THE UNIVERSITY OF HULL

The effect of intermittent exercise on mechanical loading and biomarkers of bone

metabolism

Being a thesis submitted for the degree of Doctor of Philosophy at

The University of Hull

By

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November 2016

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Acknowledgements

I would like to thank the following people for their invaluable support and contributions to the completion of this doctoral thesis.

Firstly I would like to thank my supervisors Dr Grant Abt and Dr Massimiliano Ditroilo for their guidance and patience throughout the Ph.D. I have become a better researcher, teacher and academic through your supervision. I would also like to extend my thanks to Professor Sean Carroll for his support and contributions to the thesis. A special thanks also to Dr Mark Fogarty for your writing tips, words of wisdom, and support when the going got tough.

To my friends and fellow Ph.Ds, Rich, Steph, Matt, Steve, Gallin, Simon, Al, Dan, Chris R, Chris T, Damien, Rachael, Fiona, Ben, Eleanna and James. The Ph.D can be an isolated experience but not when you're surrounded by a great bunch such as yourselves. Thanks for all the support, help with the study, and being excellent drinking buddies.

To Mum and Dad, thank you for always supporting and allowing me to rant on the phone and always making sure I didn't quit, and to Tom and Rob for the encouragement, and the great nights out.

My final thanks are reserved for Andrea. Without you I really would not have been able to complete this thesis. You always believed I could do it, your endless patience, help and support got me through. Importantly, you reminded me that there is more to life than the Ph.D. We made it together!

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Nomenclature

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BMD	Bone Mineral Density
aBMD	Areal Bone Mineral Density
BMC	Bone Mineral Content
BMR	Basal metabolic rate
Bla^-	Blood lactate
BoS	Base of support
BP	Blood pressure
$BW \cdot s^{-1}$	Body weights per second
Ca^{2+}	Calcium
CoM	Centre of mass
CoG	Centre of gravity
CTX-I	Carboxy-terminal telopeptide of type 1 collagen
hGRF	Horizontal ground reaction force
vGRF	Vertical ground reaction force
GRF	Ground reaction force
H^+	Hydrogen Ions
HIT	High-intensity interval training
HIIE	High- intensity intermittent exercise
HR	Heart rate
HR_{rest}	Heart rate at rest
HR_{max}	Maximum heart rate
HRV	Heart rate variability
$Km \cdot h^{-1}$	Kilometers per hour
$L \cdot min^{-1}$	Litres per minute
$mL \cdot kg^{-1} min^{-1}$	Millilitres per kilogram
MT	Motorised treadmill
$mM \cdot L^{-1}$	Millimoles per litre
$m \cdot s^{-1}$	Meters per second
μL	Microlitre

ng·mL^{-1}	Nanograms per millitre
NMT	Non-motorised treadmill
P1NP	Procollagen type 1 amino-terminal propeptide
PO^4	Phosphorus
PTH	Parathyroid hormone
RPE	Ratings of perceived exertion
SSG	Small- sided soccer games
$\dot{V}_E$	Minute ventilation
$\dot{V}_E \cdot \dot{V}O_2{}^{-1}$	Ventilatory equivalents for oxygen
$\dot{V}_E \cdot \dot{V}CO_2{}^{-1}$	Ventilatory equivalents for carbon dioxide
V_{T1}	Ventilatory threshold 1
V_{T2}	Ventilatory threshold 2
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen consumption
$v\dot{V}O_{2max}$	Velocity at maximal oxygen consumption

