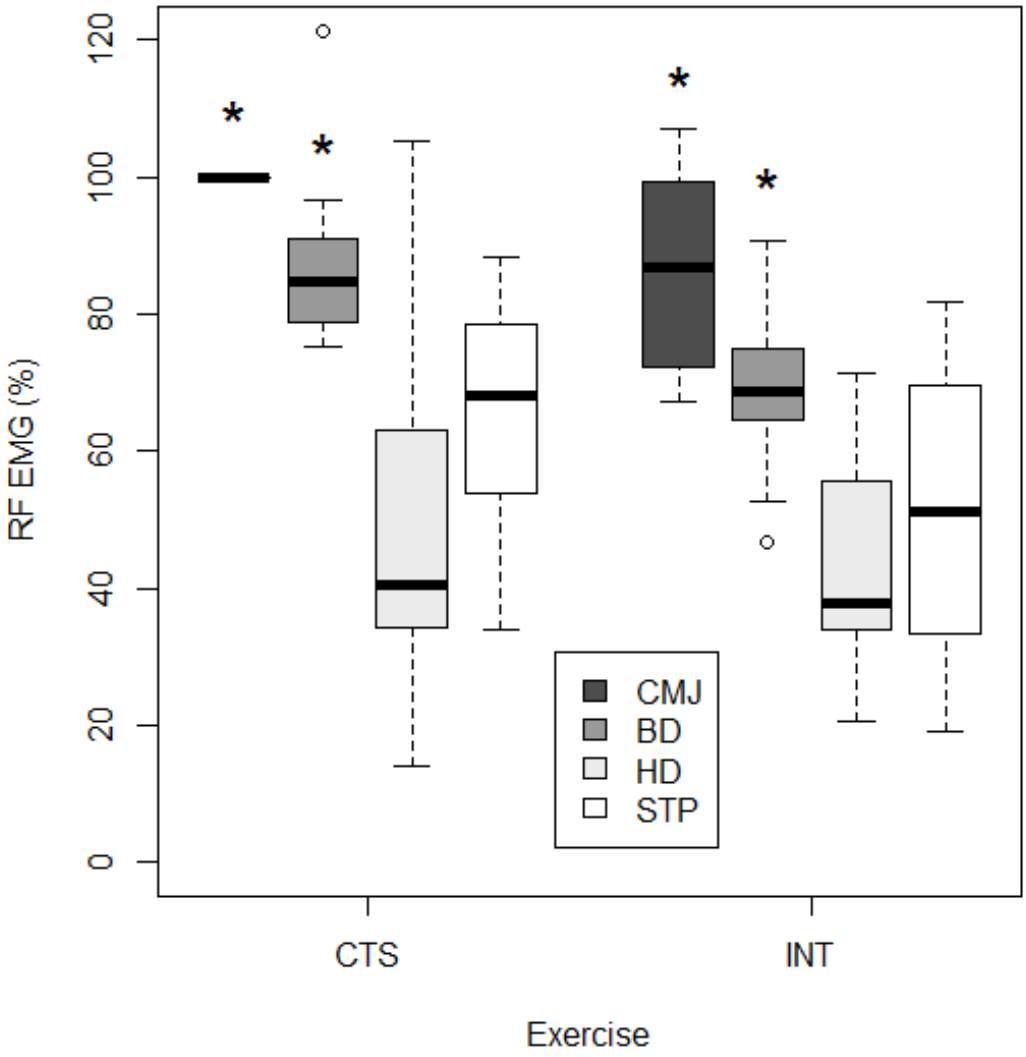


Fig. 5.3.5 Median (interquartile range, minimum and maximum) rectus femoris electromyography amplitude (RF EMG) of countermovement jumps (CMJ), box drops (BD), heel drops (HD) and stamps (STP) when performed both continuously and intermittently. Data were normalised to the continuous countermovement jump exercise and presented as a percentage. * indicates condition is statistically significantly higher than the corresponding HD and STP conditions. Circles denote outliers. n = 14

5.3.6 Semitendinosus Electromyography

There was a main effect for exercise ($P < 0.001$) and stimulus frequency ($P < 0.001$), but not for the interaction term ($P = 0.069$). CTS conditions were significantly higher than INT conditions for CMJ, BD, HD and STP exercises ($d = 1.32$ [95%CI: 0.78 to 1.86], $P < 0.001$; $d = 1.4$ [95%CI: 0.57 to 2.22], $P = 0.003$; $d = 0.58$ [95%CI: 0.19 to 0.97], $P = 0.006$; $d = 1.12$ [95%CI: 0.76 to 1.49], $P < 0.001$). For CTS conditions, CMJ, BD and STP were significantly higher than HD ($d = 1.46$ [95%CI: 1.03 to 1.89], $P < 0.001$; $d = 1.34$ [95%CI: 0.79 to 1.9], $P = 0.001$; $d = 1.01$ [95%CI: 0.45 to 1.58], $P = 0.011$). For INT conditions, CMJ and BD were significantly higher than HD ($d = 1.59$ [95%CI: 0.85 to 2.32], $P = 0.003$; $d = 1.26$ [95% CI: 0.82 to 1.7], $P = 0.001$) (Fig. 5.3.6).

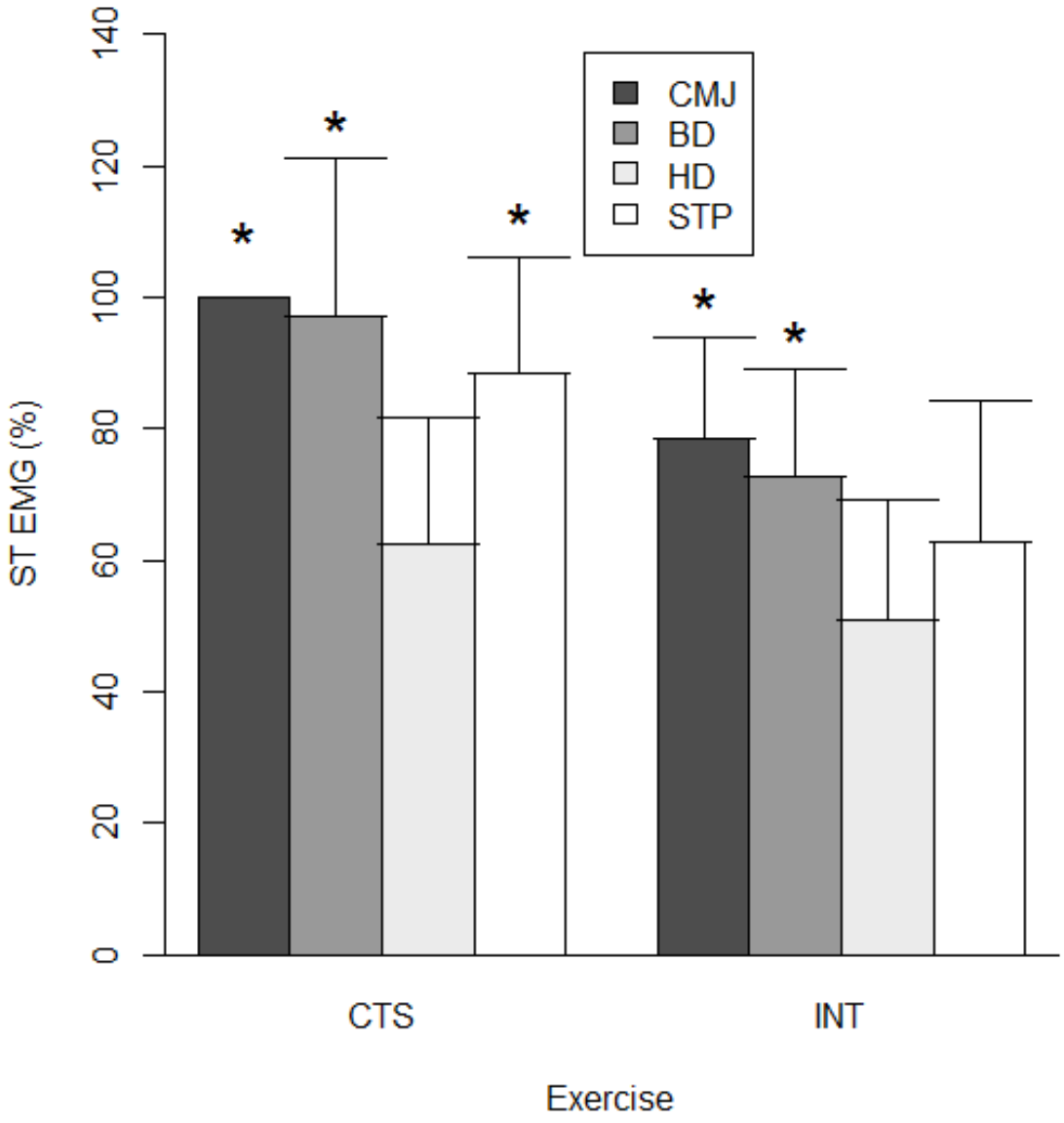


Fig. 5.3.6 Mean (±SD) semitendinosus electromyography amplitude (ST EMG) of countermovement jumps (CMJ), box drops (BD), heel drops (HD) and stamps (STP) when performed both continuously and intermittently. Data were normalised to the continuous countermovement jump exercise and presented as a percentage. * indicates condition is statistically significantly higher than the corresponding HD condition. n = 14

5.3.7 Tibialis Anterior Electromyography

There was a significant effect of exercise (P = 0.006), stimulus frequency (P = 0.029) and interaction term (P = 0.042). CTS conditions were significantly higher than INT conditions for BD and STP exercises (d = 0.68 [95%CI: 0.29 to 1.07], P = 0.002; d = 0.23 [95%CI: 0.00 to 0.46], P = 0.048). For CTS conditions, CMJ and BD were significantly higher than HD (d = 1.45 [95%CI: 0.71 to 2.19], P = 0.006; d = 1.59 [95%CI: 1.03 to 2.15], P < 0.001). For INT conditions, CMJ and BD were significantly higher than HD (d = 0.96 [95%CI: 0.5 to 1.43], P = 0.004; d = 0.71 [95%CI: 0.29 to 1.12], P = 0.016) (Fig. 5.3.7).

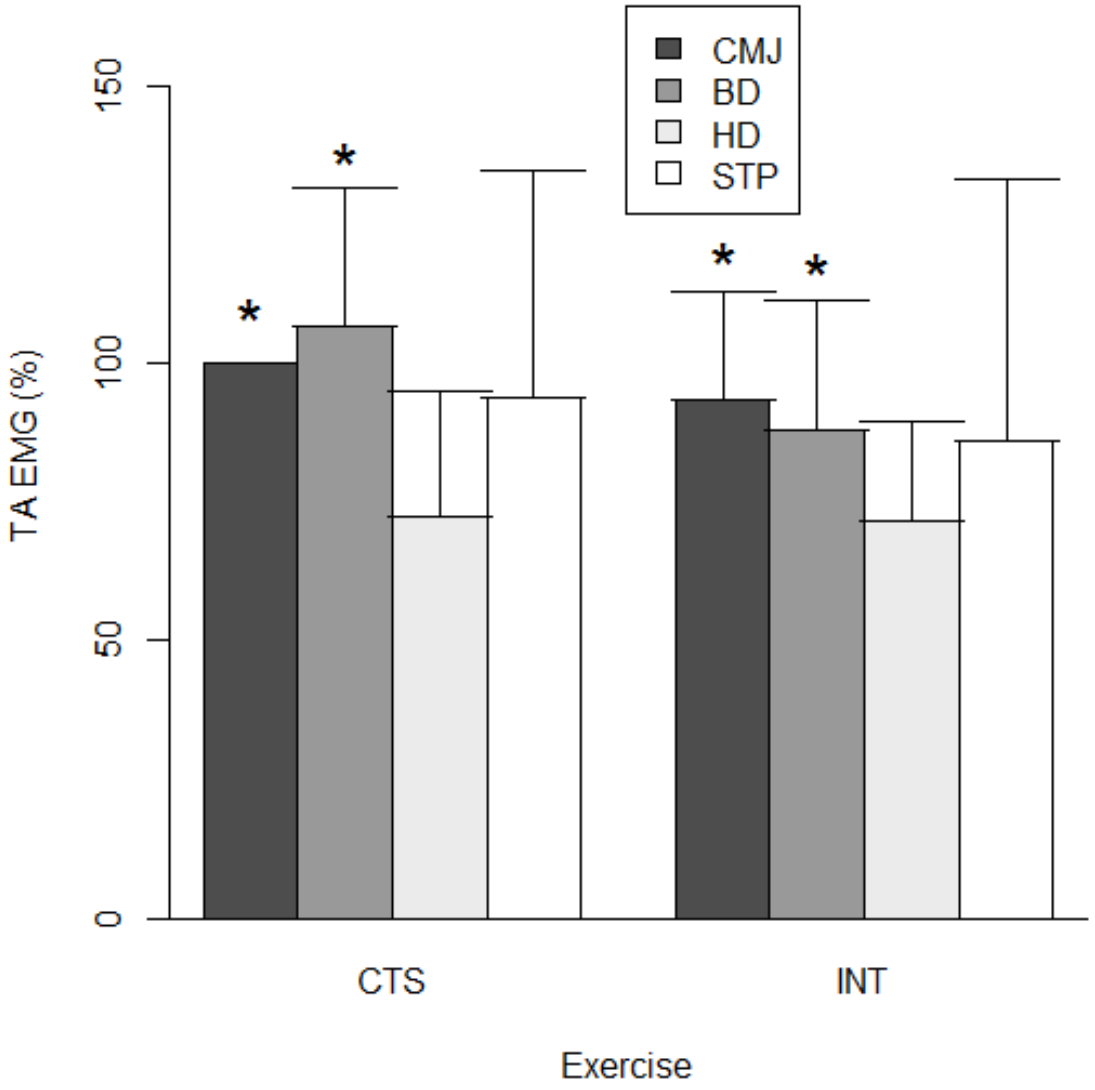


Fig. 5.3.7 Mean (±SD) tibialis anterior electromyography amplitude (TA EMG) of countermovement jumps (CMJ), box drops (BD), heel drops (HD) and stamps (STP) when performed both continuously and intermittently. Data were normalised to the continuous countermovement jump exercise and presented as a percentage. * indicates condition is statistically significantly higher than the corresponding HD condition. n = 14

5.3.8 Gastrocnemius Electromyography

There was a main effect for exercise ($P < 0.001$) and stimulus frequency ($P < 0.001$), but not for the interaction term ($P = 0.302$). CTS conditions were significantly higher than INT conditions for CMJ, BD, HD and STP ($d = 1.08$ [95%CI: 0.54 to 1.63], $P < 0.001$; $d = 1.08$ [95%CI: 0.6 to 1.57], $P < 0.001$; $d = 0.47$ [95%CI: 0.07 to 0.87], $P < 0.001$; $d = 0.42$ [95%CI: 0.07 to 0.77], $P < 0.001$). For CTS conditions, CMJ was significantly higher than BD and STP ($d = 1.4$ [95%CI: 0.86 to 1.95], $P = 0.001$; $d = 1.98$ [95%CI: 1.23 to 2.74], $P < 0.001$). HD was significantly higher than BD and STP ($d = 3.03$ [95%CI: 1.75 to 4.3], $P = 0.001$; $d = 3.61$ [95%CI: 2.57 to 4.65], $P < 0.001$). For INT conditions, CMJ was significantly higher than BD ($d = 1.05$ [95%CI: 0.33 to 1.77], $P = 0.046$), HD were significantly higher than BD and STP ($d = 3.22$ [95%CI: 1.57 to 4.87], $P = 0.006$; $d = 3.6$ [95%CI: 2.16 to 5.03], $P = 0.001$) (Fig. 5.3.8).

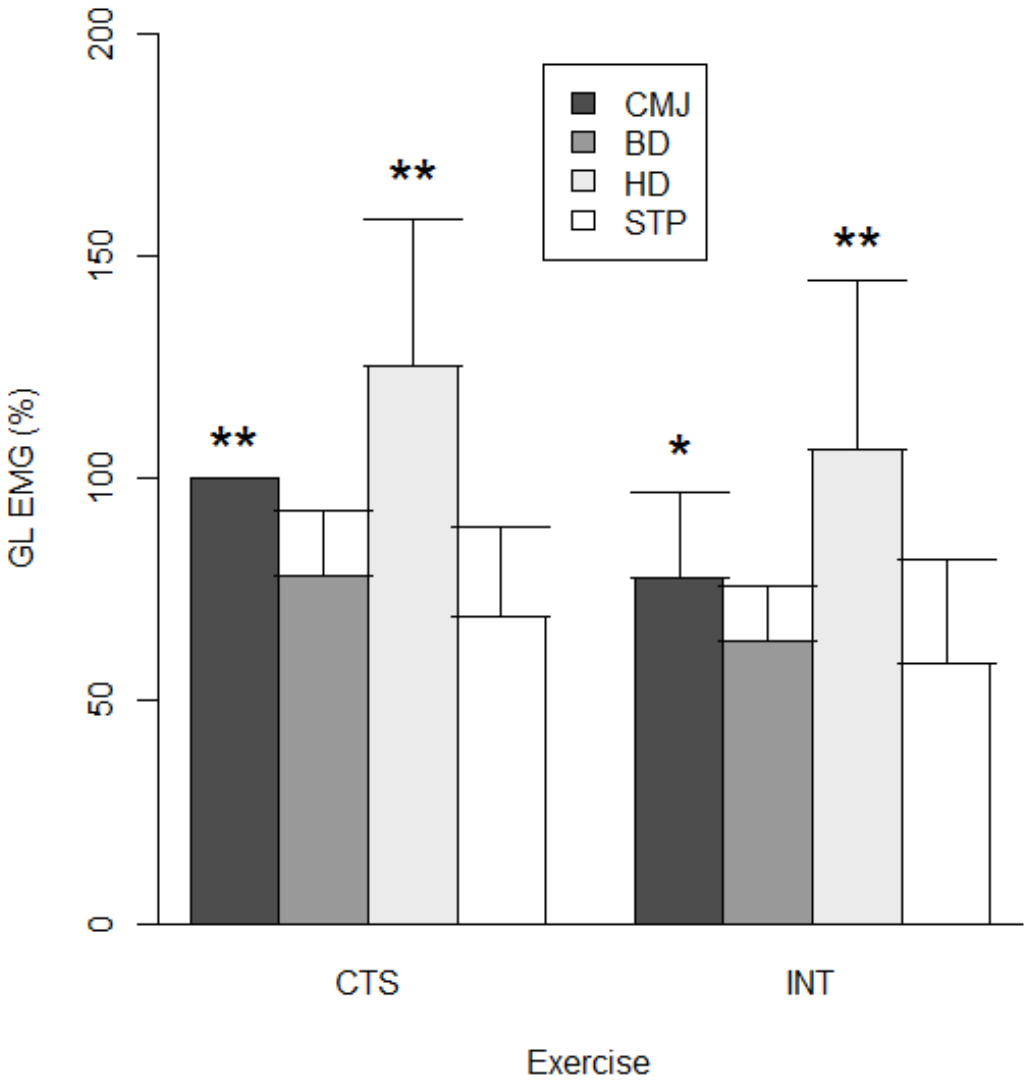


Fig. 5.3.8 Mean (±SD) gastrocnemius lateral head electromyography (GL EMG) of countermovement jumps (CMJ), box drops (BD), heel drops (HD) and stamps (STP) when performed both continuously and intermittently. Data were normalised to the continuous countermovement jump exercise and presented as a percentage. ** indicates condition is statistically significantly higher than the corresponding BD and STP condition, * indicates condition is statistically significantly higher than the corresponding BD condition. n = 14

5.4 Discussion

5.4.1 Osteogenic Index

CMJ produced the greatest OI along with BD conditions, which were not statistically significantly different from one another. CMJ, BD and HD conditions produced large-very large increases in OI when compared to STP conditions across CTS and INT trials. As both CTS and INT HD conditions displayed small but significantly different results, CMJ and BD exercises would be preferable for the evaluation of CTS and INT exercise in future interventions, additionally CMJ and BD also generated a greater OI, which provides a greater stimulus for bone adaptation (Turner and Robling, 2003). As there were no statistical differences in OI between the CTS and INT trials for CMJ and BD, these exercises would be suitable to assess the effects of stimulus frequency and the desensitisation mechanism on high impact exercise in future interventions with postmenopausal women.

5.4.2 Acceleration

Peak ACC statistics were represented in the OI calculation. The differences highlighted in Peak ACC values are meaningful in surpassing 0.17 g, the minimal

detectable change (Turcot et al, 2008). All exercises apart from STP consistently surpassed the osteogenic threshold of 4.9g (accounting for 1 g being standing) (Vainionpaa et al, 2007), which would suggest that STP exercises would be insufficient for stimulating an osteogenic response while the CMJ, BD and HD exercises appear to generate favourable loading conditions at the sacrum for maintaining postmenopausal BMD. Whilst the CMJ and BD would appear to produce higher peak ACC, it is important to note that HD were also sufficiently osteogenic and could provide a useful alternative for populations that cannot tolerate either CMJ or BD exercise.

In addition the Grad ACC data showed that all exercises surpassed the osteogenic threshold of 1000 $m \cdot s^{-3}$ (Heikkinen et al, 2007), although the CMJ and BD conditions displayed large increases in Grad ACC when compared to the STP condition across CTS and INT trials, which could indicate a greater stimulus for bone adaptation. The HD condition was the only exercise to display a difference during CTS and INT trials with the INT condition generating a small increase in Grad ACC. This lower CTS Peak ACC and Grad ACC in the HD condition could have been affected by gastrocnemius relaxation difficulties from improper relaxation during the brief rest interval in the CTS trials although this requires further investigation.

5.4.3 Electromyography

CMJ and BD conditions generated large increases in RF EMG when compared to HD and STP conditions, large increases in ST EMG when compared to HD conditions and moderate-large increases in TA EMG when compared to HD conditions across CTS and INT trials. Despite this, the HD conditions generated very large increases in GL EMG when compared to BD and STP conditions across CTS and INT trials. Greater RF EMG could indicate greater internal compressive forces on the femur during these exercises (Bassey et al, 1997). The strong linear relationship between EMG amplitude and force output would suggest that higher EMG amplitudes subject the skeleton to greater forces (Andrade and Andrade, 2012). As muscular actions have been suggested as the main stimulus for bone adaptation (Robling, 2009), CMJ and BD conditions appear to create a greater stimulus at the femur than the HD and STP exercises. HD conditions generate the lowest EMG amplitude response across the RF, ST and TA muscles but provide the greatest GL EMG. This was to be expected due to the nature of the exercises involved, and the small amount of involvement from any joint other than the ankle. While HD may not generate as higher activation in the muscles compressing the femur and spanning the hip as CMJ and BD, HD could provide a greater peripheral muscular activation on the lower limb, which could lead to greater peripheral bone adaptations (Hans et al, 2002). To date, there is little causal evidence linking higher EMG amplitude with a greater effect on

BMD adaptations and as direct internal force measurements have not been recorded, the EMG amplitude is only suggestive of internal loading. Whilst bone strain cannot be inferred from EMG measures as yet, this study could add to the current body of literature in this area and provide rationale for future investigations examining a potential relationship between EMG amplitudes and bone strain. The CTS conditions generated a statistically significantly greater RF EMG than the INT conditions during CMJ, BD and STP exercises, ST EMG for all exercises (small-large), TA EMG across BD (moderate) and STP (small) exercises and GL EMG (small-moderate) for all exercises. This might have been caused by the shorter rest interval in the CTS trials inducing greater fatigue and necessitating higher motor unit recruitment (Carneiro et al, 2010), although this would require further research. Despite the OI showing no statistical difference between CTS and INT conditions for CMJ, BD and STP exercises, the EMG amplitude data however indicates that the internal muscular activation could be substantially different as a result of altering the exercise frequency.

5.4.4 Strengths and Limitations

While the OI index reflects BMD adaptations (von Stengel et al, 2005; Rantalainen et al, 2009; Erickson and Vukovich, 2010; Rantalainen et al, 2011; Reiger and Yingling, 2015), it is largely based on loading intensity whereas other factors that also govern bone adaptation are omitted from OI calculations e.g.

stimulus frequency, acceleration gradient, muscular activation (Robling et al, 2001; Heikkinen et al, 2007; Robling, 2009;). Future research should incorporate these measures when considering mechanical loading dose and the osteogenic index. Direct comparisons with previous studies and suggested thresholds for bone adaptation are difficult due to differences in OI calculation and accelerometer processing (Jamsa et al, 2011) with low cut-off frequencies potentially having eradicated fast frequency components of the signal, which are the essential parts of the mechanical loading stimulus that bring about osteogenesis (Rantalainen et al, 2011; Kelley et al, 2014). Comparisons with self-reported data are complicated as self-reported osteogenic index measures can often be unreliable because nothing was actually measured and results can be greatly affected by personal opinions (Nilsson et al, 2012). ACC is used as an indicator of bone strain and in some cases may not accurately reflect the internal loading environment despite the two measures being highly related (Edwards et al, 2009).

5.4.5 Conclusions

CMJ and BD exercises consistently produced the highest OI, Peak ACC, Grad ACC and RF EMG, which would suggest that the CMJ and BD exercises provide a greater osteogenic stimulus than HD and STP exercises. However, the CMJ, BD and HD exercises all provide a sufficient Peak ACC osteogenic stimulus

(Vainionpaa et al, 2007). It is important to be mindful of the difference in muscular activation between the exercises and the tendency for CTS conditions to generate greater EMG amplitude than the INT conditions. This study has highlighted appropriate exercises that can inform future high impact training programmes targeted at postmenopausal BMD maintenance and can provide valuable information for exercise and osteoporosis prevention guidelines throughout the ageing process (Bassey et al, 1997; Turner and Robling, 2003; Vainionpaa et al, 2006; Heikkinen et al, 2007).

6. The effect of continuous and intermittent exercise on bone mineral density in postmenopausal women: a twelve-month randomised control trial

6.1 Introduction

Postmenopausal women experience rapid declines in bone mineral density (BMD) in the early years post-menopause (Finkelstein et al, 2008). Severe losses in BMD can increase the risk of developing osteoporosis later in life and increase the risk of experiencing a fracture (Kanis et al, 2001). In the EU, 46% of women and 22% of men over the age of 50 will experience an osteoporotic fracture (Hernlund et al, 2013) with 15% of women in the UK over the age of 50 are reported to have osteoporosis (Gauthier et al, 2011). The treatment of these fractures costs the NHS £3,496 million each year and is rising annually (Svedbom et al, 2013).

Given the social and economic cost of osteoporosis, treatments for either preventing or reducing the impact of the disease have been investigated, including exercise. For example, high-impact exercise has been shown to reduce postmenopausal bone loss and provides a cost effective alternative to drug therapies (Kelley et al, 2002; Martyn-St James and Carroll, 2009). Many exercise programmes have been reported to stimulate positive adaptations to bone tissue, which strengthen the bone structures and increase the mechanical resistance to a

sudden impact (Howe et al, 2011; Xu et al, 2016). These exercise regimes are often comprised of high-strain magnitudes along with high levels of muscular force (Bolton et al, 2012; Umemura, 2016).

The risk of developing osteoporosis increases with age, which in turn is associated with a loss in lower limb explosive power and an increased risk of falls (Skelton et al, 2002). Exercise programmes have been also shown to generate positive neuromuscular adaptations (power, balance, agility), which can decrease the likelihood of participants experiencing a fall, which in turn, decreases the chance of fracture incidence (Donath et al, 2016). Exercise interventions therefore have the potential to reduce fracture incidence through a number of different mechanisms.

Although exercise has been reported to increase BMD, it has been reported in animal models that intermittent mechanical loading generates a greater BMD response than continuous mechanical loading (Robling et al, 2001; Srinivasan et al, 2007). The greater BMD response is believed to be due to the rest interval creating a "resensitisation" effect on the mechanosensitivity of the bone tissue (Srinivasan et al, 2015). To support this, repetitive cyclic mechanical loading has reported to desensitise bone tissue (Umemura et al, 1997; Turner and Robling, 2003). However, the effect of intermittent mechanical loading has not been investigated in human populations.

Although animal studies have provided the platform to investigate the effect of

intermittent mechanical loading in controlled loading environments, this method is more complicated in humans. Animals can be anesthetised and animal bone can be stimulated with exact loading magnitudes at set loading rates with specific loading to rest ratios. With humans, exercise is commonly used as a mechanical loading stimulus for bone tissue, which is subject to individual variation in technique and anthropometric parameters that can alter the loading characteristics somewhat. Fortunately, countermovement jumping has been shown to generate adequate levels of mechanical loading for BMD adaptation in a population of early postmenopausal women and has been consistent concerning loading characteristics across both continuous and intermittent conditions (chapter 5). Countermovement jumping would therefore represent an ideal exercise mode to examine the effects of continuous and intermittent mechanical loading on postmenopausal BMD loss while controlling for mechanical loading parameters.

Although positive bone adaptations have been reported in exercise interventions in premenopausal women as early as six months after the beginning of the programme (Vainionpaa et al, 2005; Kato et al, 2006), for postmenopausal women, high impact exercise interventions have been known to require a duration greater than six months to show any response when compared to a control group (Xu et al, 2016). For this reason, a 12 month intervention would be a more appropriate duration to evaluate any potential changes in BMD.

The aim of this study was to evaluate the efficacy of 12 month continuous and

intermittent mechanical loading exercise interventions for attenuating declines in BMD in healthy early postmenopausal women. It was hypothesised that intermittent mechanical loading would reduce postmenopausal bone loss to a greater extent than continuous mechanical loading.

6.2 Methods

6.2.1 Study Design

This study was a randomised controlled exercise trial with two intervention groups and one control group. Participants performed either continuous exercise (CTS), intermittent exercise (INT) or were allocated to the control group (CON). The protocol received ethics approval by The University of Hull ethics committee and the Hull and East Yorkshire Hospitals NHS Trust ethics committee (HEY R&D Ref: R1723; REC Ref: 14/SW/1074). Written informed consent was obtained from each participant on study enrolment. Using a bespoke template (Hopkins, 2010), participants were adaptively randomised (immediately after their initial DXA scan and blood test) to one of three groups using their baseline femoral neck T-score and serum 25-hydroxy vitamin D (25 (OH) D). Measures of bone mass, bone geometry, maximal joint torques and muscle activation characteristics were recorded at baseline, after 6 months of the exercise

intervention, then finally after 12 months of the exercise intervention. The primary outcome measure was BMD (g/cm^2), whilst bone geometry, maximal joint torques and muscle activation characteristics were secondary outcome measures.

6.2.2 Participants

Study participants were healthy early postmenopausal (1 – 5 years) women 48 to 59 years of age, of white European origin and free from osteoporosis (Table. 6.3.3). Early postmenopausal women were chosen due to the levels of accelerated bone loss of 1 – 2% per year at the lumbar spine and hip sites, this population would benefit from a potential reduction in BMD loss (Finkelstein et al, 2008) and therefore reduce their risk of osteoporosis (Kanis et al, 2013). This population is not without complications however, it has been noted that early postmenopausal women with lower body mass are likely to display a faster rate of BMD loss than those with greater body mass (Finkelstein et al, 2008). The rate of bone loss can also vary depending upon the number of years post menopause. Higher rates of bone loss are seen in participants that are one year before the final menstrual cycle to two years after the final menstural cycle, whereas this rate of BMD loss is reduced from two to five years after the final menstrual cycle (Greendale et al, 2012). This would suggest that the earlier that bone loss can be reduced within close proximity to the final menstrual cycle, the more beneficial

the preservation of bone will be in terms of fracture risk. It can also mean that in postmenopausal women, the rates of bone loss can display increased variation depending upon the proximity to the menopause. It is difficult to determine these very specific stages of the lifecycle for research intervention purposes as women are not officially classed as postmenopausal until 12 months after the cessation of the final menstrual cycle (Recker, 2011). Without continual BMD monitoring prior to this stage of life, the BMD loss can only be defined after 12 months from menstrual cycle cessation. The study was estimated to require 60 participants to establish an 80% level of statistical power ($P < 0.05$). This calculation was conducted using G*Power 3.0 software (G*Power 3.0, Düsseldorf, GER), (Faul et al, 2007) and was based on an assumption that bone adaptations would be similar to that of a previously intervention from which a Cohen's *d* effect size was calculated as $d = 0.41$ using the mean group difference in lumbar spine BMD (Kelley et al, 2002). The calculation was performed with three groups, based on pre, mid and post intervention testing. In addition it was estimated that 25% of the original participants would drop out during the intervention based on previous similar research (Kemmler et al, 2013). Furthermore, it was estimated that 10% of the participants would discover that they were unknowingly osteoporotic and therefore could not be included in the current research project. Based on these estimations, a sample size of 88 initial participants was required.

Participants were all recruited from the East Riding of Yorkshire, UK, through

the following means:

a local television news programme, local newspaper articles, local newspaper/magazine adverts, local radio interviews, recruitment stalls, internal University communications, advertisement emails to 11 large local employers, advertisement material, which was distributed to three local Pilates instructors, eight health centres, four church/town halls, five rotary clubs, AGE UK, U3A, two Women's Institutes, low-impact dance classes, gardening clubs, two parish councils, an amateur dramatic society, three Zumba instructors, a reading group, local coffee shops, two secondary school publications, a church newsletter, nine further local newspapers and three University social media accounts.

The first screening was performed with telephone conversations and email communications to establish eligibility with 174 women volunteering for the project. Forty nine volunteers fit the inclusion criteria and were successfully recruited (Appendix D). Unfortunately, the recruitment target was not met due to difficulties in finding willing participants within our specific age range that were within 1 – 5 years of the menopause. Participants were included in the trial if they had either not received hormone replacement therapy (HRT) or had not been receiving HRT for at least five years. Participants had not received any form of steroids or other medication that could affect rates of bone turnover. Participants were also free from fracture incidence for at least one year prior to undertaking the current project. The included participants had not been participating in

intermittent exercise (team sports, racquet sports or other activities involving substantial impacts) or resistance exercise more than once per week and possessed sufficient serum 25-hydroxy vitamin D (25 (OH) D) along with normal calcium and phosphate levels (> 25 nmol·L^{-1}, 2.2 – 2.6 mmol·L^{-1} and 0.7 – 1.5 mmol·L^{-1} respectively). Participants were included providing they were currently non-smokers. Diet was uncontrolled providing calcium, phosphate and serum 25-hydroxy vitamin D (25 (OH) D) levels were all sufficient. For all other participant inclusion and exclusion criteria, please refer to Appendix D. Initial testing began in November 2014 with ongoing recruitment of participants in regular cohorts until September 2015 and final follow-up testing finished in November 2015 for the initial cohorts and concluded in September 2016 for the final cohort of participants.

6.2.3 Dual Energy X-Ray Absorptiometry

DXA scans were performed at the lumbar spine (L1 – L4) and right proximal femur by a trained, full-time DXA technician at Hull Royal Infirmary on a GE Lunar Prodigy DXA scanner (GE Healthcare, Madison, WI, USA), using the manufacturer's standard procedures. The DXA technician was blinded to group allocation and all scans were performed by the same technician throughout the study. DXA scans were used to calculate BMD, BMC and the following advanced HSA variables using in-built algorithms to estimate the geometric properties of

the femoral neck; cross sectional area (CSA), section modulus (SM), buckling ratio (BR), strength index (SI), cross sectional moment of inertia (CSMI) and minimum neck width (MNW). Participants received one set of DXA scans at baseline, one set after 6 months and the final set after 12 months of the investigation.

6.2.4 Exercise Intervention

Once randomly assigned to one of the three possible groups, participants were given their exercise programme, or if randomised to the control group, were instructed to keep the same level of habitual physical activity for the duration of the trial. Both the CTS and INT groups completed identical exercise programmes that lasted for 12 months and only differed in the rest interval duration between jumps. Both groups were instructed to perform 30 countermovement jumps (CMJ) for maximum height, on three separate occasions per week that were at least 48 hours apart to reduce the desensitisation effect that can occur with greater than 36 loading cycles (Umemura et al, 1997). Participants were told to complete the exercises barefoot on a hard surface. Participants were instructed to "jump as high as possible using the arms and land on the balls of the feet with bent knees" to reduce the risk of injury on landing. CMJ technique was checked and corrected if necessary during laboratory sessions. CTS participants performed their 30 CMJs at 0.25 Hz (one CMJ every 4 seconds / 15 bpm), while the INT participants

performed their 30 CMJs at 0.067 Hz (one CMJ every 15 seconds / 4 bpm). The rest interval was counted using a loud audible metronome set to either 15 bpm or 4 bpm for the CTS or INT groups respectively. Participants were advised that they could use a suitable item of furniture for light hand support, only if they felt it was absolutely necessary, and were encouraged to perform the exercise without any support as soon as they felt comfortable enough to do so. Intervention participants kept monthly exercise logs of their adherence, along with being regularly contacted via email or telephone every 3 to 4 weeks to assess any problems and to attempt to maintain interest and adherence. Participants across all groups were given an accelerometer-based activity monitor (GENEActiv Action, GENEActiv by Activinsights, Kimbolton, UK) and were instructed to wear the device for 7 days (for the entire duration apart from when sleeping). The activity monitor was worn on a hip belt that positioned the device on the superficial surface of the anterior superior iliac spine and aligned with the vertical axis. Acceleration data (±16 g) was recorded at 100 Hz for the Fz, Fy and Fx directions. The monitor was intended to record acceleration for a 7 day duration for each participant every three months, totalling 4 weeks throughout the 12 month intervention.

6.2.5 Blood Analysis

Blood was sampled at baseline by a trained phlebotomist using venepuncture and

subsequently analysed for Serum 25-hydroxy vitamin D (25 (OH) D), calcium and phosphate concentrations. Samples were batch analysed in the blood sciences laboratory at Hull Royal Infirmary. If the participant was deficient in any of the three vitamin/minerals, they were excluded from the investigation and referred to a consultant for a medical assessment.

6.2.6 Rate of Torque Development and Maximum Voluntary Isometric Torque

Maximum voluntary isometric contraction (MVIC) and rate of torque development (RTD) of the knee extensor muscles were assessed at baseline, after 6 months and after 12 months of the intervention using an isovelocity dynamometer (Biodex, System 3, Shirley, NY, USA). Participants were secured to the dynamometer to ensure a fixed hip angle of 95˚. The dynamometer was adjusted to ensure the centre of the crank arm was aligned with the knee joint centre and the crank arm was then attached to the ankle with a velcro strap so that the base of the crank arm sat on top of the medial malleolus of the tibia. Participants performed a 1 – 3 minute isometric warm up at a knee angle of 70˚ (0˚ being full knee extension) with increasing intensity.