List of terms and abbreviations

Strain: Ratio of change in length divided by the original length

Stress: Force (F) divided by the cross sectional area (A) of bone

Frequency [Hz]: Cycles per second

OI: The osteogenic index

OI_segFFT: The segmented osteogenic index

OI_FFT: The osteogenic index calculated in the frequency domain

OI_BW: The osteogenic index calculated in the time domain

Abstract

Introduction: Bone is a metabolically active tissue which plays a multifunctional role within the body. Animal models have provided evidence demonstrating that dynamic and unaccustomed mechanical loads imposed by gravity and/or muscle activation which exceed the customary strain stimulus are anabolic for bone. Moreover, because the bone's response to multiple cycles diminishes over time, the inclusion of rest periods between loading cycles has been shown to augment the osteogenic response by allowing the mechanosensory system to re-initialise. As such, rest-inserted intermittent exercise might offer a favourable environment for bone adaptation beyond traditional continuous exercise. Despite this many still engage in continuous aerobic exercise training such as continuous running, which might be deleterious to bone. The use of interval or intermittent running has been proposed as an alternative to reduce bone fatigue and maximise bone accrual more so than continuous exercise. However, evidence for the benefits of intermittent load-bearing exercise on bone adaptation is confined to animal models. Therefore, the aims of the thesis were (1) to investigate the effect of intermittent exercise of varying exercise-to-rest durations with a fixed ratio on the mechanical loading dose, assessed via components of the ground reaction force (GRF), and the osteogenic index, and (2) and to establish the magnitude of effect of intermittent load-bearing exercise on changes in bone tissue, using bone turnover markers compared to a non-exercising control condition.

Study 1 The Force 3 non-motorised treadmill (NMT) was used throughout the thesis because of its ability to measure vertical (vGRF) and anterior horizontal (hGRF) GRF continuously during exercise, and its capacity to reflect intermittent movement patterns which are not as easily replicable on a motorised treadmill (MT). However, a preliminary study to investigate; (1) the cardiorespiratory responses to running on an NMT compared to an MT, and (2) establish appropriate reference speeds to dose intermittent performance for subsequent protocols on the NMT was required. Therefore, the aim of study one was to establish the validity of peak cardiorespiratory responses to intermittent and continuous graded exercise tests (GXTs) on a NMT compared to an MT. When a continuous GXT is performed on the NMT a similar maximal oxygen uptake can be achieved ($\dot{V}O_{2max}$) compared to that achieved on the MT (P = 1.00, d = 0.01, trivial). However, there was a reduction in peak heart rate (P = 0.0001, d = 0.9, moderate), and peak speed is reduced by ~30%. When an intermittent GXT (15 x 15 s) is performed on a NMT, a similar $\dot{V}O_{2max}$ can be achieved (P = 0.701, d = 0.16, trivial), together with smaller reductions in peak HR (P = 0.170, d = 0.34, small), and peak speed (P = 0.009, d = 0.8, moderate) compared to a continuous MT running. Conversely, 30 x 30 s had a statistically significant reduction in $\dot{V}O_{2max}$ (P = 0.04, d = 0.37, small) and peak HR (P = 0.0001, d = 0.57, small) compared to the Cont-MT. As such, the shorter 15 x 15 s GXT was used to obtain reference speeds for subsequent studies (Studies 2-4).

Study 2 The osteogenic potential of impact exercise can be quantified from the analysis of kinetic (peak vGRF, load rate and vertical impulse) variables with greater peak vGRF, load rate and vertical impulse reflecting (indirectly) the magnitude and rate of the mechanical load applied to bone tissue. Our aim was to establish how manipulating the duration and frequency of exercise-to-rest intervals might change the mechanical loading environment using a fixed 1:1 ratio. Twelve healthy active males performed five 45 min intermittent running protocols on the NMT. Experiment 1: three of the intermittent protocols differed in their exercise-to-rest durations (5 s intervals [5s-Int], 20 s intervals [20s-Int] and 80 s intervals [80s-Int]). Experiment 2: three of the protocols differed in the rate of acceleration and deceleration but matched for the exercise-to-rest duration (20 s by 2 s intervals [20s2s-Int], 20 s by 4 s [20s4s-Int] and 20 s by 6 s [20s6s-Int]). The primary outcome measures for experiment 1 & 2 were mean and peak vGRF, vertical impulse, load rate and the intra-step variability assessed via the coefficient of variance (%CV) of the kinetic and kinematic data. There was no statistical difference between conditions for impulse (P = 0.175), maximum load rate (LR) (P = 0.104) or average load rate (ALR) (P =0.345). Peak vGRF data were statistically different between conditions (P = 0.023) with the 5s-Int being greater than the 80s-Int (P = 0.022). There was a statistical effect of condition on all CV data for vertical impulse (P = 0.0001), load rate (P = 0.0001), vGRF (P = 0.0001), kVert (P =0.0001) and kLeg (P = 0.0001) with the 5s-Int & 20s-Int being higher than the 80s-Int. The similarity in the mean GRF data are likely due to the higher loads generated during higher speeds being counteracted by the lower loads at the lower speeds. The variability in the data are caused by the variation between the high and low speeds.