Once ready the participants performed 5 - 10 explosive isometric knee extensions also at 70˚, with each contraction separated by 30 seconds. Participants were

instructed to push against the static crank arm "as fast and as hard as possible from a state of complete muscular relaxation" and to hold the contraction for 1 – 1.5 seconds. The "fast" element of these contractions was emphasised and reinforced during the testing protocol. Participants were told to avoid any countermovement before pushing the crank arm away. This was checked by the investigator during the warm up and continually during the testing protocol. Biofeedback was presented to the participant during the testing protocol with a monitor showing the instantaneous torque - time trace. The baseline torque and EMG activity were used to check that the participant's leg was properly relaxed before torque initiation and that there was no pre-tension that might affect the RTD values.

Participants were given 2 – 3 minutes rest before performing three maximal voluntary isometric knee extensions from the same knee angle (70˚), lasting 3 - 5 seconds each that were separated by 1 minute. They were instructed to push against the static crank arm "as hard as possible". Loud verbal encouragement was given during these efforts. The torque and EMG data were automatically synchronously recorded at 3000 Hz via Noraxon hardware (Noraxon Telemyo 2400T, Scottsdale, Arizona, USA), (EMG: input impedance > 100 MΩ, CMRR > 100 dB, baseline noise < 1μV RMS, base gain = 200, final gain = 500), and stored in the Noraxon data acquisition system (Noraxon Telemyo 2400T, Scottsdale, Arizona, USA).

6.2.7 Electromyography

Surface EMG electrodes (Ambu Blue Sensor N, Ambu, Cambridgeshire, UK) were placed over the vastus lateralis and biceps femoris muscles of the participant's right leg in accordance with SENIAM surface electromyography recommendations (Hermens et al, 1999). Prior to electrode attachment, the skin was shaved, abraded and cleansed with a 70% alcohol swab. EMG wearable hardware were secured with surgical tape to reduce unwanted movement and signal artefacts.

All signal processing was completed using a bespoke MATLAB programme (MATLAB R2011a, Mathworks, Cambridge, UK). All torque data were gravity-corrected to account for the baseline torque. MVIC was determined as the maximum torque value achieved across each of the maximal efforts. For the RTD contractions, the identification of all torque onsets was completed manually by the same investigator, as the manual identification method has been shown to be more sensitive and reliable than automated methods (Tillin et al, 2010). The torque signal was low-pass filtered at 21 Hz to remove high frequency noise. RTD data were analysed as individual contractions with the initial view of each individual torque - time graph beginning with a y-axis resolution of ± 400 N and an x-axis of 2 seconds. The investigator manually zoomed to a resolution of ± 0.5 N (y-axis) and a 0.002 second window along the x-axis to allow manual identification of the onset of the point of torque application. To assist the

investigator, the first derivative of the torque - time graph was added to the graph to further highlight the point at which the torque data deflected away from the baseline noise, this point was manually determined as the torque onset (Tillin et al, 2010). From the point of torque onset, the RTD was established at time points of 50 ms, 100 ms and 150 ms (RTD50, RTD100, RTD150), which provided information on the initial increases in torque development as it usually takes greater than 300 ms to produce a maximal torque (Thorstensson et al, 1976; Tillin et al, 2010; Tillin et al, 2012). The three highest RTD efforts (based on the highest torque at 100 ms) were selected for analysis and were averaged to get a single RTD value at each time point (50 ms, 100 ms, 150 ms). All trials with greater than ± 1 Nm of pretension or countermovement in the 2 seconds before torque onset, were automatically eliminated from the analysis during the data processing. For reliability purposes, one RTD contraction was reanalysed per participant to establish the effect of investigator error on torque onset.

6.2.8 Statistical Analysis

All data were log-transformed to adjust for the small sample size, normality was tested using a Shapiro-Wilk test before analysis was performed. Using a bespoke template (Hopkins, 2006), absolute changes in BMD pre and post intervention are presented as mean difference (g/cm^2) and 95% confidence intervals, percentage changes in BMD pre and post intervention are presented as mean

difference (%) and 95% confidence intervals. Cohen's *d* effect size was reported and evaluated using the following scale: 0 - 0.19 trivial, 0.2 - 0.59 small, 0.6 - 1.19 moderate, 1.2 - 1.99 large, 2.0 - 3.99 very large. Uncertainty in the population estimates are expressed as 95% confidence intervals along with the likelihood that the effect is substantially positive, trivial or substantially negative.

Using R (R Foundation for Statistical Computing 3.2.1, Vienna, Austria), between-group baseline variables were analysed with Welch's t-tests due to unequal group variances. A two-way (3 group x 3 time points) repeated measures ANOVA with a type 3 correction for unequal sample sizes was used to examine the effect of the intervention for group (CTS, INT and CON), time (pre, mid and post) and group × time interaction. Post-hoc within-group pairwise comparisons were completed with paired t-tests adjusted for multiple comparisons using the Holm-Sidak method (Holm, 1979). Main effects for group (CTS, INT and CON) are presented, as are main effects for time (pre and post) and group × time interaction. The significance level was set at $P < 0.05$.

6.3 Results

6.3.1 Adherence, Withdrawals and Adverse Events

From the 49 participants that were initially recruited, four participants were excluded as a result of already having osteoporosis and were referred to a consultant for medical treatment. Similarly, four participants were excluded due to having serum 25-hydroxy vitamin D (25 (OH) D) levels below 25 OH nmol·L^{-1} and were also referred to a consultant for treatment. Two participants dropped out of the study after the DXA scan but before baseline neuromuscular testing, one of which had no available free time, and the other experienced back problems. Four participants dropped out due to back, hip and foot pain from previous conditions that were undisclosed at the initial recruitment interview and medical questionnaire. One participant dropped out due to a change in employment, another dropped out but gave no reason as to why. One participant experienced a fall and subsequent hip fracture whilst carrying heavy shopping bags, which was unrelated to the exercise intervention and was removed from the trial as a result. Four participants were uncontactable during follow-up (Fig. 6.3.1).

28 participants completed the study (nine CTS, eight INT, 11 CON).

Of the 17 intervention participants that completed the study, only 13 participants returned exercise logs. The four participants from which no exercise records

could be obtained, were subsequently eliminated from the final analysis. Of the 13 participants that provided completed exercise logs, 10 participants had greater than 70% adherence to the exercise programme. Three participants had particularly poor adherence (< 70%) and were eliminated from the final analysis. The final 10 intervention participants that were included in the final analysis had no reported difference in recreational activity levels. Four CON group participants were excluded from the final analysis due to drastically increasing their physical activity above their habitual levels during the yearlong follow up period. These ranged from beginning a 10 km running programme, starting a resistance training programme, and participating in a separate University lead exercise intervention involving a substantial amount of resistance training (this remained undisclosed until after the study).

The CTS group had 98.2 ± 5.9% adherence and the INT group had 87.3 ± 8.7% adherence. Due to problems with data corruption, each participant only wore a physical activity monitor on one occasion for a 7 day duration. Monitors lost power prematurely and failed to register with the charger cradle. Data could not be retrieved and after 4 months of discussions with the manufacturer, the devices were recalled.

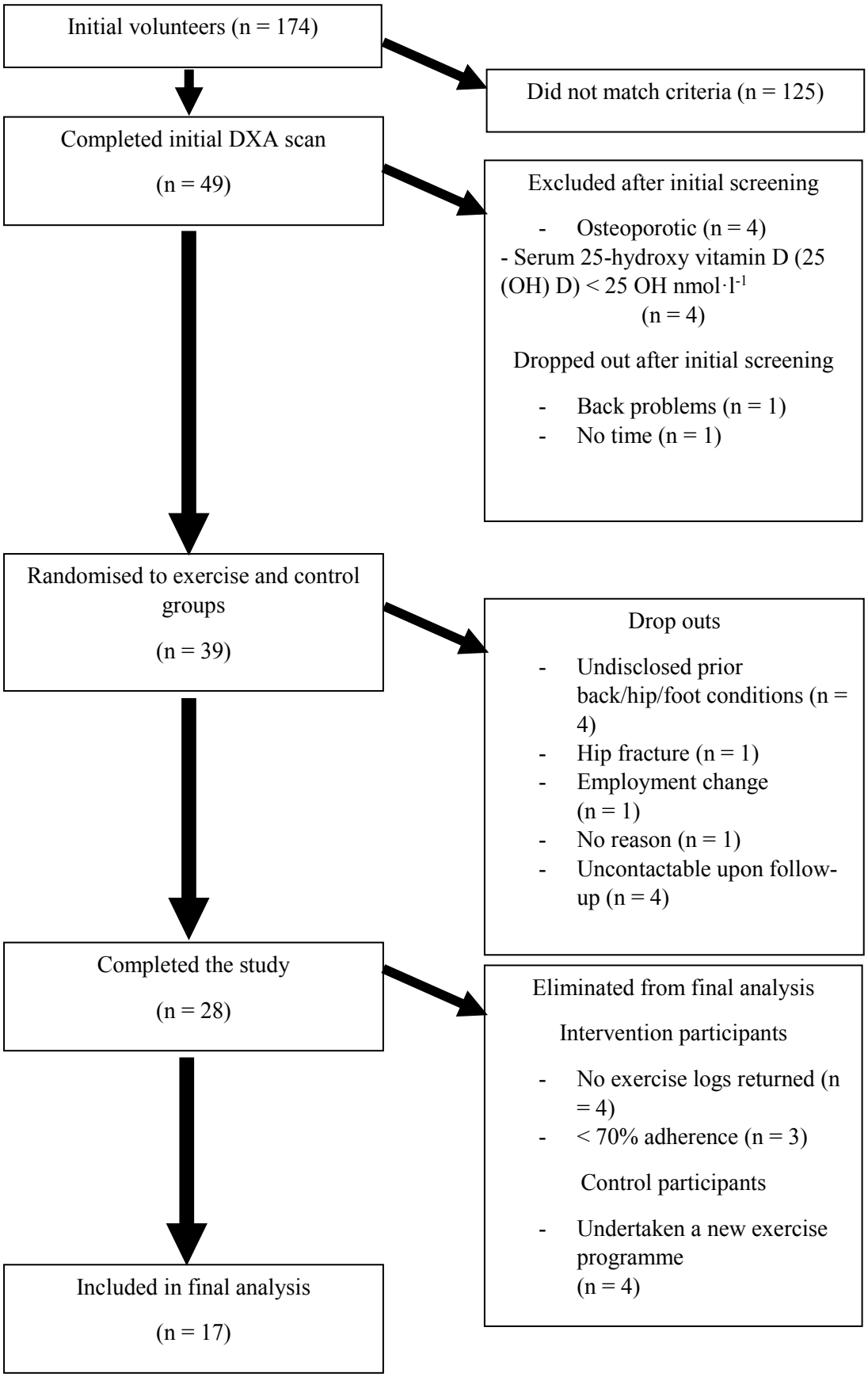


Fig. 6.3.1 Participant inclusion flow diagram

6.3.2 Dual Energy X-Ray Absorptiometry Precision Error

DXA precision error was given as root mean square % coefficient of variation (RMS % CV), which was 0.9% and 1.4% for the lumbar spine and femoral neck respectively (Steel, 2009). The least significant change for lumbar spine and femoral neck BMD was calculated as 2.5% and 3.9% for the lumbar spine and femoral neck regions respectively in accordance with current recommendations from the International Society for Clinical Densitometry (Baim et al, 2015).

6.3.3 Participant Baseline Characteristics

Of the 28 participants that completed the study, activity levels ranged from sedentary to active. Only one participant engaged in a form of resistance training once per week whilst no others completed any regular high impact or resistance exercise. Seven participants were ex-smokers that all quit a minimum of seven years ago. Only one participant consumed more than the recommended intake of 21 units of alcohol per week. No participants had previously received any HRT therapy. Serum 25-hydroxy vitamin D (25 (OH) D) levels were greater than 25 $nmol \cdot l^{-1}$ (53.3 ± 18.8 $nmol \cdot l^{-1}$), serum calcium levels were within the normal range (2.3 ± 0.1 $mmol \cdot l^{-1}$), as were serum phosphate levels (1.2 ± 0.1 $mmol \cdot l^{-1}$).

No statistical differences were found in any of the BMD, BMC, HSA, blood profile or age, height and weight characteristics across the intervention and

control groups (Table 6.3.3) with the exception of the femoral neck strength index, which was statistically greater in the CTS group than the INT group (d = 1.72 [95%CI: 0.23 to 3.22], 98% positive; P = 0.021). Lumbar spine T-Scores were -0.6 ± 1.4, -1.0 ± 0.8 and -0.5 ± 0.9 whilst femoral neck T-Scores were -0.4 ± 0.6, -0.7 ± 0.7 and -0.2 ± 0.4 for the CTS, INT and CON groups respectively.

Table 6.3.3 Descriptive and inferential statistics for bone mineral density **(**BMD), bone mineral content (BMC) and hip structural analysis (HSA) parameters at baseline

	Mean ± SD			Cohen's d [95% confidence intervals]		
	CONTINUOUS (n = 5)	**INTERMITTENT (n = 5)**	**CONTROL (n = 7)**	**CTS - CON**	**INT - CON**	**CTS - INT**
BMD						
Lumbar Spine BMD (g/cm^2)	1.105 ± 0.168	1.061 ± 0.095	1.117 ± 0.116	*d* = -0.12 [-1.73 to 1.49], 46% negative; *P* = 0.907	*d* = -0.38 [-1.45 to 0.69], 64% negative; *P* = 0.426	*d* = 0.16 [-0.86 to 1.18], 46% positive; *P* = 0.663
Femoral Neck BMD (g/cm^2)	0.936 ± 0.075	0.889 ± 0.087	0.959 ± 0.049	*d* = -0.4 [-2.11 to 1.30], 61% negative; *P* = 0.605	*d* = -1.23 [-3.40 to 1.94], 86% negative; *P* = 0.197	*d* = 0.48 [-0.88 to 1.85], 68% positive; *P* = 0.436
Trochanter BMD (g/cm^2)	0.801 ± 0.106	0.722 ± 0.141	0.789 ± 0.083	*d* = 0.1 [-1.35 to 1.54], 43% positive; *P* = 0.847	*d* = -0.8 [-3.03 to 1.44], 74% positive; *P* = 0.428	*d* = 0.62 [-1.00 to 2.24], 72% negative; *P* = 0.397
BMC						
Femoral Neck BMC (g)	4.68 ± 0.52	4.51 ± 0.6	4.5 ± 0.57	*d* = -0.01 [-1.67 to 1.65], 39% negative; *P* = 0.967	*d* = -0.86 [-2.83 to 1.1], 79% negative; *P* = 0.315	*d* = 0.52 [-0.78 to 1.81], 71% positive; *P* = 0.377
HSA						
CSA (mm^2)	140.8 ± 17.8	140.8 ± 18.5	140.4 ± 18.7	*d* = -0.4 [-2.46 to 1.66], 59% negative; *P* = 0.694	*d* = -1.03 [-3.25 to 1.19], 81% negative; *P* = 0.299	*d* = 0.31 [-0.95 to 1.56], 58% positive; *P* = 0.585
Section Modulus (mm^3)	621.7 ± 108.2	602.7 ± 120.7	613.4 ± 96.9	*d* = -0.19 [-2.68 to 2.30], 50% negative; *P* = 0.950	*d* = -1.72 [-4.49 to 1.05], 90% negative; *P* = 0.140	*d* = 0.6 [-0.61 to 1.82], 77% positive; *P* = 0.274
Buckling Ratio	3.7 ± 0.8	4.1 ± 1.1	3.7 ± 0.9	*d* = 0.34 [-0.61 to 1.29], 63% positive; *P* = 0.542	*d* = -0.39 [-2.06 to 1.28], 60% negative; *P* = 0.815	*d* = 1.00 [-1.29 to 3.30], 79% positive; *P* = 0.547
Strength Index	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	*d* = 0.74 [-0.11 to 1.60], 91% positive; *P* = 0.075	*d* = -0.07 [-0.99 to 0.86], 37% negative; *P* = 0.772	*d* = 1.72 [0.23 to 3.22], 98% positive; *P* = 0.021 *
CSMI (mm4)	10512.4 ± 2285.7	10124.4 ± 2514.4	10287 ± 2282.3	*d* = -0.07 [-2.12 to 1.97], 44% negative; *P* = 0.965	*d* = -1.08 [-3.22 to 1.06], 83% negative; *P* = 0.264	*d* = 0.5 [-0.73 to 1.73], 71% positive; *P* = 0.369
Minimum Neck Width (mm)	31.1 ± 1.4	30.9 ± 1.8	30.8 ± 1.4	*d* = 0.46 [-1.07 to 1.99], 66% positive; *P* = 0.474	*d* = -0.54 [-2.80 to 1.71], 64% negative; *P* = 0.586	*d* = 0.68 [-0.92 to 2.28], 75% positive; *P* = 0.342
Blood						
25-hydroxy vitamin D (25 (OH) D) ($nmol \cdot l^{-1}$)	50.7 ± 15.6	48.0 ± 16.1	46.2 ± 11.6	*d* = 0.23 [-1.31 to 1.78], 52% positive; *P* = 0.637	*d* = 0.05 [-1.51 to 1.61], 41% positive; *P* = 0.848	*d* = 0.12 [-1.08 to 1.33], 44% positive; *P* = 0.818
Calcium ($mmol \cdot l^{-1}$)	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	*d* = 0.11 [-0.87 to 1.09], 42%	*d* = 0.09 [-1.03 to 1.21], 42%	*d* = 0.03 [-1.36 to 1.43], 39%

				positive; $P = 0.811$	positive; $P = 0.864$	positive; $P = 0.969$
Phosphate ($mmol \cdot l^{-1}$)	1.3 ± 0.0	1.2 ± 0.2	1.1 ± 0.1	d = 0.75 [-0.07 to 1.97], 92%	d = 0.04 [-1.36 to 1.45], 40%	d = 2.24 [-2.22 to 6.70], 86%
				positive; $P = 0.070$	positive; $P = 0.917$	positive; $P = 0.254$
Age (y)				d = 0.21 [-0.66 to 1.08], 51%	d = -0.15 [-1.21 to 0.91], 46%	d = 0.65 [-1.07 to 2.36], 73%
	55.6 ± 1.9	54.0 ± 3.0	54.7 ± 3.6	positive; $P = 0.623$	negative; $P = 0.743$	positive; $P = 0.400$
Height (m)	1.62 ± 0.04	1.65 ± 0.02	1.64 ± 0.04	d = -0.44 [-1.49 to 0.60], 70%	d = 0.14 [-0.76 to 1.04], 77%	d = -0.65 [-1.67 to 0.38], 83%
				negative; $P = 0.353$	positive; $P = 0.746$	negative; $P = 0.181$
Mass (kg)	64.7 ± 6.3	65.0 ± 6.8	65.0 ± 6.9	d = -0.24 [-1.19 to 0.71], 54%	d = 0.18 [-1.62 to 1.97], 49%	d = -0.58 [-3.09 to 1.93], 64%
				negative; $P = 0.535$	positive; $P = 0.728$	negative; $P = 0.494$
Body Mass Index ($kg \cdot m^{-2}$)	24.8 ± 2.2	24.8 ± 2.5	25.0 ± 2.4	d = -0.10 [-1.13 to 0.93], 42%	d = 0.16 [-2.17 to 2.49], 48%	d = -0.3 [-2.94 to 2.34], 54%
				negative; $P = 0.793$	positive; $P = 0.744$	negative; $P = 0.656$

* indicates significant difference between groups at baseline $P < 0.05$.

There was no statistical change in mass (CTS: mean difference (kg) = 0.30 [95% CI: -1.11 to 1.71], *d* = 0.03 [95%CI: -0.11 to 0.17], 98% trivial, *P* = 0.609; INT: mean difference (kg) = -2.24 [95% CI: -10.84 to 6.36], *d* = -0.05 [95%CI: -0.35 to 0.25], 84% trivial, *P* = 0.646; CON: mean difference (kg) = 0.96 [95% CI: -1.39 to 3.31], *d* = 0.06 [95%CI: -0.11 to 0.23], 95% trivial, *P* = 0.440) or body mass index during the intervention across all groups (CTS: mean difference (BMI) = 0.15 [95% CI: -0.37 to 0.67], *d* = 0.04 [95%CI: -0.10 to 0.19], 97% trivial, *P* = 0.464; INT: mean difference (BMI) = -0.82 [95% CI: -3.93 to 2.28], -0.05 [95%CI: -0.34 to 0.24], 85% trivial, *P* = 0.646; CON: mean difference (BMI) = -3.43 [95% CI: -12.89 to 6.03], -8.9 [95%CI: -30.94 to 13.13], 81% negative, *P* = 0.361).

6.3.4 Lumbar Spine Bone Mineral Density (L1 – L4)

From baseline testing to final testing after 12 months, for lumbar spine BMD there was no main effect for group (*P* = 0.750), but a significant main effect for time was found, showing a reduction across groups (*P* = 0.006), the interaction term was not significant (*P* = 0.307), (Table 6.3.8).

The CTS group experienced a very likely trivial within-group change in lumbar spine BMD over 12 months (mean difference (g/cm^2) = 0.000 [95%CI: -0.030 to 0.031]), (% difference = -0.1 [95%CI: -3.2 to 3.0]), (*d* = -0.01 [95%CI: -0.15 to

0.14], 98% very likely trivial; P = 0.999), (Fig. 6.3.4). The INT group experienced a possibly small within-group loss in BMD over 12 months, although there was no statistically significant difference (mean difference (g/cm^2) = -0.032 [95%CI: -0.086 to 0.021]), (% difference = -3.2 [95%CI: -8.1 to 1.8]), (*d* = -0.26 [95%CI: -0.67 to 0.15], 66% possibly small; *P* = 0.296). The CON group experienced a statistically significant small within-group loss in lumbar spine BMD over 12 months, (mean difference (g/cm^2) = -0.029 [95%CI: -0.042 to -0.016]), (% difference = -2.7 [95%CI: -3.9 to -1.4]), (*d* = -0.21 [95%CI: -0.30 to -0.11], 55% possibly small; *P* = 0.006). When compared to the least significant change of 2.5%, it was clear that 20% of the CTS participants experienced clinically detectable reductions in lumbar spine BMD whereas 60% of the INT participants and 57% of the CON participants experienced clinically detectable reductions in lumbar spine BMD.

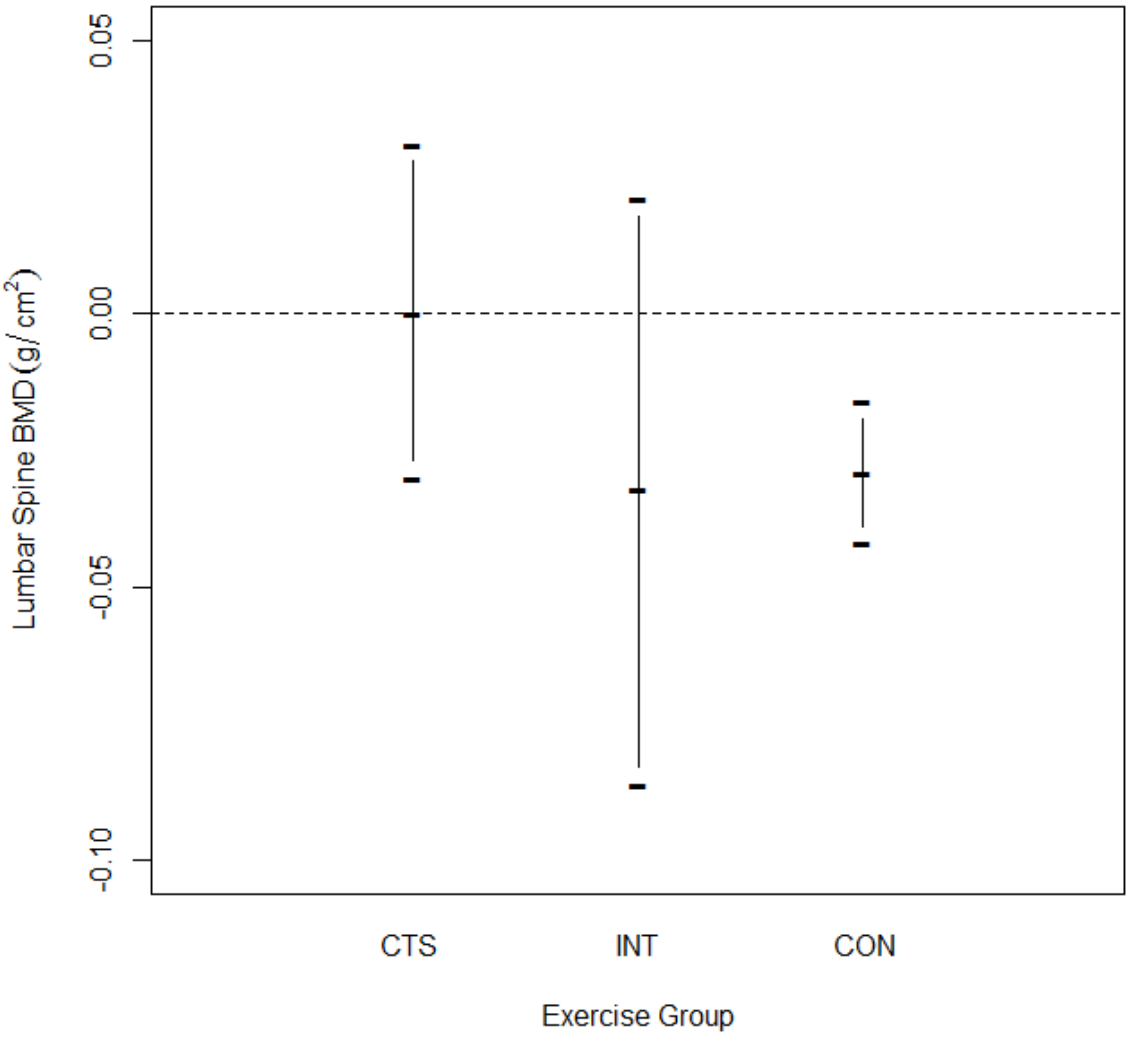


Fig. 6.3.4 Changes in lumbar spine (L1 – L4) bone mineral density (BMD) after 12 months. Data are mean differences ± 95% CI; continuous group (CTS) n = 5, intermittent group (INT) n = 5, control group (CON) n = 7

6.3.5 Femoral Neck Bone Mineral Density

No main statistical effect was found upon femoral neck BMD for group (P = 0.307) but a statistically significant main effect for time was found, showing a

reduction across groups ($P < 0.001$),), the interaction term was not significant ($P = 0.970$), (Table 6.3.8).

The CTS group experienced an unclear within-group change in femoral neck BMD over 12 months with no statistically significant difference (mean difference (g/cm^2) = -0.019 [95%CI: -0.074 to 0.036]), (% difference = -2.2 [95%CI: -7.7 to 3.6]), (d = -0.20 [95%CI: -0.72 to 0.32]), 51% possibly small; $P = 0.680$), (Fig. 6.3.5). The INT group experienced a possibly negative within-group change in femoral neck BMD over 12 months, although there was no statistically significant difference (mean difference (g/cm^2) = -0.022 [95%CI: -0.045 to 0.001]), (% difference = -2.3 [95%CI: -4.7 to 0.1]), (d = -0.17 [95%CI: -0.34 to 0.01], 68% possibly trivial; $P = 0.111$). The CON group experienced a small statistically significant within-group loss in femoral neck BMD over 12 months (mean difference (g/cm^2) = -0.029 [95%CI: -0.050 to -0.007]), (% difference = -3.0 [95%CI: -5.1 to -0.8]), (d = -0.47 [95%CI: -0.81 to -0.12], 95% likely small; $P = 0.048$). When compared to the least significant change of 3.9%, it was clear that, 20% of the CTS participants, 20% of the INT participants and 57% of the CON participants experienced clinically detectable reductions in femoral neck BMD.

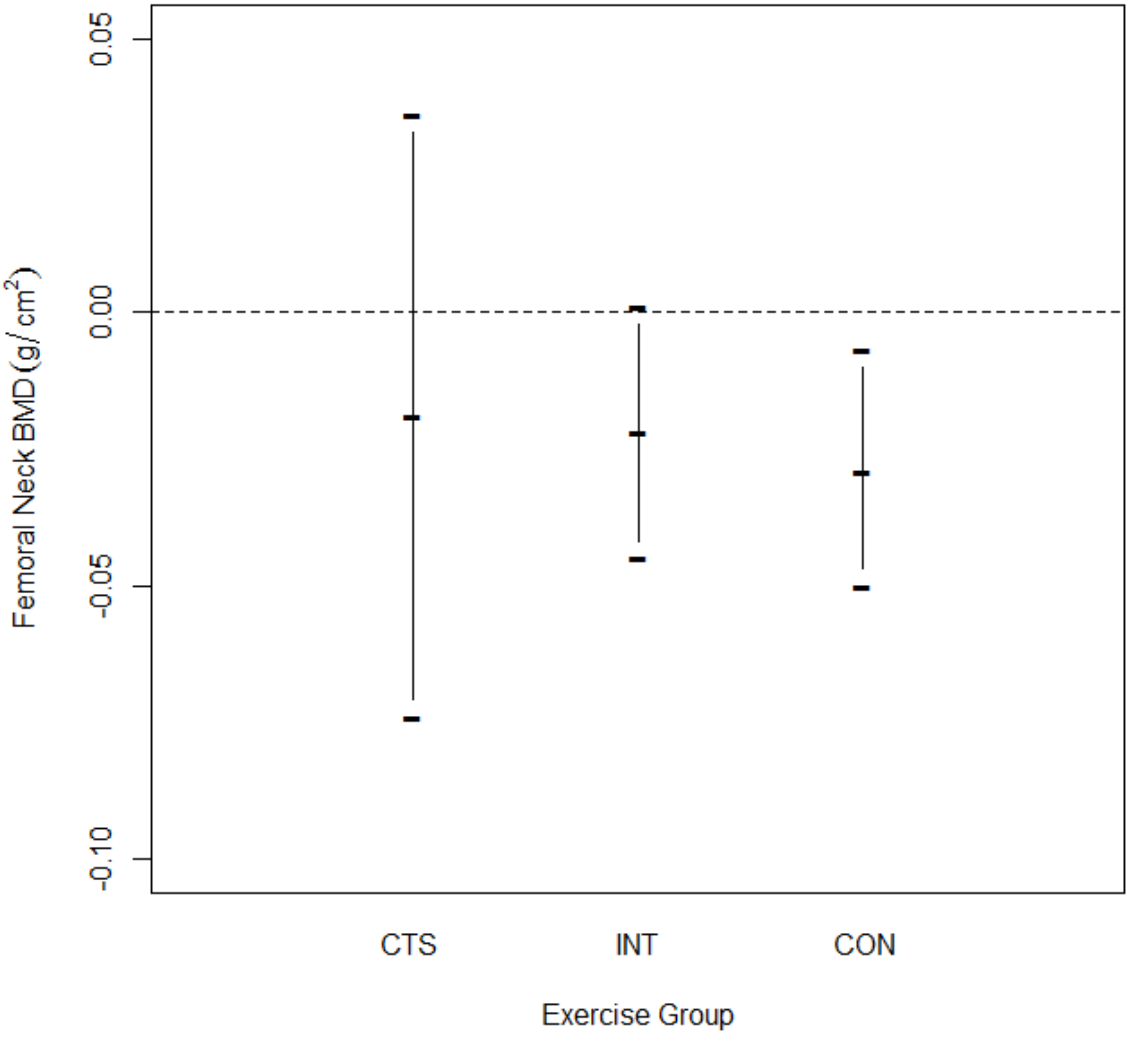


Fig. 6.3.5 Changes in femoral neck bone mineral density (BMD) after 12 months. Data are mean differences ± 95% CI; continuous group (CTS) n = 5, intermittent group (INT) n = 5, control group (CON) n = 7

6.3.6 Trochanter Bone Mineral Density

No statistical main effect on trochanter BMD was found for group or time (P = 0.530; P = 0.091), the interaction term was also not significant (P = 0.090) (Table

6.3.8).

6.3.7 Femoral Neck Bone Mineral Content

No statistical main effect on femoral neck BMC was found for group ($P = 0.426$) but a statistically significant effect for time was found showing a loss in BMC over the 12 months across all groups ($P = 0.038$), the interaction term was not significant ($P = 0.652$) (Table 6.3.8). The CTS group experienced an unclear within-group change in femoral neck BMC over 12 months with no statistically significant difference (mean difference (g) = -0.18 [95%CI: -0.65 to 0.29]), (% difference = -4.0 [95%CI: -13.6 to 6.8]), (d = -0.25 [95%CI: -0.92 to 0.41]), 58% possibly small; $P = 0.950$). The INT group experienced a very likely trivial within-group change in femoral neck BMC over 12 months with no statistically significant difference (mean difference (g) = -0.09 [95%CI: -0.19 to 0.02]), (% difference = -1.9 [95%CI: -4.1 to 0.3]), (d = -0.11 [95%CI: -0.23 to 0.01]), 95% very likely trivial; $P = 0.220$). The CON group experienced an unclear within-group change in femoral neck BMC over 12 months with no statistically significant difference (mean difference (g) = -0.07 [95%CI: -0.18 to 0.04]), (% difference = -1.5 [95%CI: -3.6 to 0.7]), (d = -0.15 [95%CI: -0.38 to 0.08]), 68% possibly trivial; $P = 0.410$).

6.3.8 Hip Structural Analysis

There were no main statistical effects for group in any HSA variable over the 12 month intervention period (Table 6.3.8). There were also no main statistical effects for time and no statistically significant interaction terms for any HSA variable, during the 12 month intervention.

Table 6.3.8 Descriptive and inferential statistics for bone mineral density **(**BMD), bone mineral content (BMC) and hip structural analysis (HSA) parameters pre, mid and post 12 month exercise intervention

	Continuous (n =5)			Intermittent (n = 5)			Control (n = 7)			Cohen's *d* [95% confidence intervals] PRE – POST		
	Mean ± SD											
	PRE	**MID**	**POST**	**PRE**	**MID**	**POST**	**PRE**	**MID**	**POST**	**CTS - CON**	**INT - CON**	**CTS - INT**
BMD												
Lumbar Spine L1-L4 (g/cm^2)	1.105 ± 0.168	1.112 ± 0.165	1.105 ± 0.179	1.061 ± 0.095	1.044 ± 0.090	1.028 ± 0.115	1.117 ± 0.116	1.112 ± 0.125	1.088 ± 0.118	*d* = 0.16 [-0.04 to 0.37], 67% trivial; *P* = 0.085	*d* = -0.05 [-0.47 to 0.37], 73% trivial; *P* = 0.766	*d* = 0.19 [-0.13 to 0.51], 51 % trivial; *P* = 0.189
Femoral Neck (g/cm^2)	0.936 ± 0.075	0.922 ± 0.088	0.916 ± 0.092	0.889 ± 0.087	0.875 ± 0.081	0.867 ± 0.074	0.959 ± 0.049	0.944 ± 0.038	0.930 ± 0.043	*d* = 0.10 [-0.61 to 0.80], 47% trivial; *P* = 0.743	*d* = 0.06 [-0.21 to 0.33]), 83% trivial; *P* = 0.607	*d* = 0.01 [-0.46 to 0.47], 68% trivial; *P* = 0.965
Trochanter (g/cm^2)	0.801 ± 0.106	0.820 ± 0.094	0.792 ± 0.109	0.722 ± 0.141	0.735 ± 0.114	0.743 ± 0.121	0.789 ± 0.083	0.791 ± 0.080	0.787 ± 0.075	*d* = -0.08 [-0.28 to 0.12], 89% trivial; *P* = 0.459	*d* = 0.18 [-0.10 to 0.45], 57% trivial; *P* = 0.104	*d* = -0.20 [-0.43 to 0.03], 51% trivial: *P* = 0.072
BMC												
Femoral Neck (g)	4.68 ± 0.52	4.51 ± 0.60	4.50 ± 0.57	4.32 ± 0.56	4.26 ± 0.52	4.23 ± 0.53	4.67 ± 0.36	4.64 ± 0.28	4.59 ± 0.28	*d* = -0.22 [-1.15 to 0.71], 52% negative; *P* = 0.569	*d* = -0.04 [-0.23 to 0.16], 94% trivial; *P* = 0.825	*d* = -0.12 [-0.75 to 0.51], 52% trivial; *P* = 0.614
HSA												
CSA (mm^2)	140.8 ± 17.8	140.8 ± 18.5	140.4 ± 18.7	133.6 ± 18.0	131.6 ± 15.7	133.8 ± 19.6	144.9 ± 9.9	144.4 ± 7.9	144.7 ± 9.1	*d* = -0.02 [-0.41 to 0.36], 73% trivial; *P* = 0.933	*d* = 0.00 [-0.47 to 0.47], 64% trivial; *P* = 0.934	*d* = -0.02 [-0.38 to 0.34], 78% trivial; *P* = 0.868
Section Modulus (mm^3)	621.7 ± 108.2	602.7 ± 120.7	613.4 ± 96.9	537.6 ± 93.5	557.3 ± 94.3	558.3 ± 95.0	625.5 ± 49.5	627.7 ± 40.4	627.9 ± 37.7	*d* = -0.10 [-0.84 to 0.64], 46% trivial; *P* = 0.706	*d* = 0.18 [-0.26 to 0.62], 50% trivial; *P* = 0.397	*d* = -0.20 [-0.68 to 0.29], 49% negative; *P* = 0.370

Buckling Ratio	3.7 ± 0.8	4.1 ± 1.1	3.7 ± 0.9	3.1 ± 1.8	3.7 ± 0.9	3.8 ± 1.5	3.3 ± 1.1	3.2 ± 1.0	3.6 ± 1.0	*d* = -0.26 [-1.16 to 0.64], 56% negative; *P* = 0.601	*d* = 0.34 [-0.72 to 1.39], 62% positive; *P* = 0.550	*d* = -0.50 [-1.50 to 0.51], 77% negative; *P* = 0.292
Strength Index	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.4 ± 0.2	1.3 ± 0.3	1.5 ± 0.3	1.4 ± 0.3	1.6 ± 0.4	1.5 ± 0.4	*d* = -0.18 [-0.84 to 0.49], 47% negative; *P* = 0.356	*d* = -0.08 [-0.99 to 0.82], 39% negative; *P* = 0.744	*d* = -0.12 [-1.06 to 0.81], 42% negative; *P* = 0.560
CSMI (mm^4)	10512.4 ± 2285.7	10124.4 ± 2514.4	10287.0 ± 2282.3	9066.2 ± 2000.8	9388.4 ± 2187.8	9406.0 ± 2312.0	10455.0 ± 1284.4	10604.0 ± 1078.5	10652.3 ± 966.3	*d* = -0.21 [-0.80 to 0.37], 52% negative; *P* = 0.390	*d* = 0.04 [-0.37 to 0.46], 69% trivial; *P* = 0.780	*d* = -0.18 [-0.59 to 0.24], 52% trivial; *P* = 0.334
Minimum Neck Width (mm)	31.1 ± 1.4	30.9 ± 1.8	30.8 ± 1.4	29.9 ± 2.0	30.0 ± 2.0	29.7 ± 1.9	30.5 ± 1.0	30.7 ± 1.0	30.7 ± 1.2	*d* = -0.36 [-0.86 to 0.15], 75% negative; *P* = 0.131	*d* = -0.20 [-0.56 to 0.15], 51% negative; *P* = 0.205	*d* = -0.06 [-0.27 to 0.16], 91% trivial; *P* = 0.518

6.3.9 Maximum Voluntary Isometric Torque

No statistical main effect on MVIC was found for group (P = 0.426), but a statistically significant main effect for time was found indicating higher MVIC values post intervention (P = 0.038), the interaction term was not significant (P = 0.652). The CTS group experienced a possibly small within-group improvement in MVIC over 12 months, although this was not statistically significant (mean difference (Nm) = 12.9 [95%CI: -6.8 to 32.6]), (% difference = 9.8 [95%CI: -3.6 to 25.0]), (d = 0.28 [95%CI: -0.11 to 0.66], 70% possibly small; P = 0.350) (Table 6.3.10). The INT group experienced a likely trivial within-group change in MVIC over 12 months (mean difference (Nm) = 3.1 [95%CI: -3.2 to 9.5]), (% difference = 2.7 [95%CI: -2.3 to 7.8]), (d = 0.08 [95%CI: -0.07 to 0.24], 94% likely trivial; P = 0.640). The CON group experienced an unclear effect on MVIC over 12 months (mean difference (Nm) = 4.0 [95%CI: -6.1 to 14.1]), (% difference = 2.6 [95%CI: -6.6 to 12.7]), (d = 0.20 [95%CI: -0.51 to 0.91], 50% possibly small; P = 0.600).