Study 3 The magnitude, rate and frequency of the mechanical load are proportional to the amount of bone adaptation. These components were considered in isolation in study 2. However, the components can be combined into one mathematical algorithm, the osteogenic index (OI), to assess the osteogenic potential of the exercise. However, because we demonstrated no statistical effect on mean loading due to the higher loads being counteracted by the lower loads, it is unlikely that the traditional OI can distinguish between the variable loading environments of more intermittent exercise. A novel approach has been developed which incorporates the magnitude and rate of the loading dose across a frequency spectra. The number of loading segments of a particular exercise can then be categorised into the magnitude, intensity and frequency. As yet this method has only been utilised with accelerometers and only during steady-state conditions. Experiment one: There was a statistically significant difference between conditions for the OI_fft ($P = 0.0001$). The OI_fft was highest for the 80s-Int being 28% greater than the 5s-Int and 23% greater than the 20s-Int. There was a statistically significant 3-way interaction for condition*frequency band*intensity ($P = 0.012$) with the more intermittent conditions having a higher loading dose at higher frequencies. Experiment two: There was a significant main effect for condition for the mean differences of the OI_fft between conditions ($P = 0.033$). There was no significant effect of condition for the OI_BW ($P = 0.572$). There was no significant 3-way interact for condition*frequency band*loading intensity ($P = 0.870$). When the magnitude, intensity and frequency of multiple loading segments are considered, intermittent locomotion allows the individual to obtain higher magnitudes of loading dose, shifting towards a higher frequency band. It is unclear whether the intermittent protocol might offer a more favourable loading environment due to the more variable loading patterns with greater magnitudes of load at higher frequencies.

Study 4: Whilst brief continuous load-bearing exercise increases bone remodelling in favour of resorption, it is unclear how intermittent exercise effects acute bone remodelling. Our aim was to investigate the effect of varying degrees of intermittent exercise, with a fixed exercise-to-rest ratio, on acute bone remodelling, as measured by bone turnover biomarkers. It was hypothesised that the more intermittent protocol would result in a greater bone turnover compared to the non-exercising control and less intermittent conditions. The same exercise protocols from study 2 and 3 were used. Venous blood samples were collected at the same time of day following a 12 h fast at baseline, 1 h, 2 h and 24 h post-exercise. Carboxyterminal crosslinked telopeptide (CTX-1) and procollagen type 1 amino terminal propeptide (P1NP) were used as markers of bone resorption and formation, respectively. There was a significant main effect for time ($P = 0.0001$), condition ($P = 0.032$) and a significant condition by time interaction ($P = 0.001$) for CTX-I. At 1 h the 20s-Int and 5s-Int were higher than the control condition (20s-Int: $P = 0.0001$, 5s-Int: $P = 0.010$). There was no significant condition by time interactions for P1NP. The results confirm that bone remodelling is stimulated acutely by load-bearing exercise. Very short and short interval intermittent exercise results in greater bone turnover compared to longer interval intermittent exercise.

Conclusion: The effect of different exercise-to-rest intervals, and therefore different frequency of intermittency did not have an effect on the mean vGRF, load rate, vertical impulse or osteogenic index. However, as expected there was a greater intra-step variability in these measures leading to a more variable mechanical loading environment which might offer a more favourable loading environment for bone. Indeed, when bone tissue turnover was assessed using traditional bone turnover markers all exercise conditions demonstrated an increase in bone resorption compared to a non- exercising control condition at 1 h. However, only the short and very short intervals were statistically elevated above control. Therefore, very short and short intermittent exercise might cause greater bone resorption in the prevailing hours following exercise which could stimulate an increase bone formation.

CHAPTER 1:

General Introduction

1.1 Introduction

Bone is a metabolically active tissue adapting its structure in response to the changing mechanical environment (Robling & Turner, 2009). This process of bone modelling optimises the geometry of the bone to cope with the evolving mechanical demands such as during childhood growth (Frost, 1987). Bone modelling occurs through two processes; resorption via osteoclast activation or through formation via osteoblast activation. These processes occur in isolation and once skeletal maturity is reached, modelling activity rate declines (Robling & Turner, 2009). Unlike modelling, bone remodelling follows an activation → resorption → formation sequence whereby the cells (osteoclasts, osteoblasts and osteocytes) of the basic multicellular unit are closely coupled through a cascade of signalling events (Hughes & Petit, 2010). The controlled coupling between the cells ensures that the amount of bone removed equals the amount of bone replaced. Perturbations in bone balance can occur in disease states such as osteoporosis or osteopenia where more bone is resorbed creating a negative bone balance (Delaisse, 2014). The negative bone balance can also occur in situations where the mechanical loading environment changes, such as during times of reduced gravity (e.g. bed rest or space flight) (Goodship et al., 1998), sedentary lifestyles, or during times of multiple high intensity loading cycles (Turner & Robling, 2003). Continued perturbations in the homeostasis of bone tissue can lead to reductions in bone mineral density (BMD), quality and strength, reducing the structural integrity of bone and increasing the risk of fractures (Delaisse, 2014).

Exercise is associated with improvements in BMD (Snow-Harter & Robert, 1991; Kohrt, 2001), particularly at load-bearing sites (Brahm et al., 1997c; Calbet et al., 2001; Fredericson et al., 2007) and can improve both balance and stability (Howe et al., 2011a) which when reduced are known to contribute to risk of falling, and increasing fractures in high risk populations (e.g. the elderly and obese children). As such, participation in exercise training programmes is advocated for the prevention and treatment of poor bone health (Bonaiuti et al., 2002; Howe et al., 2011b) and thus decreasing the risk of developing osteoporosis in later life. The role exercise plays in preventing bone metabolic diseases such as osteoporosis is suggested to be due either to increased peak BMD

during childhood, which reaches its peak by the 3rd decade, or by reducing the rate of decline in BMD associated with aging (Kohrt, 2001). Therefore, participating in exercise interventions from an early age, and continuing to exercise is recommended to maximise bone mass and skeletal health throughout life. As such the development of interventions to maximise bone health in the young is warranted. Hence, the National Health Service recommend young people participate in at least 3 sessions per week of bone building exercise. Moreover, given the extent of bone losses during space flight in astronaut's (~1-3% per month) (Carmeliet & Bouillon, 2001), exercise protocols which maximise bone adaptation are a key preventative measure for long term space travel (Hackney et al., 2015). However the specific mechanism(s) by which exercise exerts its osteogenic effects on bone, and the most osteogenic mode of exercise, remains elusive. This is not surprising given the myriad of factors that vary between exercise modalities, such as intensity, duration, and the type of mechanical loading pattern (e.g. impact vs. non- impact) (Kohrt et al., 2009).

The exercise-induced mechanical loads imparted on bone originate either through gravitational forces (impact) and/or through muscle activity (Judex & Carlson, 2009). As yet no study has demonstrated unequivocally which of these two mechanisms (gravity or muscle activity) is most important for bone. Regardless, the mechanical load induces deformation or strain on the bone which is the ratio of change in length, divided by the original length (Skerry, 2006). Bone remodelling is then activated at the sites of the greatest strain, but only if the strain stimulus surpasses its customary threshold (Frost, 1987). The optimal strain threshold is currently unknown and likely differs between different loading sites. Moreover, bone responses to exercise are population specific such that bone responses are governed by age, gender and genetics (Frost, 2003), making it challenging and unlikely that a universal exercise therapy exists to improve bone health across populations.

Nevertheless, pioneering animal models have unravelled the types of mechanical stimuli which govern bone adaptation and the osteogenic potential of exercise-induced mechanical loads depends on the magnitude, frequency and rate of the mechanical strain (Lanyon & Rubin, 1984;

Turner et al., 1995; Turner, 1998; Turner & Robling, 2005b). The duration of the loading cycle is also a key factor, as bone cell [osteocyte] sensitivity becomes saturated by prolonged mechanical loads after approximately 100 loading cycles (Umemura et al., 1997; Turner, 1998; Umemura et al., 2002). The relationship between the increase in the number of loading cycles (N) and the decrease in mechanosensitivity of bone tissue follows a monoexponential decay, proportional to $1/(N + 1)$ (Umemura et al., 1997). This desensitisation of the osteocyte network leads to a reduction in the osteogenic potential of the applied mechanical load (Robling & Turner, 2009).