6.3.10 Rate of Torque Development

RTD50

For RTD50 over 12 months, there was no main effect of group (P = 0.426) but there was a main effect for time showing increased RTD50 (P = 0.038), the

interaction term was not significant ($P = 0.652$). The CTS group had a likely small within-group improvement in RTD50 although this was not significant (mean difference (Nm) = 1.7 [95%CI: -0.9 to 4.2]), (% difference = 45.0 [95%CI: -3.5 to 117.7]), ($d = 0.44$ [95%CI: -0.04 to 0.91], 92% positive; $P = 0.370$). The INT group had an unclear effect on RTD50 (mean difference (Nm) = 2.6 [95%CI: -4.9 to 10.1]), (% difference = 35.3 [95%CI: -39.0 to 200.2]), ($d = 0.48$ [95%CI: -0.79 to 1.76], 73% positive; $P = 0.630$). The CON group had a likely small within-group improvement in RFD50 although this was not significant (mean difference (Nm) = 1.8 [95%CI: -0.2 to 3.7]), (% difference = 28.6 [95%CI: -4.1 to 72.4), ($d = 0.51$ [95%CI: -0.09 to 1.11], 89% positive; $P = 0.230$).

RTD100

For RTD100 over 12 months, there was no main effect of group ($P = 0.426$) but there was a main effect for time showing increased RTD100 ($P = 0.038$), the interaction term was not significant ($P = 0.652$).

The CTS group had an unclear within-group effect on RFD100 (mean difference (Nm) = 8.1 [95%CI: -12.4 to 28.6]), (% difference = 18.6 [95%CI: -18.9 to 73.4]), ($d = 0.36$ [95%CI: -0.45 to 1.17], 72% positive; $P = 0.500$). The INT group had a possibly positive within-group effect on RFD100 although this was not statistically significant (mean difference (Nm) = 4.1 [95%CI: -3.9 to 12.1), (% difference = 7.0 [95%CI: -6.3 to 22.1]), ($d = 0.19$ [95%CI: -0.19 to 0.57], 50% trivial; $P = 0.610$). The CON group had an unclear effect on RFD100 (mean

difference (Nm) = 3.1 [95%CI: -3.0 to 9.1]), (% difference = 4.9 [95%CI: -10.0 to 22.3]), (*d* = 0.19 [95%CI: -0.43 to 0.82], 49% positive; *P* = 0.960).

RTD150

For RTD150 over 12 months, there was no main effect of group (*P* = 0.426) but there was a main effect for time showing increased RTD150 (*P* = 0.038), the interaction term was not significant (*P* = 0.652).

The CTS group had an unclear within-group effect on RTD150 (mean difference (Nm) = 3.9 [95%CI: -13.4 to 21.2]), (% difference = 6.5 [95%CI: -16.0 to 35.1), (*d* = 0.15 [95%CI: -0.42 to 0.72], 52% trivial; *P* = 0.910). The INT group had a likely trivial within-group effect on RFD150 (mean difference (Nm) = 2.1 [95%CI: -3.8 to 8.1]), (% difference = 2.2 [95%CI: -5.4 to 10.4]), (*d* = 0.07 [95%CI: -0.19 to 0.33], 87% trivial; *P* = 0.890). The CON group had an unclear within-group effect on RFD150 (mean difference (Nm) = 2.0 [95%CI: -5.0 to 9.0]), (% difference = 1.1 [95%CI: -11.6 to 15.6]), (*d* = 0.05 [95%CI: -0.53 to 0.62], 60% trivial; *P* = 0.999).

The mean difference in investigator-determined force onset error was 0.7 ± 2.0 ms.

Table 6.3.10 Descriptive and inferential statistics for rate of torque development (RTD) at 50, 100 and 150 ms from force onset and maximum voluntary isometric contraction (MVIC) (Nm) parameters pre, mid and post 12 month exercise intervention

	Continuous (n = 5)			Intermittent (n = 5)			Control (n = 6)					
	Mean Torque (Nm) ± SD									Cohen's *d* [95% confidence intervals] PRE - POST		
	PRE	**MID**	**POST**	**PRE**	**MID**	**POST**	**PRE**	**MID**	**POST**	**CTS - CON**	**INT - CON**	**CTS - INT**
RTD												
50 ms	3.8 ± 1.3	7.2 ± 3.8	5.6 ± 2.2	6.7 ± 2.8	5.3 ± 0.8	9.3 ± 3.9	6.1 ± 2.2	5.2 ± 2.2	7.9 ± 2.8	*d* = 0.17 [-0.35 to 0.69], 49% likely trivial; *P* = 0.433	*d* = 0.10 [95%CI: -1.35 to 1.54], 43% positive; *P* = 0.861	*d* = 0.10 [95%CI: -1.10 to 1.29], 40% positive; *P* = 0.810
100 ms	39.2 ± 10.5	45.7 ± 14.4	47.4 ± 15.2	48.5 ± 10.6	48.4 ± 9.5	52.6 ± 14.3	44.0 ± 7.8	41.2 ± 8.3	47.1 ± 11.3	*d* = 0.36 [-0.71 to 1.43], 65% positive; *P* = 0.502	*d* = 0.07 [-0.54 to 0.68], 53% trivial; *P* = 0.760	*d* = 0.26 [-0.77 to 1.29], 57% positive; *P* = 0.595
150 ms	64.5 ± 16.5	68.2 ± 20.6	68.4 ± 16.7	75.1 ± 15.3	77.4 ± 13.9	77.3 ± 17.7	69.3 ± 11.7	65.7 ± 12.2	71.3 ± 16.3	*d* = 0.17 [-0.57 to 0.92], 46% positive; *P* = 0.771	*d* = 0.04 [-0.51 to 0.60], 59% trivial; *P* = 0.972	*d* = 0.12 [-0.61 to 0.85], 49% trivial; *P* = 0.778
MVIC	120.9 ± 29.1	125.2 ± 34.8	133.7 ± 38.6	116.7 ± 26.1	118.5 ± 25.8	119.9 ± 27.6	117.2 ± 11.9	113.2 ± 15.9	118.9 ± 18.1	*d* = 0.31 [-0.34 to 0.95], 65% positive; *P* = 0.312	*d* = 0.00 [-0.46 to 0.46], 66% trivial; *P* = 0.856	*d* = 0.22 [-0.20 to 0.64], 54% positive; *P* = 0.250

6.4 Discussion

6.4.1 Main Findings

The main findings showed that there appeared to be no beneficial effects of either CTS or INT exercise programmes on reducing postmenopausal BMD loss in healthy early postmenopausal women. However, this study was underpowered and it is not possible to appropriately determine the effect of CTS or INT exercise on postmenopausal BMD loss. The CTS and INT groups appeared to maintain lumbar spine and femoral neck BMD levels whereas the control group experienced a statistically significant loss in both lumbar spine BMD (% difference = -2.7 [95%CI: -3.9 to -1.4]) and femoral neck BMD (% difference = -3.0% [95%CI: -5.1 to -0.8]). When comparing the magnitude of change between groups however, all findings were either trivial or unclear. Interestingly, the percentage of CON participants experiencing clinically meaningful reductions in both lumbar spine and femoral neck BMD was almost three times higher than that of the CTS and INT groups. The overall group statistics however, were not statistically significant and would not advocate the use of CTS or INT exercise for the reduction of postmenopausal BMD loss when compared to a CON group. The CON group displayed a 2.4 times higher level of postmenopausal bone loss when compared to a longitudinal study involving 47 to 63 year old women

(Shipman et al, 1999), this may be due to participants being in closer proximity to the menopause where rates of bone loss are known to be higher (Greendale et al, 2012). The present study showed comparable lumbar spine postmenopausal BMD loss when compared to a similar population of early postmenopausal women (Finkelstein et al, 2008). Our study supports the rapid rates of BMD loss that have been found in close proximity to the menopause.

Of the participants that completed the exercise protocol (5 CTS group, 5 INT group), the level of adherence to the sessions was excellent (98.2 ± 5.9% and 87.3 ± 8.7%). However, the high drop out rate and the high level of exclusion during the final analysis due to insufficient adherence, raises some questions over the sustainability of a countermovement jumping based programme for early postmenopausal women. The low sample size, which could be due to the initial under recruitment as a result of the narrow inclusion range of 1-5 years postmenopausal, makes any findings less meaningful and less applicable to the wider population. It appeared that some of the more active participants undertook extra exercise training during the study, which consequently meant elimination from the analysis, if the exercise programme were more demanding, it is possible that these participants would have maintained their interest longer and not sought other forms of training. Despite the frequent checks, future programmes may benefit from individual or group training sessions with an instructor, where adherence can be objectively accounted for by the research team and a group

environment could potentially increase quality of life and bolster adherence (Yorks et al, 2017).

Although high impact interventions have stimulated trochanter BMD adaptations in the absence of lumbar spine and femoral neck adaptations (Bassey and Ramsdale, 1994; Winters et al, 2000; Marques et al, 2011b), the current findings would not support the use of CTS or INT CMJ exercise for the reduction of postmenopausal BMD loss. The previous studies that have found trochanter BMD adaptations have either used a much younger population of premenopausal women or used a higher volume exercise programme with mixed loading exercises in older women (> 60 years) (Marques et al, 2011b).

Unsurprisingly, the HSA variables also showed no beneficial effect of either CTS or INT exercise when compared to the CON group. It is important to note that these measures have been reported to be highly variable and are not used clinically (Broy et al, 2015), which could potentially mean that any changes that might have arisen during the intervention could have been undetectable when considering the relatively high variance that commonly occurs in these measures (Khoo et al, 2005).

The main effect for time on RTD data could be due to the familiarisation effect of using the isovelocity dynamometer, particularly as no notable within-group improvements were found at any of the measured timepoints (De Caravalho Froufe Andrade et al, 2013). Errors in force onset determination were comparable

to previous research using the same method (Tillin et al, 2010).

The current findings are supported by previous studies that also found no effect of a countermovement jumping programme on postmenopausal BMD loss (Bassey et al, 1998; Sugiyama et al, 2002; Newstead et al, 2004). This could possibly be the result of decreased oestrogen bioavailability, which increases bone demineralisation (Cauley, 2015). However, a variety of different exercise programmes have found positive bone adaptations in oestrogen depleted postmenopausal women (Martyn-St James and Carroll, 2006b; Maddalozzo et al, 2007; Martyn-St James and Carroll, 2009; Howe et al, 2011; Marques et al, 2012; Xu et al, 2016). Furthermore, with a known osteogenic exercise stimulus in premenopausal women, the addition of oestrogen in the form of HRT for a population of postmenopausal women has not had any effect on BMD status, which suggests the influence of factors other than oestrogen for the blunted BMD response (Bassey et al, 1998). Oestrogen depletion may well be a contributing factor to the none-response in BMD to the CTS and INT programmes, though there may be more than one cause of this phenomenon, which warrants further investigation into the decreased mechanosensitivity that occurs in postmenopausal women.

With no statistically meaningful difference in BMD, BMC, HSA, MVIC or RTD variables between the CTS, INT and CON groups after 12 months of intervention it is unclear if the exercise programmes have had any beneficial effects on

musculoskeletal parameters. Therefore, the question of whether intermittent exercise could provide a greater osteogenic stimulus than continuous exercise in postmenopausal women as has been previously demonstrated in animals unfortunately remains unanswered in this case (Robling et al, 2001).

6.4.2 Strengths and Limitations

These findings could be due to a number of reasons. The main limitation in this study is the low sample size, which has reduced the likelihood of detecting small changes in BMD parameters. The study was powered anticipating that 60 volunteers would complete the intervention, unfortunately only 17 remained in this case. The reported benefits of exercise for reducing postmenopausal bone loss at the lumbar spine and femoral neck have previously been small and have ranged from 0.005 to 0.025 g/cm^2 (Martyn-St James and Carroll, 2009), similarly, the benefits of an intervention over that of a control group have been reported to be ~2% (Kelley et al, 2002). To detect a change of this relatively small magnitude would potentially require a larger number of participants than are available in the current study. It is important to note that a small 5.4% change in DXA derived BMD can equate to a 64% increase in force tolerance (Robling et al, 2002), which could mean that even minimal changes in BMD could have large beneficial effects in increasing bone strength. It is also possible that the mechanical strength of the bones that were measured could have improved without any change in the

BMD and that the HSA was not sensitive enough to detect any potential changes to bone architecture (Felsenberg and Boonen, 2005). The CTS group showed a 3.2% improvement in lumbar spine BMD when compared to the CON group and a 2.6% improvement in lumbar spine BMD when compared to the INT group although these changes were not statistically significant. This is a trend that may require further investigation with a larger population that could have larger implications for the mechanical strength of the bone sites measured.

DXA scans are limited in their ability to determine any geometric adaptations to the bone. DXA BMD measures account for 60 - 70% of the bone strength parameters (Ammann and Rizzoli, 2003). It is consequently possible that some level of bone adaptation has occurred but was undetectable with the current methods. DXA and the addition of MRI or CT scans have been advocated for combined use in determining bone strength parameters at the distal radius, lumbar spine and femoral neck regions as opposed to purely DXA alone due to the incorporation of the proportions of cortical and trabecular bone along with the added knowledge of the geometrical shape of the bone (Krug et al, 2010; Baum et al, 2013; Johnston et al, 2014; Bandirali et al, 2015).

In addition to the low sample size, the exercise interventions may not have been sufficiently stressful enough to stimulate a statistical reduction in postmenopausal bone loss although this would have been difficult to determine. The countermovement jump exercise for the current population has shown to generate

accelerations well in excess of the osteogenic threshold of 4.9 g (Vainionpaa et al, 2007) and acceleration gradient of 1000 $m \cdot s^{-3}$ (Heikkinen et al, 2007) (chapter 5). It is difficult to determine the intensity of the home based programme when participants are left unsupervised and it is possible that at times, exercise intensity could have been reduced and therefore may have reduced the bone stimulus (i.e. submaximal countermovement jumping).

The exercise programmes were designed to minimise the time commitment (< 8 minutes, 3 times per week), minimise the need for exercise equipment (none required), minimise the need for travel (home-based) and maximise the bone adaptations to the relatively brief loading bouts (30 CMJs). The programme was intended to be easy to complete and to be accessible to all. It is obvious from the high dropout rate that this programme was not easy to adhere to however. Some of the more successful interventions with the current population have involved either resistance exercise or mixed loading protocols and could indicate that adherence is improved with an increased variety of exercise (Martyn-St James and Carroll, 2009; Howe et al, 2011; Xu et al, 2016). Anecdotally, participants particularly in the INT group sometimes expressed a sense of boredom during the longer rest intervals. This could have been a contributing factor to the dropout rate. Unfortunately, despite attempting to get activity monitor data for each of the participants, due to data corruption problems with activity monitors, only exercise logs were used to record adherence. Upon the return of the devices following the

first seven days of recording, the devices would not register in their charging unit, which prevented the retrieval of any data. When returned to the manufacturer, the delay was such that weeks two to four of monitoring were missed. In addition, the manufacturer failed to return any of the activity data. The self-reported activity logs that were used instead, can be more subjective than physical activity monitors.

A large proportion of the participants were serum 25-hydroxy vitamin D (25 (OH) D) deficient at baseline. Seasonal fluctuations in serum 25-hydroxy vitamin D (25 (OH) D) status could have seen further reductions in the bioavailability, which could have blunted the osteogenic potential of the exercise programmes through a reduction in calcium absorption leading to reduced bone mineral accrual (Morris et al, 2010). The average T-score of the femoral neck for an age-matched population to the current study is -1.0 (Looker et al, 1998). The participants for the current study had noticeably higher T-scores at baseline of -0.4 ± 0.6, -0.7 ± 0.7 and -0.2 ± 0.4 for the CTS, INT and CON groups respectively. For that reason, it is possible that for participants with a higher level of BMD, a greater level of mechanical stimulation is needed to initiate an adaptive response. The current exercise intervention may have shown a statistically detectable benefit for individuals with lower initial T-scores and may have reduced postmenopausal BMD loss. The current exercise volume was relatively low at 30 CMJ, performed three times per week. Despite the fact that many more than 36

loading cycles has shown little extra benefit for BMD adaptation in animal populations, it is possible that human bone requires a greater number of loading cycles (Rubin and Lanyon, 1984; Umemura et al, 1997). This necessitates further research to establish potential thresholds for the adaptation of human bone.

6.4.3 Conclusions

To conclude, CTS and INT CMJ exercise have an unclear effect on reducing postmenopausal BMD loss at the lumbar spine and femoral neck. With the number of participants that completed the intervention, it was not possible to determine if there was an effect of the exercise interventions.