In vivo animal studies have demonstrated that inserting rest phases between loading cycles can re-sensitise the osteocyte network prior to the next mechanical stimuli (Robling et al., 2000; Srinivasan et al., 2002; Umemura et al., 2002; Srinivasan et al., 2015). The duration of this intervening recovery period may be a key factor in restoring the mechanosensitivity of bone (Srinivasan et al., 2015). As such, the recovery phenomenon has been explored both *in vivo* and *in vitro* by varying the duration of the inserted rest periods (Robling et al., 2000), with rest periods as short as 10 s augmenting the osteogenic response (Srinivasan et al., 2002). Whilst these animal studies, often performed in rodents (Umemura et al., 1997), have been fundamental in advancing knowledge in bone physiology, there are fundamental differences between human and rat skeletons. Therefore, the osteogenic effect of inserted rest on bone in humans requires further investigation.

Currently intermittent exercise is recommended as part of the exercise programme to improve bone health (Kohrt et al., 2004), and interval running (a form of intermittent exercise) has been promoted as an intervention strategy offering more potent health benefits for bone adaptation (Boudenot et al., 2016). However, continuous aerobic endurance exercise is predominantly engaged in and prescribed by clinicians (Boudenot et al., 2015). Moreover, continuous modes of aerobic training, such as road marching, are often undertaken by the military leading to high injury rates (Jones & Hauschild, 2015). Furthermore, continuous moderate intensity exercise is still used as an exercise intervention during space exploration. Therefore, investigating the potential bone building properties of intermittent forms of exercise in a healthy population is warranted.

The external validity of studying exercise with inserted rest periods, or brief bouts of lower intensity exercise is high, as this type of activity reflects both human and animal movement patterns performed in their natural habitats (Weinstein, 2001). This type of locomotion is defined as 'intermittent exercise' (ÅStrand et al., 1960; Weinstein, 2001; Gibala et al., 2012). While a large body of research examining the physiology of intermittent exercise already exists (Laursen & Jenkins, 2002), more recent research has demonstrated that intermittent exercise is a potent stimulus for health and wellbeing, often evoking positive adaptations beyond that of continuous exercise (Gibala & McGee, 2008). As such, intermittent exercise is used as a popular training method for professional athletes, recreational exercisers, and children. Despite this, the efficacy and effectiveness of intermittent exercise as an approach for enhancing bone health in humans has received only limited and indirect scholarly attention (Lin et al., 2012; Tolly et al., 2014; Mezil et al., 2015). The national aeronautics and space administration (NASA) (Ploutz-Snyder et al., 2014) have asked the question 'run far or fun fast' which is better for maintaining bone and muscle in space? Clearly, there is a need to investigate the effectiveness of intermittent exercise for enhancing bone health.

In support of the *in vivo* animal models (Robling et al., 2001), both cross-sectional (Calbet et al., 2001) and training studies (Helge et al., 2010; Krustrup et al., 2010b) in humans, have demonstrated that games play, such as soccer, which is characterised by brief periods of high-intensity exercise, interspersed with periods of low-intensity exercise, or complete rest (Bangsbo, 1994), results in positive anabolic effects on BMD (Krustrup et al., 2009; Helge et al., 2010; Krustrup et al., 2010b). The aforementioned studies by Krustrup et al, (2010, 2009) compared the utility of small-sided soccer games (SSGs) against a continuous aerobic running intervention to assess the improvement in multiple health and fitness parameters including blood pressure, aerobic capacity, resting heart rate, leg strength and BMD. These authors (Krustrup et al., 2010b) reported a 2.3% increase from baseline in whole body BMD in pre-menopausal females after 16 months of SSGs compared to a 0.2% change in the running group.

However, the training studies have a number of limitations. Firstly, the authors failed to appropriately control the internal physiological load, opting to use the percentage of one's maximum heart rate (% HR_{max}) to prescribe training intensity, which is prone to large inter-and intra-individual variability (Morton, 2007). The use of individualised speed thresholds or percentages of the velocity at one's maximal oxygen consumption ($v\dot{V}O_{2max}$) are argued to be more favourable methods to dose intermittent exercise (Buchheit & Laursen, 2013b). Secondly, the unpredictable exercise-to-rest intervals elicited by SSGs make it difficult to match the external load between intermittent and continuous exercise conditions (Buchheit & Laursen, 2013b; 2013a). The external load here refers to measures such as distance covered, mean speed, and work done which is the product of the force and the distance over which the force is applied (Winter, 1979). Therefore, it is unclear from these training studies whether the intermittent nature of the exercise itself (peaks and troughs) contributed to the reported osteogenic effects, or whether there was greater external load (distances covered, higher mean speeds, and a greater amount of external work) in the intervention group compared to the control group. Furthermore, the varying exercise-to-rest intervals attributed to SSGs makes it difficult to establish any dose-response relationships between the magnitude of the osteogenic effect and the duration and frequency of changes of the exercise-to-rest intervals. Therefore, it is unclear whether longer rest intervals are needed following higher intensity exercise bouts in order to maximise the osteogenic response, or whether a greater frequency of changes of acceleration and deceleration phases offers a more osteogenic environment. The answer to these questions will also be dependent on the study population being investigated.

Finally, the mechanical loads induced by the different exercise conditions (intermittent vs. continuous) were not quantified in the aforementioned training studies (Helge et al., 2010; Krustrup et al., 2010b). This is understandable as it is methodologically difficult to assess mechanical load in field-based training programmes. However, without this quantification of the mechanical load, it is not possible to assess how intermittent loading patterns may deliver a greater osteogenic stimulus. Given the nature of intermittent exercise, the high, yet brief, mechanical loads experienced during high-force actions might be counteracted by the low-forces experienced

during periods of low intensity exercise and/or rest. Conversely, due to the multiple changes in speed during intermittent running, there is a greater emphasis on the acceleration and deceleration phases of the running cycle (Bangsbo, 1994; Greig & Siegler, 2009), potentially leading to cumulative mechanical stresses, and greater mechanical strains (Greig et al., 2006) for intermittent exercise involving a higher frequency of exercise-to-rest intervals.

To aid in quantifying the osteogenic potential of a specific exercise mode, Turner and Robling (2003) developed a mathematical formula known as the osteogenic index (OI). The OI estimates bone formation using the intensity of the loading cycle, which is quantified via the peak vertical ground reaction force (vGRF) and the number of loading cycles (Turner & Robling, 2003). The ground reaction force is the force exerted by the ground when the whole body is in contact with it, and can be used as a surrogate measure of strain on the skeletal system (Cavagne & Lafortune, 1979).

The OI is expressed mathematically as:

$$OI = I * Ln(N + 1) \qquad (1)$$

Where; OI is the osteogenic index, I is the intensity of exercise (e.g. vGRF), Ln is the natural log used to adjust for the diminishing returns of multiple loading cycles, N is the number of cycles, and $N + 1$ representing the rate of change of the logarithmic relationship increasing by a factor of 1 (Umemura et al., 1997).

The rate and frequency of the load can be incorporated into the equation using Fourier analysis:

$$k \cdot \sum_{i-1}^{n} \varepsilon_i f_i \qquad (2)$$

Where k is a proportional constant such as the number of steps or jumps, ε is the peak to peak strain and f is the frequency of the loading waveform. This equation demonstrates an important

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phenotype key to bone in that static loading would not result in adaptation because $f = 0$ (Turner 1998), therefore bone only adapts to dynamic loading (Lanyon & Rubin, 1984).

Several studies have utilised the OI during both acute (Rantalainen et al., 2009; Tolly et al., 2014) and chronic (Lester et al., 2009; Rantalainen et al., 2011a) exercise in humans. Studies have used a variety of techniques to estimate vGRF such as ; force plates (Rantalainen et al., 2009), accelerometers (Chahal et al., 2014; Kelley et al., 2014) and pressure pads (Tolly et al., 2014). A characteristic of the OI is to use one single representative intensity value (i.e vGRF) to reflect the loading dose of the entire exercise period. Whilst the OI is useful to distinguish the osteogenic potential of discrete bouts of exercise (Weeks & Beck, 2008), it is unclear how the OI may distinguish between intermittent exercise which varies in the frequency of the exercise-to-rest intervals, and involves both high and low forces. Moreover, due to limitations of static force plates, many studies have not quantified the mechanical load experienced for every loading cycle (e.g. each step) in more ecologically valid environments (Rantalainen et al., 2009; Lin et al., 2012). Therefore, studies may have failed to observe the nuances of the mechanical loading patterns imparted by more intermittent exercise modes. Given that variability of the loading dose during intermittent exercise may be a key factor in enhancing bone adaptation (Moreno et al., 2008), it is important to quantify the intra-individual variability of each loading cycling (Giakas & Baltzopoulos, 1997) during the exercise condition. Therefore, all loading cycles must be measured to establish the effect of the varied mechanical loading environment.