7. General Discussion

There is a growing rise in osteoporosis and osteoporotic fracture incidence worldwide (Hernlund et al, 2013; Ballane et al, 2017; Center, 2017). This problem is highly likely to get progressively worse due to our ageing population and will increase the demand for future medical resources and care (Svedbom et al, 2013). Postmenopausal women are at a greater risk of developing osteoporosis and experiencing a subsequent higher fracture risk due to the greater level of postmenopausal bone loss that is attributed to the loss of oestrogen levels (Shipman et al, 1999; Kanis et al, 2001; Kanis et al, 2009). The normal level of BMD loss in postmenopausal women is 1 – 2% per year (Finkelstein et al, 2008), however, appropriately designed exercise interventions involving high mechanical loading stimuli have shown that this postmenopausal BMD loss can be somewhat attenuated, therefore leading to BMD maintenance throughout the ageing process (Martyn-St James and Carrol, 2006b, Martyn-St James and Carroll, 2009; Howe et al, 2011; Kemmler et al, 2015). There still remains some debate over the most beneficial form of exercise however (Xu et al, 2016).

Studies with animals have shown that intermittent mechanical loading can provide a more potent osteogenic stimulus than continuous mechanical loading (Robling et al, 2001; Srinivasan et al, 2007). The greater stimulus from intermittent loading is thought to occur due to the restoration of bone mechanosensitivity to repeated loading cycles, as with continuous mechanical

loading, bone can become desensitised to the loading stimulus (Umemura et al, 1997). This phenomenon could potentially benefit human populations and assist in providing more effective exercise programmes, particularly postmenopausal women that are in a state of rapid BMD loss. However, the effects of continuous and intermittent exercise have never been properly examined in human populations in a controlled environment.

Therefore, this thesis sought to examine potentially beneficial forms of exercise for postmenopausal women in terms of mechanical loading characteristics. In addition, the effects of continuous and intermittent exercise were investigated to establish whether the greater bone adaptations that have been discovered as a result of intermittent exercise in animal studies could potentially present in a population of postmenopausal women.

The ensuing subsections recapitulate the main outcomes of the four investigations that were accomplished as part of the present thesis.

7.1 Chapter 3, The effects of continuous and intermittent exercise upon changes in bone mineral density in humans: a systematic review

The review aimed to evaluate the existing literature in the areas of the effects of continuous and intermittent exercise on human bone mineral density. Surprisingly few studies were adequately controlled for the exercise intervention

in terms of the exercise protocol volume and sufficiently quantified the exercise to rest intervals. The review discovered only highlighted seven continuous exercise studies and eight intermittent studies that met the stringent criteria. Comparisons were complicated due to the inconsistencies in the reporting standards of BMD throughout the interventions. Absolute changes or absolute pre and post values are preferred as the ISCD reporting standard for BMD (Baim et al, 2015). Three intermittent interventions showed statistical benefits of the exercise programme on BMD at the femoral neck and distal arm sites (Allison et al, 2013; Bailey and Brooke-Wavell, 2010; Bhatia et al, 2015) whereas one continuous exercise study found a statistical benefit of the exercise programme on BMD at the calcaneus site (Brooke-Wavell et al, 1997). It was not possible to determine if continuous or intermittent exercise would be superior in stimulating improvements in BMD from the evidence available. The large discrepancies in the types of exercises used have further vetoed continuous and intermittent comparisons. The review also showed that seven out of fifteen studies failed to provide any measure of precision error for the chosen method of BMD measurement. BMD reporting standards did not follow current recommendations however (Baim et al, 2015). The review exposed knowledge gaps in the current literature and showed that there is a need to investigate the effects of continuous and intermittent exercise in a load matched randomised control trial in a human population. Using the areas that were highlighted in the review as requiring investigation, the effects of continuous and intermittent exercise were

investigated in chapter 6.

7.2 Chapter 4, Tibial impacts and muscle activation during walking, jogging and running when performed overground, on a motorised and non-motorised treadmill

In order to examine the effect of continuous and intermittent exercise in human populations, firstly a form of exercise that can be easily manipulated to incorporate both a continuous and intermittent condition whilst keeping exercise volume constant must be identified. Walking, jogging and running on a NMT (Woodway Force 2.0. Woodway, Weil an Rhein, Germany) seemed to provide an attractive and viable option in this case. This type of freely moveable treadmill is well known for enabling the performance of intermittent exercise protocols (Brown et al, 2007; Highton et al, 2012; Aldous et al, 2014; Tofari et al, 2015). The device also permits the quantification of instantaneous GRF measurement throughout an exercise protocol, which would quantify mechanical loading characteristics and allow for the control of these variables during continuous and intermittent protocols. The NMT loading parameters have not been well established or compared to that of overground or motorosed treadmill conditions previously. This study highlighted that there are large reductions in peak acceleration during NMT locomotion when compared to the overground and motorised treadmill equivalents across all walking (δ = -0.56 [95%CI: -0.81 to -

0.13], P = 0.004; , δ = -0.58 [95%CI: -0.83 to -0.15], P = 0.002), jogging (δ = -0.64 [95%CI: -0.85 to -0.23], P = 0.001; δ = -0.78 [95%CI: -0.92 to -0.45], P = 0.001) and running conditions (δ = -0.51 [95%CI: -0.77 to -0.11], P = 0.004; δ = -0.51 [95%CI: -0.78 to -0.01], P = 0.001). The peak acceleration did not consistently meet the osteogenic threshold of 4.9 g across participants (Vainionpaa et al, 2007), and ranged from 3.4 to 10.8 g, which would potentially reduce or prevent osteogenic adaptations from developing in some participants should this device be used for an intervention study. This is further emphasised when compared to the range of peak acceleration values from the overground and motorised treadmill conditions, which were 5.6 to 12.0 g and 5.8 to 12.3 g respectively. This would suggest that there is greater confidence that these conditions would initiate osteogenic adaptations as the 4.9 g threshold is consistently surpassed in all participants. It is also evident that the habitual muscular activation patterns are altered when using a NMT due to the small to very large increases in rectus femoris, semitendinosus and soleus EMG amplitude (d = 0.57 to 2.17; δ = 0.2 to 0.71). This indicates that for NMT locomotion, a higher level of muscular activity is created when using overground matched velocities. This raises questions as to whether an older population would experience difficulties completing NMT based continuous and intermittent running protocols and whether this would be an appropriate osteogenic form of exercise to perform. For the stimulation of BMD adaptation, it is clear that from these findings, other forms of exercise require evaluation for adequate

musculoskeletal loading characteristics in order to conduct an intervention study. Using the information presented in chapter 4, a range of exercises were then evaluated for their loading parameters in chapter 5, in order to establish an appropriately osteogenic form of mechanical loading that could be used for a population of early postmenopausal women.

7.3 Chapter 5, The osteogenic index of four common continuous and intermittent exercises used in osteoporosis prevention in an at-risk population

This study aimed to determine a suitably osteogenic form of exercise to be used in a successive intervention study for postmenopausal women. It was important that the exercise that was selected for the intervention was consistent in terms of mechanical loading parameters when performed either continuously or intermittently. Countermovement jumps and box drops gave the greatest loading characteristics in terms of; osteogenic index, peak acceleration, acceleration gradient and rectus femoris EMG amplitude. These findings would advocate the use of both countermovement jumping and box drops for an intervention study in postmenopausal women as the osteogenic thresholds for peak acceleration (4.9 g) and acceleration gradient (1000 $m \cdot s^{-3}$) were easily met during both continuous (10.7 ± 4.8 g for CMJ; 9.6 ± 4.1 g for BD) and intermittent conditions (10.0 ± 5.0 g for CMJ; 9.5 ± 4.0 g for BD) (Vainionpaa et al, 2007; Heikkinen et al, 2007).

There was also no statistical difference between the continuous and intermittent conditions of either countermovement jumps or box drops for peak acceleration or acceleration gradient. This showed that there could be some consistency in loading parameters for the evaluation of continuous and intermittent exercise with CMJ and BD exercises.

Of interest is the finding that heel drop exercise created an osteogenic level of loading for the majority of participants (7.3 ± 3.8 g and 8.6 ± 4.4 g for continuous and intermittent conditions). This could potentially benefit populations that have difficulty in performing CMJ or BD exercises and generate bone adaptation from a relatively simple action that can be performed anywhere. This would require further investigation however.

Countermovement jump and box drop conditions produced large increases in rectus femoris EMG amplitude when compared to heel drop and stamp conditions, large increases in semitendinosus EMG amplitude when compared to heel drop conditions and moderate-large increases in tibialis anterior EMG amplitude when compared to heel drop conditions across continuous and intermittent trials. Although there is currently no evidence linking EMG amplitude to greater bone adaptations, muscle forces have been suggested to be the greatest stressor on bone tissue (Frost, 2003; Robling, 2009), with femoral neck BMD adaptation having been suggested to be proportional to GRF (Kohrt et al, 1997). This suggests greater musculoskeletal loading in these conditions

and greater EMG amplitude has been linked with higher compressive forces for muscles acting on the femur (Bassey et al, 1997). Previous research has estimated internal muscle forces equivalent to 7 bodyweights during running and hopping tasks, further emphasising the effect of muscle action on bone tissue (Gerus et al, 2012). Although muscles provide a great stimulus for bone adaptation and the relationship between EMG amplitude and force output is linear (Andrade and Andrade, 2012), the evidence for EMG amplitude and bone adaptation is yet to be quantified and currently requires investigation before any future programmes seek to maximise EMG amplitude in order to stimulate increases in BMD. Countermovement jumping and box drops certainly generate adequate loading conditions for bone adaptation and there is the potential for these conditions to stimulate greater bone adaptations than the heel drop and stamp exercises. This would need to be assessed in an intervention study to investigate the longitudinal effects of these exercises however. Countermovement jumping has been advocated as an osteogenic exercise that generates sufficient stimulus and strain energy for bone adaptation (Martelli et al, 2014; Zhao et al, 2014). Countermovement jumping interventions have been used before in postmenopausal women and have shown no effect on BMD. However, one of these studies only lasted six months (Sugiyama et al, 2002), which has been suggested to be of an inadequate duration for DXA derived BMD changes to be detected (Kemmler and Engelke, 2004). Another countermovement jump intervention in postmenopausal women was conducted with participants that were

slightly later post menopause and the intervention was performed on mats, which could reduce the rate of strain required for BMD adaptations in this population (Bassey et al, 1998). Furthermore, a countermovement jump intervention study with postmenopausal women found no beneficial effect on BMD when compared to a control group, although neither groups experienced a reduction that exceeded the DXA least significant change value at any site and the control group experienced a slightly improved BMD in some areas, which was hypothesised to be due to extra calcium supplementation (Newstead et al, 2004). Further investigation is therefore warranted into countermovement jumping interventions in postmenopausal women. There is no requirement for additional equipment and can be performed anywhere, making it an ideal exercise for a home-based intervention. Intermittent countermovement jumping also has the potential for a greater osteogenic response than continuous countermovement jumping (Robling et al, 2001), although this requires evaluation. These findings would advocate the use of countermovement jumping for BMD maintenance in intervention studies and assessing the differences between continuous and intermittent mechanical loading on BMD in humans. As the countermovement jump provided the greatest osteogenic index, peak accelerations, acceleration gradients and muscular activation, it was used during a 12 month randomised control trial to investigate the effects of continuous and intermittent exercise on postmenopausal BMD loss in chapter 6.

7.4 Chapter 6, The effect of continuous and intermittent exercise on bone mineral density in postmenopausal women: a twelve-month randomised control trial

Continuous and intermittent exercise groups maintained lumbar spine and femoral neck BMD levels whereas the control group experienced a statistically significant loss in both lumbar spine (-2.7% [95%CI: -3.9 to -1.4]) and femoral neck (-3.0% [95%CI: -5.1 to -0.8]) BMD. When comparing the magnitude of change between exercise and control groups however, all findings were either trivial or unclear, which is likely due to the small sample size, despite the % difference when comparing the CTS group to both the INT and CON groups at the lumbar spine showing +3.2% and +2.6% respectively. These statistics were not statistically significant and would suggest that the use of solely CTS exercise may not reduce postmenopausal BMD loss. However, it is interesting to note that 20% of the CTS participants experienced clinically meaningful reductions in lumbar spine BMD whereas 60% of the INT participants and 57% of the CON participants experienced clinically meaningful reductions in lumbar spine BMD. For the femoral neck site, 20% of the CTS participants, 20% of the INT participants and 57% of the CON participants experienced clinically meaningful reductions in femoral neck BMD.

Despite this and most likely due to the small sample size, the current study found little evidence of an effect of either continuous exercise or intermittent exercise

on BMD when compared to the control group or even when comparing continuous to intermittent exercise. As the study was underpowered, it was not possible to determine if the exercise interventions had any effect.

It is clear that previous exercise programmes have stimulated BMD adaptations in postmenopausal women (Martyn-St James and Carroll, 2006b; Martyn-St James and Carroll, 2009; Howe et al, 2011; Xu et al, 2016), but a minimum exercise threshold for bone adaptation has not been established in human populations, as has been demonstrated in animals (Turner et al, 1994; Umemura et al, 1997). The current study may not have included a sufficient exercise volume for bone adaptations and with the blunted mechanosensitivity due to a reduced serum oestrogen level, older populations may require a greater level of mechanical stimulation to produce improvements in BMD (Klein-Nulend et al, 2015).

The large drop out number and the relatively poor adherence data would suggest that the current intervention is not conducive for maintaining the interest of participants. It is possible that even if the exercise was potentially osteogenic in this population, if the participants struggle to maintain interest and adherence then the exercise intervention becomes immediately less effective. The mixed loading approach may present a more interesting alternative to stimulate both BMD adaptations and also greater adherence in the current population (Xu et al, 2016).

One limitation of this study was that a known osteogenic stimulus for

postmenopausal women was not used. The exercise programmes should have been osteogenic in theory but in practice, this may have been different. It is very difficult to establish a single osteogenic stimulus for this population as many of the previous exercise programmes that have shown to benefit postmenopausal women have included a variety of different exercises (Howe et al, 2011). Very few interventions have used a single exercise that could have been manipulated to enable the evaluation of both continuous and intermittent exercise. It may be that one single form of exercise (e.g. countermovement jumping) does not give a sufficient stimulus on the musculoskeletal system to provide BMD benefits and that the reason that many of the studies with positive BMD adaptations include various exercises is that the variety of exercises and loading patterns are required for bone adaptation (Xu et al, 2016). There is not a current minimum threshold for bone adaptation in humans, therefore it is not possible to determine whether a threshold was reached with the current exercise programmes. The current findings support previous investigations evaluating countermovement jumping based protocols with postmenopausal women (Bassey et al, 1998; Sugiyama et al, 2002; Newstead et al, 2004; Martyn-St James and Carroll, 2009). The current intervention may have held a greater osteogenic potential for BMD adaptations if combined with resistance exercise in the form of a mixed loading programme (Martyn-St James and Carroll, 2009; Kemmler et al, 2015). This would complicate the design of future interventions aiming to establish the effects of continuous and intermittent exercise however.

Fatigue may have played a role in the reduced response to the current exercise protocol and may have reduced the jump height during repeated efforts, this could have reduced the magnitude of impacts experienced by the participants, although this would require further investigation.

The current intervention lasted 12 months in duration, which has been suggested as adequate for assessing BMD changes (Kemmler and Engelke, 2004). With such relatively small BMD changes present in similar investigations, the current intervention may require longer than 12 months to display detectable changes in BMD from DXA scans (Snow et al, 2000). If the current trends for the lumbar spine were maintained for a longer duration in the exercise groups (CTS: -0.1% [95%CI: -3.2 to 3.0]; INT: -3.2% [95%CI: -8.1 to 1.8]) for continuous and intermittent respectively) when compared to the control group (CON: -2.7% [-3.9 to -1.4]), then it is possible that this relationship may reach statistical significance at some point although this may well be limited by the number of participants.

8. Future Work

The current studies have provided information that may contribute to future projects involving the evaluation of continuous and intermittent exercise in human populations or when designing exercise programmes aimed at reducing postmenopausal BMD loss. A number of areas have been outlined for future research projects.

8.1 Exercises

The undertaking of a supervised exercise programme may help to minimise the risk of poor adherence and ensure that the exercise programme is completed at an adequate intensity throughout. This would agree with recent research that has shown a 59% dropout rate when transferring from a supervised exercise programme to an unsupervised exercise programme and of those that began the unsupervised programme, 66.6% failed to complete the programme (Carpenter and Gilleland, 2016). If the current study was supervised then more participants would have potentially completed the programme as previous research has shown improved attrition rates with supervised exercise programmes when compared with non-supervised exercise programmes (Gettman et al, 1983; Courneya et al, 2012). Any discrepancies in exercise intensity could have been eliminated if participants were observed or even measured with the use of accelerometry to

check that sufficient loading was being experienced as was anticipated with the current study and has been shown with previous literature (Ahola et al, 2010; Chahal et al, 2014).

A minimum loading intensity and loading volume threshold for bone adaptation in postmenopausal women have yet to be determined. Attempts have been made to find a minimal effective exercise dose for premenopausal women, but it is unclear if these suggestions transfer to postmenopausal populations (Vainionpaa et al, 2006; Jamsa et al, 2006; Heikkinen et al, 2007). Many exercise programmes have found beneficial effects on BMD but as with previous animal based research studies that have identified minimum effective loading intensities and loading volumes, human interventions have been less well controlled for these factors (Rubin and Lanyon, 1984; Turner et al, 1994; Umemura et al, 1997). Future projects should evaluate a range of different loading intensities and exercise volumes in postmenopausal women in order to quantify a minimum effective loading threshold in terms of intensity and volume and also an optimal loading threshold in terms of intensity and volume. These parameters could then provide a platform for further research comparing continuous and intermittent exercise on BMD adaptations.

There is the potential to use different exercise modes to evaluate the effects of continuous and intermittent exercise on BMD. Resistance training that specifically targets loading at the hip has been shown to reduce postmenopausal

BMD loss at the femoral neck (Howe et al, 2011), whereas reductions in lumbar spine postmenopausal BMD loss have also been found with resistance training exercise (Martyn-St James and Carroll, 2009). Also of potential benefit are mixed loading exercise programmes involving both high impact activity in combination with resistance training exercises (Kemmler et al, 2015; Watson et al, 2015; Xu et al, 2016). Musculoskeletal and finite-element model research has supported the use of resistance exercise for greater site-specific loading at the femoral neck (Martelli et al, 2014). Exercises involving maximal hip extension and knee flexion were found to show the greatest femoral neck loading parameters along with one-legged long jumps (Martelli et al, 2014). This would suggest that in order for optimal loading, near maximal resistance training must be undertaken to ensure that the femoral neck is appropriately stressed. Older men can also benefit from high impact and resistance exercise, it has been shown that one-legged hopping activity has effectively improved femoral neck BMD in older men when compared with controls, and therefore may have the potential to reduce postmenopausal BMD loss in women at the hip, if applied to this population (Allison et al, 2013). Combined high impact and resistance exercise has demonstrated preservative effect on BMD in older osteopenic men when compared to controls. It seems that whilst resistance training has had beneficial effects on femoral neck BMD, the addition of jumping exercise has stimulated lumbar spine BMD adaptations (Hinton et al, 2015). The use of heavy resistance exercise has been found to stimulate postmenopausal BMD adaptations whereas

lighter load resistance exercise has shown no BMD response (Kerr et al, 1996). It appears that maximal or near maximal resistance exercise (8 RM) is necessary for BMD adaptation in postmenopausal women, which has been supported by a number of studies involving higher intensity resistance exercise (Howe et al, 2011; Watson et al, 2015; Watson et al, 2017). In order to further investigate the effects of continuous and intermittent exercise on BMD adaptations in a population of postmenopausal women, it might be more effective to use these forms of activity instead of solely countermovement jumping as has been used in the current intervention.

8.2 Duration

The current intervention lasted 12 months, which has been suggested as a desirable duration for the investigation of postmenopausal BMD changes (Kemmler and Engelke, 2004; Nikander et al, 2010; Xu et al, 2016). Often, the greater the duration of the intervention, the stronger the statistical difference in BMD between the exercise and control groups (Engelke et al, 2006; Kemmler et al, 2015). Should this relationship apply to the current data set, it may have taken longer for the relatively small differences in BMD to reach statistical significance and may have required much longer than 12 months. Previous research with premenopausal women that has shown beneficial effects of exercise on BMD, has demonstrated that after an 18 month intervention when exercise has ceased, the

benefits for BMD are still present for the following five years when compared to a control group (Kontulainen et al, 2004). A similar exercise intervention with premenopausal women that showed improved trochanter BMD over 12 months when compared to a control group also showed that a six month detraining period caused the BMD adaptation to return to pre-intervention levels (Winters and Snow, 2000). The detraining effect has also been shown to return previously improved BMD levels to baseline levels with a 12 month walking and gymnastic training intervention and subsequent 12 month detraining period with osteoporotic postmenopausal women (Iwamoto et al, 2001). This evidence further strengthens the argument for a longer exercise intervention as there is potential for longer lasting benefits to BMD levels. As there were no apparent benefits of the current intervention, it is unlikely that there will be any differences between the intervention and control groups for any future follow up testing. Due to the low sample size, this would be unlikely to yield any interesting results.

8.3 Population

In order to determine the effects of continuous and intermittent exercise on BMD, it may prove useful to examine a known stimulus for BMD adaptation in a younger population of premenopausal women to assess for potential continuous and intermittent differences. A variety of prior osteogenic programmes could be manipulated to allow the continuous and intermittent comparisons from 10

countermovement jumps for 3 days per week (Kato et al, 2006) to 50 countermovement jumps for six days per week (Bassey et al, 1998) or 50 daily multidirectional hops (Bailey and Brooke-Wavell, 2010), as jumping type exercise has been shown to be an effective osteogenic stimulus for premenopausal women (Zhao et al, 2014). The same type of programme could be advocated for a population of young or older men as they have also shown to display a response to high impact exercise (Allison et al, 2013; Hinton et al, 2015). Another potential population to examine is those with osteoporosis, as exercise has been shown to maintain BMD in postmenopausal women with osteoporosis and the due to the low level of initial BMD in this population, the current exercise delivery may create a greater osteogenic stimulus (Iwamoto et al, 2001; Watson et al, 2015).

8.4 Measures

Future research would ideally use a range of bone measures to give a greater understanding of potential adaptations. DXA scans are inherently limited in the sense that only 2D results are returned whereas with pQCT for instance, a 3D bone image is returned from which the proportions of cortical and trabecular bone can be assessed along with the geometric adaptations that would contribute to bone strength and also muscular size adaptations (Polidoulis et al, 2012). MRI scans have been suggested to complement DXA scans and the use of both has been supported in favour of DXA alone due to the potential for 3D geometric

imaging and the determination of cortical and trabecular bone (Baum et al, 2013).

8.5 Lifestyle factors

High levels of dietary calcium have been recommended to optimise bone health in postmenopausal women (Compston et al, 2013). Calcium and serum 25-hydroxy vitamin D (25 (OH) D) have both been recommended for the reduction in fracture risk in populations at risk of inadequate intake (Harvey et al, 2017).

Vitamin D supplementation of 10 g/day is currently recommended in the UK to ensure serum 25-hydroxy vitamin D (25 (OH) D) concentration remains above 25 nmol/L (Public Health England, 2016). For future research in this area, it would seem necessary to supplement an intervention with both vitamin D3 and calcium to ensure that these are not limiting factors in the bone adaptation process. At baseline the current participants were mostly vitamin D replete but this was not re-measured throughout the year and has been known to be subject to seasonal variations. Supplementation could assist in the retention of optimal calcium and vitamin D levels.

9. Conclusions

The effects of continuous and intermittent exercise on mechanical bone remodelling in human populations is a largely unexplored area with the potential for identification of greater osteogenic exercise programmes.

Non-motorised treadmill locomotion is unlikely to stimulate substantial BMD adaptations due to the reduced loading characteristics that arise from perturbations in the habitual locomotion parameters. Overground and motorised treadmill running are likely to be osteogenic forms of exercise but do not easily permit the evaluation of continuous and intermittent exercise on BMD adaptations in a well-controlled intervention. Countermovement jumps and box drops provide a level of mechanical loading that is considered osteogenic and both forms of exercise are consistent during continuous and intermittent conditions when performed by postmenopausal women.

Continuous and intermittent countermovement jumping exercise when performed in a 12 month exercise intervention have little effect on reducing postmenopausal BMD loss and appear difficult for participants to adhere to. It was not possible to determine if the exercise interventions had any effect due to the very small sample size. Continuous and intermittent countermovement jumping exercise require further investigation in a much larger intervention study.

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11. Appendices

11.1 Appendix A - Search Strategy for “The effects of continuous and intermittent exercise upon changes in bone mineral density in humans: a systematic review”

PubMed – 1375 results

("continuous exercise" OR "intermittent exercise" OR "interval exercise" OR “Mechanical loading” OR “loading” OR “rest inserted exercise” OR “rest-inserted exercise” OR “rest inserted loading” OR “rest-inserted loading” OR “high-impact exercise” OR “high impact exercise” OR “impact exercise” OR "Exercise/classification"[Mesh] OR "Exercise/epidemiology"[Mesh] OR "Exercise/methods"[Mesh] OR "Exercise/physiology"[Mesh] OR "Exercise/therapeutic use"[Mesh] OR "Exercise/utilization"[Mesh] OR "Circuit-Based Exercise/methods"[Mesh] OR "Plyometric Exercise/methods"[Mesh] OR "Plyometric Exercise/therapeutic use"[Mesh] OR "Resistance Training/classification"[Mesh] OR "Resistance Training/methods"[Mesh] OR "Resistance Training/therapeutic use"[Mesh] OR "Exercise Movement Techniques/methods"[Mesh] OR "Exercise Movement Techniques/nursing"[Mesh] OR "Exercise Movement Techniques/therapeutic use"[Mesh] OR "Exercise Movement Techniques/therapy"[Mesh] OR "Exercise

Movement Techniques/utilization"[Mesh] OR "Exercise Therapy/classification"[Mesh] OR "Exercise Therapy/epidemiology"[Mesh] OR "Exercise Therapy/etiology"[Mesh] OR "Exercise Therapy/methods"[Mesh] OR "Exercise Therapy/nursing"[Mesh] OR "Exercise Therapy/therapeutic use"[Mesh] OR "Exercise Therapy/therapy"[Mesh] OR "Exercise Therapy/utilization"[Mesh] OR "Exercise Test/epidemiology"[Mesh] OR "Exercise Test/methods"[Mesh] OR "Exercise Test/nursing"[Mesh] OR "Exercise Test/therapeutic use"[Mesh] OR "Exercise Test/therapy"[Mesh] OR "Exercise Test/utilization"[Mesh])

AND

(“Bone mineral density” OR “Bone mineral content” OR “BMD” OR “BMC” OR "Bone and Bones/analysis"[Mesh] OR "Bone and Bones/anatomy and histology"[Mesh] OR "Bone and Bones/classification"[Mesh] OR "Bone and Bones/diagnosis"[Mesh] OR "Bone and Bones/epidemiology"[Mesh] OR "Bone and Bones/etiology"[Mesh] OR "Bone and Bones/metabolism"[Mesh] OR "Bone and Bones/microbiology"[Mesh] OR "Bone and Bones/physiology"[Mesh] OR "Bone and Bones/physiopathology"[Mesh] OR "Bone and Bones/radiography"[Mesh] OR "Bone and Bones/radionuclide imaging"[Mesh] OR "Bone and Bones/therapeutic use"[Mesh] OR "Bone and Bones/therapy"[Mesh] OR "Bone and Bones/ultrasonography"[Mesh] OR "Bone and Bones/ultrastructure"[Mesh] OR "Leg Bones/analysis"[Mesh] OR "Leg

Bones/anatomy and histology"[Mesh] OR "Leg Bones/diagnosis"[Mesh] OR "Leg Bones/epidemiology"[Mesh] OR "Leg Bones/etiology"[Mesh] OR "Leg Bones/metabolism"[Mesh] OR "Leg Bones/microbiology"[Mesh] OR "Leg Bones/pathology"[Mesh] OR "Leg Bones/physiology"[Mesh] OR "Leg Bones/physiopathology"[Mesh] OR "Leg Bones/radiography"[Mesh] OR "Leg Bones/radionuclide imaging"[Mesh] OR "Leg Bones/therapy"[Mesh] OR "Leg Bones/ultrasonography"[Mesh] OR "Leg Bones/ultrastructure"[Mesh] OR "Femur Neck/analysis"[Mesh] OR "Femur Neck/anatomy and histology"[Mesh] OR "Femur Neck/diagnosis"[Mesh] OR "Femur Neck/etiology"[Mesh] OR "Femur Neck/metabolism"[Mesh] OR "Femur Neck/microbiology"[Mesh] OR "Femur Neck/pathology"[Mesh] OR "Femur Neck/physiology"[Mesh] OR "Femur Neck/physiopathology"[Mesh] OR "Femur Neck/radiography"[Mesh] OR "Femur Neck/radionuclide imaging"[Mesh] OR "Femur Neck/therapy"[Mesh] OR "Femur Neck/ultrasonography"[Mesh] OR "Femur Neck/ultrastructure"[Mesh] OR "Lumbar Vertebrae/analysis"[Mesh] OR "Lumbar Vertebrae/anatomy and histology"[Mesh] OR "Lumbar Vertebrae/etiology"[Mesh] OR "Lumbar Vertebrae/metabolism"[Mesh] OR "Lumbar Vertebrae/microbiology"[Mesh] OR "Lumbar Vertebrae/pathology"[Mesh] OR "Lumbar Vertebrae/physiology"[Mesh] OR "Lumbar Vertebrae/physiopathology"[Mesh] OR "Lumbar Vertebrae/radiography"[Mesh] OR "Lumbar Vertebrae/radionuclide imaging"[Mesh] OR "Lumbar Vertebrae/therapy"[Mesh] OR "Lumbar

Vertebrae/ultrasonography"[Mesh] OR "Lumbar Vertebrae/ultrastructure"[Mesh])

AND

("Absorptiometry, Photon/classification"[Mesh] OR "Absorptiometry, Photon/methods"[Mesh] OR "Absorptiometry, Photon/nursing"[Mesh] OR "Absorptiometry, Photon/standards"[Mesh] OR "Absorptiometry, Photon/statistics and numerical data"[Mesh] OR "Absorptiometry, Photon/therapeutic use"[Mesh] OR "Absorptiometry, Photon/therapy"[Mesh] OR "Absorptiometry, Photon/utilization"[Mesh] OR "Tomography Scanners, X-Ray Computed/classification"[Mesh] OR "Tomography Scanners, X-Ray Computed/etiology"[Mesh] OR "Tomography Scanners, X-Ray Computed/therapeutic use"[Mesh] OR "Tomography Scanners, X-Ray Computed/utilization"[Mesh] OR “pQCT” OR “DXA” OR “Dual energy X ray absorptiometry” OR “pQCT” OR “Peripheral Quantitative Computed Tomography” OR “QCT” OR “Quantitative Computed Tomography” OR “Micro Computed Tomography” OR “Computed Tomography” OR “Micro CT” OR “Dual Photon Absorptiometry” OR “DPA”)

NOT

("Child"[Mesh])

NOT

("Body Composition"[Mesh])

NOT

(“animal”[Mesh])

Web of Science – 467 results

TS=(“continuous exercise” OR "intermittent exercise" OR "interval exercise" OR "Mechanical loading" OR "rest inserted exercise" OR "high impact exercise" OR "impact exercise" OR "Circuit-Based Exercise" OR "Plyometric Exercise" OR "Resistance Training")

AND

TS=("Bone mineral density" OR "Bone mineral content" OR "BMD" OR "BMC" OR "Bone" OR "Bones" OR "Leg Bones" OR "Femur Neck" OR "Lumbar Vertebrae")

AND

TS=(“Absorptiometry” OR "Tomography Scanners" OR “pQCT” OR “DXA” OR "Dual energy X ray absorptiometry" OR “pQCT” OR "Peripheral Quantitative Computed Tomography" OR “QCT” OR "Quantitative Computed Tomography" OR "Micro Computed Tomography" OR "Computed

Tomography" OR "Micro CT" OR "Dual Photon Absorptiometry" OR “DPA”)

NOT

TS=(“child”)

NOT

TS=("body composition" OR "body fat")

NOT

TS=(“animal”)

Cochrane Library – 273 results

#1 "continuous exercise" or "intermittent exercise" or "interval exercise" or "Mechanical loading" or "loading" or "rest inserted exercise" or "rest-inserted exercise" or "rest inserted loading" or "rest-inserted loading" or "high-impact exercise" or "high impact exercise" or "impact exercise"

#2 MeSH descriptor: [Exercise] explode all trees

#3 "Bone mineral density" or "Bone mineral content" or "BMD" or "BMC"

#4 MeSH descriptor: [Bone Density] explode all trees

#5 "pQCT" or "DXA" or "Dual energy X ray absorptiometry" or "pQCT" or

"Peripheral Quantitative Computed Tomography" or "QCT" or "Quantitative Computed Tomography" or "Micro Computed Tomography" or "Computed Tomography" or "Micro CT" or "Dual Photon Absorptiometry" or "DPA"

#6 MeSH descriptor: [Absorptiometry, Photon] explode all trees

#7 MeSH descriptor: [Tomography Scanners, X-Ray Computed] explode all trees

#8 #1 or #2

#9 #3 or #4

#10 #5 or #6 or #7

#11 #8 and #9 and #10

#12 MeSH descriptor: [Child] explode all trees

#13 MeSH descriptor: [Body Composition] 1 tree(s) exploded

#14 MeSH descriptor: [Animals] explode all trees

#15 #11 not #12 not #13 not #14

All database searches were performed on 1st November 2016

11.2 Appendix B – Participant Information Sheet and Consent Form for Chapter 4 - "Tibial impacts and muscle activation during walking, jogging and running when performed overground, on a motorised and non-motorised treadmill"

UNIVERSITY OF Hull
Faculty of Science

Participant Letter of Invitation 

Project title	Impact forces and muscle activation during walking and running performed over-ground, on a motorised and non-motorised treadmill
Principal investigator	Name: Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 3859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 07796270654

04/06/2013

Dear Sir or Madam

This is a letter of invitation to enquire if you would like to take part in a research project at Hull University.

Before you decide if you would like to take part it is important for you to understand why the project is being done and what it will involve. Please take time to carefully read the Participant Information Sheet on the following pages and discuss it with others if you wish. Ask me if there is anything that is not clear, or if you would like more information.

If you would like to take part please complete and return the Informed Consent Declaration form.

Please do not hesitate to contact me if you have any questions.

Yours faithfully,

Gallin Montgomery

Participant Information Sheet

Project title	Impact forces and muscle activation during walking and running performed over-ground, on a motorised and non-motorised treadmill
Principal investigator	Name: Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 3859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 07796270654

What is the purpose of this project?

This study will analyse the participant whilst walking, jogging and running over-ground, on a motorised treadmill and on a non-motorised treadmill. The study will quantify the forces involved and muscular activity in each condition. The analysis will determine how each condition could relate to exercise training to improve bone health.

Why have I been chosen?

We believe that you may meet our subject criteria which is as follows;

You must not be suffering from any existing medical condition. You must not have a past history of cardiovascular, renal, hepatic, thyroid disease, have a history of physical disability and any family history of sudden cardiac death. You have been sent this information because we feel you may fit the requirements for this study. Your eligibility for the study will be checked through the use of a pre-exercise medical questionnaire. .

What happens if I volunteer to take part in this project?

First, it is up to you to decide whether or not to take part. If you decide to take part you will be given this Participant Information Sheet to keep and asked to complete the Informed Consent Declaration at the back. You should give the Informed Consent Declaration to the investigator at the earliest opportunity. You will also have the opportunity to ask any questions you may have about the project. If you decide to take part you are still free to withdraw at any time and without needing to give a reason.

What will I have to do?

On arrival you will be met by the investigator who will brief you on the testing procedures and answer any questions or concerns that you might have. After signing a consent form, the investigator will ask you to complete a pre-exercise medical questionnaire requesting some information on your present state of health.

You will firstly attend a familiarisation session to practice walking jogging and running at self selected speeds whilst on the laboratory floor and then both on a motorised treadmill and a non motorised treadmill. The session will begin in the biomechanics lab and then move to the physiology lab to use the treadmills. Changing facilities will be available and you will be provided with running footwear in your own size.

For the main test, again arrival you will be met by the investigator who will brief you on the testing procedures and answer any questions or concerns that you might have. You will sign another consent form and the investigator will ask you to complete a pre-exercise medical questionnaire requesting some information on your present state of health.

Four electrodes will be attached the right leg which includes the, shin, calf, thigh and back of the leg. Attaching these electrodes will measure muscular activity and will entail shaving a small patch on the leg before cleaning with alcohol wipes and then securing the electrodes with surgical tape and elasticated bandages.

Two movement sensors will be placed on the trunk and right leg which includes the lower leg and lower back. These will be secured with surgical tape and elasticated bandages.

You will perform two maximal leg extensions, leg flexions, dorsi flexions and plantar flexions on a dynamometer to give your maximum muscle activation. Efforts are separated by 1 minute.

You will then perform a 30 second walk, jog and run whilst normally travelling overground at a self selected speed in the biomechanics lab. The speeds will be quantified and then you will replicate each trial in the physiology lab at a matched speed on a motorised treadmill and after that on a non-motorised treadmill (where you have to drive the belt). Throughout these trials the sensors will remain placed on your body until the protocol is over.

Will I receive any financial reward or travel expenses for taking part?

No

Are there any other benefits of taking part?

A mild sense of wellbeing afterwards.

If requested we can provide a brief overview of the forces experienced during running which may predispose individuals to injury.

Will participation involve any physical discomfort or harm?

There are general risks associated with the participation in exercise activities including cardiovascular complications, musculoskeletal injuries, again precautions have been made to minimize these risks. Your physical state will be monitored throughout exercise, and you may stop at anytime during the trial.

Will I have to provide any bodily samples (e.g. blood or saliva)?

No

Will participation involve any embarrassment or other psychological stress?

As long as you are comfortable with walking, jogging, running and having sensors attached to your legs and lower back then no. The only persons present in the laboratory at the time will be the primary investigator and a fellow research colleague during the exercise test sessions. A laboratory technician will be available for any emergencies that may arise.

What will happen once I have completed all that is asked of me?

You will be provided with a participant debrief sheet explaining what the purpose of the study was and how you may find out about the results of the study if you so wish. You will have the opportunity to ask any further questions regarding the testing protocol. You will then be free of any requirements and not be required for further testing in this study.

How will my taking part in this project be kept confidential?
You will be allocated with an anonymous participant code that will be used to identify any data that you may provide. Nobody other than the principle supervisor and student investigator have knowledge of this code. All data from the trials will be kept on a password encrypted computer, and a back up kept on a password encrypted laptop and memory stick. Informed consent forms and pre-exercise medical questionnaires will be kept separate from trial data in a locked office. All information and data gathered during this research will be stored in line with the 1988 Data Protection Act and will be destroyed 5 years following the conclusion of the study. During the time the data may be used by members of the research team only for purposes appropriate top the research question, but at no point will your personal information or data be revealed.