To date, the only study (Tolly et al., 2014) to have measured all loading cycles during intermittent exercise to assess the OI, used pressure sensitive insoles to capture the vGRF during the sport of cyclocross, which was performed outside the control of a laboratory environment. These authors used the traditional calculation for OI (equation 1) which does not incorporate all components of the mechanical load (frequency, magnitude and rate). A novel method utilising Fourier analysis (equation 2) has been developed to incorporate the frequency across multiple loading cycles using an accelerometer (Kelley et al., 2014). The inclusion of multiple loading segments over the entire loading period may help to distinguish between the intermittent and more continuous loading

environments. As yet this novel approach has not been investigated using a force measuring treadmill in different non-steady state loading environments.

To assess the ground reaction forces of multiple steps within a laboratory environment requires the use of a fully instrumented treadmill. Fully instrumented motorised treadmills (MTs) have been shown to be valid and reliable ergometers for use in controlled laboratory studies (Kluitenberg et al., 2012) to assess the vGRF during locomotion. However, fully instrumented MTs are expensive and do not allow the exerciser to accelerate and decelerate at their own pace (Davies et al., 1984), making them less suitable for simulating intermittent locomotion. Moreover, several studies have demonstrated that MTs do not replicate the stresses of over-ground running (Nigg et al., 1995; Schache et al., 2001).

Given these limitations, an alternative is to use a non-motorised treadmill (NMT), an ergometer that allows the exerciser to change intensity (speed) under their own volition. Non-motorised treadmills have been previously used to simulate the physiological responses and activity profile observed during intermittent team sports (Sirotic & Coutts, 2008; Hopker et al., 2009) and repeated sprinting (Highton et al., 2012). Comparative studies between MTs and NMTs have observed greater energy expenditure at lower speeds (Davies et al., 1984; Everett et al., 2010), reduced time to exhaustion (De Witt et al., 2009), similar peak vGRF, and an attenuated/absent impact peaks (Hagan et al., 2006; Everett et al., 2010) when running on NMTs. As such, long term training on the NMT could result in different physiological effects (Davies et al., 1984; De Witt et al., 2009), and the lessening/absence of impact transients may produce an attenuated osteogenic response (Rantalainen et al., 2011a). Therefore, the response of bone remodelling to exercise on NMTs remains unclear and requires further examination.

Nonetheless, the ability to quantify both internal and external load in a controlled laboratory environment on the NMT offers a unique opportunity to assess the effect of intermittent exercise on bone remodelling and overcome some of the limitations of the aforementioned training studies using SSGs (Helge et al., 2010; Krustrup et al., 2010b). Moreover, the ability to measure the

vertical and anterior horizontal components of the ground reaction force on every loading cycle to assess the differences (if any) and intra-step variability in kinetic (peak vGRF, impulse, load rate) and kinematic (step frequency, step length) parameters during intermittent running may help to establish the nuances in loading patterns between intermittent and less intermittent exercise.

Currently, the 'gold standard' measurement of BMD is obtained through duel-energy X-ray absorptiometry (DXA). The sensitivity of DXA is limited however, as it can take several (3-6) months, but often in excess of 12 months, to see discernible changes in BMD (Kemmler & Engelke, 2004) at least in post-menopausal females. The time required to observe these changes is largely due to the length of the bone remodelling processes (Vasikaran et al., 2011b). An alternative to DXA that allows more rapid changes in bone remodelling to be observed is the use of bone turnover markers (BTMs). Unlike the static densiometric techniques, BTMs are ideal tools to detect the metabolic uncoupling between formation and resorption (Seibel, 2005; Banfi et al., 2010). Exercise-induced mechanical loads initiate mechanical deformation of osteoblasts and osteoclasts (Bloomfield, 2001) and bone cells have been shown to respond rapidly to mechanical stimuli as evidenced by gene expression (Raab-Cullen et al., 1994; Mantila Roosa et al., 2011) and protein expression of BTMs (Bonnet et al., 2009). Furthermore, advances in technology have greatly enhanced the accuracy and reliability of BTMs with rapid, low cost assays. Thus examining the acute changes in the circulating concentrations of bone turnover markers is an important step in identifying the mechanisms by which exercise exerts its effects on bone.