How will my data be used?
Data will be totally anonymised and shall be used to form analyses along with the other participants' data to see if there is an overall response to exercise. Data will be kept on a password encrypted computer , and a backup stored on a password encrypted memory stick and personal laptop belonging to the student investigator. This data will only be used by members of the research team. Results from the study will be used as data for a Ph.D thesis and an undergraduate dissertation. Should the data be published or presented in any form you will not be identified. If you wish to receive a copy of any potential publication/presentation this will be made available at the earliest possible opportunity.

Who has reviewed this study?
This project has undergone full ethical scrutiny and all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Ethics Committee at the University of Hull.

What if I am unhappy during my participation in the project?
You are free to withdraw from the project at any time. During the study itself, if you decide that you do not wish to take any further part then please inform the person named in Section 18 and they will facilitate your withdrawal. You do not have to give a reason for your withdrawal. Any personal information or data that you have provided (both paper and electronic) will be destroyed or deleted as soon as possible after your withdrawal. After you have completed the research you can still withdraw your personal information and data by contacting the person named in Section 18. If you are concerned that regulations are being infringed, or that your interests are otherwise being ignored, neglected or denied, you should inform Dr Lee Ingle, Chair of the Department of

Sport, Health and Exercise Research Ethics Committee, who will investigate your complaint (Tel: 01482 463141; Email: l.ingle@hull.ac.uk

How do I take part?

Contact the investigator using the contact details given below. He or she will answer any queries and explain how you can get involved.

Name: Gallin Montgomery Email: G.Montgomery@2012.hull.ac.uk Phone: 07796270654

UNIVERSITY OF Hull
Faculty of Science

Informed Consent Declaration

Project title	Impact forces and muscle activation during walking and running performed over-ground, on a motorised and non-motorised treadmill
Principal investigator	Name: Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 3859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 07796270654

Please Initial

☐ I confirm that I have read and understood all the information provided in the Informed Consent Form (EC2) relating to the above project and I have had the opportunity to ask questions.

☐ I understand this project is designed to further scientific knowledge and that all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Research Ethics Committee at the University of Hull. Any questions I have about my participation in this project have been answered to my satisfaction.

☐ I fully understand my participation is voluntary and that I am free to withdraw from this project at any time and at any stage, without giving any reason. I have read and fully understand this consent form.

☐ I agree to take part in this project.

.....	.....	.....
Name of participant	Date	Signature
.....	.....	.....
Person taking consent	Date	Signature

11.3 Appendix C – Inclusion and Exclusion Criteria for "The osteogenic potential of four common exercises used in osteoporosis prevention for postmenopausal women"

Inclusion Criteria

- Women aged 46 years and over
- Post-menopausal for 1-5 years
- Able to participate in an exercise programme

Exclusion Criteria

- Premature menopause (at age 45 or younger) and not treated with HRT [Explanation: then bone density may be considerably low, percentage changes are exaggerated]
- Vertebral fractures
- Spinal disease precluding impact exercises
- Organ failures: heart failure, liver failure, renal failure, respiratory failure
- Unable to undertake exercise for any reason
- Any history of heart conditions
- Family history of sudden cardiac death under 50 years of age
- Any musculoskeletal and/or orthopaedic conditions
- Current injury

- History of fracture within the last year
- Uncorrected visual impairment
- Recent history of dizziness or fainting
- Vestibular disorders
- Systolic blood pressure of more than 180 mm Hg or diastolic blood pressure of more than 120 mm Hg
- Pregnancy
- Pre-existing severe physical disability
- Resting heart rate of more than 120 BPM
- Shortness of breath with minimum exertion

11.4 Appendix D – Participant Information Sheet and Consent Form for Chapter 5 - "The osteogenic index of four common continuous and intermittent exercises used in osteoporosis prevention in an at-risk population"

Department of Sport, Health & Exercise Science

UNIVERSITY OF Hull
Faculty of Science

Participant Letter of Invitation 

Project title	The Osteogenic Index of a Single Bout of Exercise and a Week Long Exercise Programme (Commonly Used Exercises to Prevent Osteoporosis)
Principal investigator	Name: Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 01482 463859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 07796270654

04/06/2013

Dear Sir or Madam

This is a letter of invitation to enquire if you would like to take part in a research project at Hull University.

Before you decide if you would like to take part it is important for you to understand why the project is being done and what it will involve. Please take time to carefully read the Participant Information Sheet on the following pages and discuss it with others if you wish. Ask me if there is anything that is not clear, or if you would like more information.

If you would like to take part please complete and return the Informed Consent Declaration form.

Please do not hesitate to contact me if you have any questions.

Yours faithfully,

Gallin Montgomery

Participant Information Sheet

Project title	The Osteogenic Index of a Single Bout of Exercise and a Week Long Exercise Programme (Commonly Used Exercises to Prevent Osteoporosis)
Principal investigator	Name: Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 01482 463859
Student investigator (if applicable)	Name: Gallin Montgomery and Connor Cox (Undergraduate) Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 07796270654

What is the purpose of this project?

This study will quantify the level of importance of common high, impact interventions to assess which could prove the most beneficial for improving or maintaining bone health.

Why have I been chosen?

We believe that you may meet our subject criteria which is as follows;

You must not be suffering from any existing medical condition. You must not have a past history of cardiovascular, renal, hepatic, thyroid disease, have a history of physical disability and any family history of sudden cardiac death. You have been sent this information because we feel you may fit the requirements for this study. Your eligibility for the study will be checked through the use of a pre-exercise medical questionnaire. .

What happens if I volunteer to take part in this project?

First, it is up to you to decide whether or not to take part. If you decide to take part you will be given this Participant Information Sheet to keep and asked to complete the Informed Consent Declaration at the back. You should give the Informed Consent Declaration to the investigator at

the earliest opportunity. You will also have the opportunity to ask any questions you may have about the project. If you decide to take part you are still free to withdraw at any time and without needing to give a reason.

What will I have to do?

On arrival you will be met by the investigator who will brief you on the testing procedures and answer any questions or concerns that you might have. After signing a consent form, the investigator will ask you to complete a pre-exercise medical questionnaire requesting some information on your present state of health.

You will have sensors placed on your thigh, hamstring, calf and shin to detect the muscular activity. These are small and are stuck with double sided tape. Attaching these electrodes will measure muscular activity and will entail shaving a small patch on the leg before cleaning with alcohol wipes and then securing the electrodes with surgical tape and elasticated bandages. They do not hurt and are not uncomfortable.

You will also have a sensor attached to your lower back in a similar fashion to record overall body movement. This is also painless and not uncomfortable.

Once the sensors are attached you will be required to perform a series of jumping, stepping and stamping exercises, this will not be fatiguing and will be performed in sets of 10 with plenty of rest in between. The entire protocol will only be 2 hours (including sensor attachment).

Will I receive any financial reward or travel expenses for taking part?

No

Are there any other benefits of taking part?

You will get a small insight into your general health (blood pressure, resting heart rate, BMI).

You will be greatly helping with research to improve bone health throughout the ageing process.

If requested we can provide a brief overview of the forces experienced during exercise which may predispose individuals to injury.

Will participation involve any physical discomfort or harm?

There are only very general risks that are associated with any form of physical activity. Our research will not expose you to any greater risk than any other light, easy physical activity. Your physical state will be monitored throughout exercise, and you may stop at anytime during the trial.

Will I have to provide any bodily samples (e.g. blood or saliva)?
No

Will participation involve any embarrassment or other psychological stress?
As long as you are comfortable with performing jumping/stepping/stamping exercise and having sensors attached to your legs and lower back then no. The only persons present in the laboratory at the time will be the primary investigator and a fellow research colleague during the exercise test sessions. A laboratory technician will be available for any emergencies that may arise.

What will happen once I have completed all that is asked of me?
You will be provided with a participant debrief sheet explaining what the purpose of the study was and how you may find out about the results of the study if you so wish. You will have the opportunity to ask any further questions regarding the testing protocol. You will then be free of any requirements and not be required for further testing in this study.

How will my taking part in this project be kept confidential?
You will be allocated with an anonymous participant code that will be used to identify any data that you may provide. Nobody other than the principle supervisor and student investigator have knowledge of this code. All data from the trials will be kept on a password encrypted computer, and a back up kept on a password encrypted laptop and memory stick. Informed consent forms and pre-exercise medical questionnaires will be kept separate from trial data in a locked office. All information and data gathered during this research will be stored in line with the 1988 Data Protection Act and will be destroyed 5 years following the conclusion of the study. During the time the data may be used by members of the research team only for purposes appropriate top the research question, but at no point will your personal information or data be revealed.

How will my data be used?
Data will be totally anonymised and shall be used to form analyses along with the other participants' data to see how the human body responds to each exercise in terms of muscular

activation and overall body movement. This will inform the researchers of which exercises will be the most beneficial in improving a participant's bone health if they were to put each exercise into an exercise programme to improve bone health during the ageing process. Data will be kept on a password encrypted computer , and a backup stored on a password encrypted memory stick and personal laptop belonging to the student investigator. This data will only be used by members of the research team. Results from the study will be used as data for a Ph.D thesis and an undergraduate dissertation. Should the data be published or presented in any form you will not be identified. If you wish to receive a copy of any potential publication/presentation this will be made available at the earliest possible opportunity.

Who has reviewed this study?

This project has undergone full ethical scrutiny and all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Ethics Committee at the University of Hull.

What if I am unhappy during my participation in the project?

You are free to withdraw from the project at any time. During the study itself, if you decide that you do not wish to take any further part then please inform the person named in Section 18 and they will facilitate your withdrawal. You do not have to give a reason for your withdrawal. Any personal information or data that you have provided (both paper and electronic) will be destroyed or deleted as soon as possible after your withdrawal. After you have completed the research you can still withdraw your personal information and data by contacting the person named in Section 18. If you are concerned that regulations are being infringed, or that your interests are otherwise being ignored, neglected or denied, you should inform Dr Lee Ingle, Chair of the Department of Sport, Health and Exercise Research Ethics Committee, who will investigate your complaint (Tel: 01482 463141; Email: l.ingle@hull.ac.uk

How do I take part?

Contact the investigator using the contact details given below. He or she will answer any queries and explain how you can get involved.

Name: Gallin Montgomery Email: G.Montgomery@2012.hull.ac.uk Phone: 07796270654

UNIVERSITY OF Hull
Faculty of Science

Informed Consent Declaration

Project title	The Osteogenic Index of a Single Bout of Exercise and a Week Long Exercise Programme (Commonly Used Exercises to Prevent Osteoporosis)
Principal investigator	Name: Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 01482 463859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 07796270654

Please Initial

☐ I confirm that I have read and understood all the information provided in the Informed Consent Form (EC2) relating to the above project and I have had the opportunity to ask questions.

☐ I understand this project is designed to further scientific knowledge and that all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Research Ethics Committee at the University of Hull. Any questions I have about my participation in this project have been answered to my satisfaction.

☐ I fully understand my participation is voluntary and that I am free to withdraw from this project at any time and at any stage, without giving any reason. I have read and fully understand this consent form.

☐ I agree to take part in this project.

.....	.....	.....
Name of participant	Date	Signature
.....	.....	.....
Person taking consent	Date	Signature

11.5 Appendix E – Inclusion and Exclusion Criteria for "The effect of continuous and intermittent exercise on bone mineral density in postmenopausal women: a twelve-month randomised control trial"

Inclusion criteria

- Women aged 46 years to 60 years of age.
- Post-menopausal for 1-5 years (defined as; cessation of menstrual cycle for more than 12 months)
- If previously on hormone replacement therapy (HRT), off HRT for at least 5 years.
- Bone density in normal (T-score > -1) or osteopenic (T-score -1 to -2.4) range
- Able to participate in an exercise programme

Exclusion criteria

- Osteoporosis: T-score at or <-2.5 at lumbar spine and/or femoral neck
- Untreated vitamin D deficiency (25-OH vitamin D lower than 25 nmol/L)
- Blood calcium level outside the normal range (2.2-2.6 mmol/L)
- Premature menopause (at age 45 or younger) and not treated with HRT [Explanation: then bone density may be considerably low, percentage changes are exaggerated]
- Vertebral fractures
- Spinal disease precluding impact exercises

- Medical conditions likely to affect physiological rate of bone turnover: (a) endocrine conditions - thyrotoxicosis, hyperprolactinaemia, primary ovarian failure, Cushing's disease, acromegaly, (b) rheumatological conditions - rheumatoid disease, SLE, mixed connective tissue disorder, polymyalgia rheumatica, (c) inflammatory bowel disease - Crohn's disease, ulcerative colitis, (d) respiratory conditions - asthma or COPD with or without inhaled steroid therapy

- Medications likely to affect physiological rate of bone turnover: (a) steroids - systemic or inhaled, (b) aromatase inhibitors, (c) selective oestrogen-receptor modifier - tamoxifen

- Organ failures: heart failure, liver failure, renal failure, respiratory failure

- History of fracture within the last year

- Unable to undertake an exercise programme for any reason.

- Participation in intermittent exercise (i.e. those sports involving substantial impacts such as: team sports, racket sports, etc) and/or resistance exercise more than once per week as determined from a validated physical activity questionnaire.

11.6 Appendix F – Participant Information Sheet and Consent Form for Chapter 6 - "The effect of continuous and intermittent exercise on bone mineral density in postmenopausal women: a twelve-month randomised control trial"

Department of Sport, Health & Exercise Science

UNIVERSITY OF Hull
Faculty of Science

Participant Letter of Invitation 

Project title	**Bone Health Intermittent and Continuous Exercise Intervention**
Principal investigator	Name: Dr Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 01482 463859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 0796270654

Click here to enter a date.

Dear Madam

This is a letter of invitation to enquire if you would like to take part in a research project at the University of Hull.

Before you decide if you would like to take part it is important for you to understand why the project is being done and what it will involve. Please take time to carefully read the Participant Information Sheet on the following pages and discuss it with others if you wish. Ask me if there is anything that is not clear, or if you would like more information.

If you would like to take part please complete and return the Informed Consent Declaration form.

Please do not hesitate to contact me if you have any questions.

Yours faithfully,

Gallin Montgomery

Participant Information Sheet

Project title	**Bone Health Intermittent and Continuous Exercise Intervention**
Principal investigator	Name: Dr Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 01482 463859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 01482 466080

What is the purpose of this project?

The project aim is to assess the effects of continuous and intermittent exercise upon bone health in postmenopausal women (1-5 years) over a 12 month intervention.

Why have I been chosen?

You fit our criteria.

What happens if I volunteer to take part in this project?

First, it is up to you to decide whether or not to take part. If you decide to take part you will be given this Participant Information Sheet to keep and asked to complete the Informed Consent Declaration at the back. You should give the Informed Consent Declaration to the investigator at the earliest opportunity. You will also have the opportunity to ask any questions you may have about the project. If you decide to take part you are still free to withdraw at any time and without needing to give a reason.

You will be required to have an initial blood test and bone density scan to assess your suitability for involvement. If everything shows that you meet our criteria you will participate in a 12 month home-based exercise intervention and will receive a further 2 bone density scans to show any

possible improvements in bone health. You will be required to complete 3 testing sessions spread evenly throughout the year to assess muscular function and balance. At evenly spread out times during the year; you will also be required to wear a hip mounted activity monitor for the duration of up to a week at a time.

What will I have to do?

You will need to complete 3 impact training sessions per week, each lasting less than 8 minutes in duration for 12 months. You will need to attend the University for an initial blood test, muscle strength, muscle stiffness, tendon stiffness and balance tests. You will need to attend the Centre for Metabolic Bone Disease at Hull Royal Infirmary for a bone density scan. The tests at the University and Centre for Metabolic Bone Disease will be repeated after 6 months (apart from the blood test) and again after 12 months (apart from the blood test).

Will I receive any financial reward or travel expenses for taking part?

Reimbursement for travel expenses is available upon request.

Are there any other benefits of taking part?

Benefits depend upon the individual. It is possible for the intervention groups that bone density and muscular function may improve. For non-exercising control groups the benefits will be the knowledge of how the exercise intervention can help healthy ageing once the study has been completed.

Will participation involve any physical discomfort or harm?

No, physical discomfort will be no more than is associated with very light exercise. During the muscular function assessments there is a very small chance of a minor skin reaction to the alcohol swab or adhesive gel which is used.

Will I have to provide any bodily samples (e.g. blood or saliva)?

Yes, a one-off blood sample will be taken before you are allowed to participate in the study.

Will participation involve any embarrassment or other psychological stress?
No, participation in all protocols will be conducted in accordance with standardised procedures.

What will happen once I have completed all that is asked of me?
Once the study has been completed, the research team will notify you of the outcomes and findings.

How will my taking part in this project be kept confidential?
You will be assigned a participant number so that when your data is analysed no names or identifiable information will be used.

How will my data be used?
Your data will be analysed and will form part of a PhD thesis at the University of Hull. If sufficient findings are present then the results will be submitted to a scientific journal for publication.

Who has reviewed this study?
This project has undergone full ethical scrutiny and all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Ethics Committee at the University of Hull.

What if I am unhappy during my participation in the project?
You are free to withdraw from the project at any time. During the study itself, if you decide that you do not wish to take any further part then please inform the person named in Section 18 and they will facilitate your withdrawal. You do not have to give a reason for your withdrawal. Any personal information or data that you have provided (both paper and electronic) will be destroyed or deleted as soon as possible after your withdrawal. After you have completed the research you can still withdraw your personal information and data by contacting the person named in Section 18. If you are concerned that regulations are being infringed, or that your interests are otherwise being ignored, neglected or denied, you should inform Dr Lee Ingle, Chair of the Department of Sport, Health and Exercise Research Ethics Committee, who will investigate your complaint (Tel: 01482 463141; Email: l.ingle@hull.ac.uk

If for any reason you lose the capacity to give consent during the study, you will be removed from the study and your data will be destroyed.

How do I take part? 

Contact the investigator using the contact details given below. He or she will answer any queries and explain how you can get involved.

Name: Gallin Montgomery Email: g.montgomery@2012.hull.ac.uk Phone: 01482 466080

UNIVERSITY OF Hull
Faculty of Science

Informed Consent Declaration

Project title	**Bone Health Intermittent and Continuous Exercise Intervention**
Principal investigator	Name: Dr Max Ditroilo Email address: M.Ditroilo@hull.ac.uk Contact telephone number: 01482 463859
Student investigator (if applicable)	Name: Gallin Montgomery Email address: G.Montgomery@2012.hull.ac.uk Contact telephone number: 01482 466080

Please Initial

☐ I confirm that I have read and understood all the information provided in the Informed Consent Form (EC2) relating to the above project and I have had the opportunity to ask questions.

☐ I understand this project is designed to further scientific knowledge and that all procedures have been risk assessed and approved by the Department of Sport, Health and Exercise Science Research Ethics Committee at the University of Hull. Any questions I have about my participation in this project have been answered to my satisfaction.

☐ I fully understand my participation is voluntary and that I am free to withdraw from this project at any time and at any stage, without giving any reason. I have read and fully understand this consent form.

☐ I agree to take part in this project.

.....	.....	.....
Name of participant	Date	Signature
.....	.....	.....
Person taking consent	Date	Signature