The acute effect of continuous exercise on bone turnover markers has been extensively investigated using a variety of exercise modes including running (Scott et al., 2010; 2011; 2013), cycling (Guillemant et al., 2004; Herrmann et al., 2007), resistance exercise (Rong et al., 1997), whole body vibration (Bemben et al., 2015; Harrison et al., 2015), and jumping (Lin et al., 2012). Bone turnover markers appear to be intensity (Scott et al., 2011), duration (Kristoffersson et al., 1995; Woitge et al., 1998), population (Kish et al., 2015) and exercise type specific (Lin et al., 2012) hence responses in the literature are non-uniform. Moreover, studies have used a range of

different BTMs to assess the effect of exercise, and some of these are less specific to changes in bone than others (Banfi et al., 2010; Wheater et al., 2013). Finally, there is a paucity of studies in the current literature including a non-exercising control condition when comparing the effects of acute exercise on BTMs (Rogers et al., 2011; Lin et al., 2012). Therefore, the true effect of exercise may be lessened by the effects of circadian variation.

Recently, the effect of acute high-intensity intermittent exercise on bone turnover marker responses, in a group of healthy active males has been investigated (Mezil et al., 2015), demonstrating an increase in bone turnover despite the short exercise duration (~10 minutes). However, the study failed to include a control condition. Therefore, it is likely that the post-exercise increase in bone alkaline phosphatase (BAP) simply reflects the circadian variation in BTMs. Moreover, the research design did not include a comparison between different models of intermittent exercise. Intermittent exercise models can be manipulated in a variety of ways which will stimulate different stresses on the body (Buchheit & Laursen, 2013b). These factors are: (1) the exercise-to-rest ratio (e.g. 15 s : 15 s [1:1 ratio] vs 30 s : 15 s [2:1 ratio]); (2) the delta exercise intensity - the magnitude of change between higher and lower intensities (e.g. alternating between 8 km·h^{-1} and 12 km·h^{-1} vs 6 km·h^{-1} and 14 km·h^{-1}); (3) the absolute exercise intensity; (4) exercise duration; (5) exercise intensity change frequency - the rate at which the exercise changes from lower to higher intensity (e.g. a change every 3 s vs every 6 s); and (6) the exercise mode (running vs cycling). Establishing how manipulation of the intermittent model affects physiological and biomechanical strain will aid in the development of appropriate targeted training models to maximise bone accrual for the specific population in the intervention (e.g. young, healthy, active males). It is likely the intensity and duration of the exercise stimulus given prior to the rest phase will dictate the duration of the rest phase, although this has not been established in human studies. Therefore, assessing the response to different exercise-to-rest durations is warranted.

Finally, Mezil et al. (2015) used a non-impact mode of exercise (cycle ergometry) which has been attributed to deleterious effects on BMD (Nagle & Brooks, 2011). Cycling produces mainly

concentric muscle activation whereby the muscle shortens during its action (Bijker et al., 2002). However, running intermittently involves more eccentric muscle activation where the muscle lengthens (Greig & Siegler, 2009), potentially increasing forces acting on the bone (Hawkins et al., 1999; Bodor & Jarosz, 2015). Therefore, the non-impact exercise mode would not be comparable to intermittent high-impact activities, such as soccer, and would not impart the same mechanical strain on bone. The authors suggested the use of cycling would reflect only systemic changes in bone rather than site-specific changes as a result of mechanical load. However, BTMs do not reflect a site-specific response, and by their nature reflect systemic changes (Banfi et al., 2010; Garnero, 2014). As such, the effect of high-intensity intermittent exercise with varying exercise-to-rest ratios on bone requires further investigation.

In summary, mechanical loading with inserted rest periods has been well established in animal models and appears to enhance the osteogenic potential of the mechanical loads. However, the efficacy of intermittent exercise as an anabolic stimulus for bone has received limited attention in human studies. Whilst both cross sectional and training studies investigating the effects of soccer on BMD have shown positive findings, these studies failed to provide clear mechanisms of action as to why intermittent exercise may be more osteogenic. Moreover, the unpredictable exercise-to-rest ratios performed in soccer makes it impossible to establish a dose-response relationship (if any) between the response of bone and the exercise-to-rest ratio. Bone turnover markers have been shown to provide useful information on the acute changes in bone remodelling in response to various modes of exercise. Several studies have demonstrated greater bone turnover, in favour of resorption, for continuous exercise modes. However, the acute metabolic responses of intermittent high-impact exercise, of varying exercise-to-rest ratio, remain unclear

1.2 Statement of the Problem

The existing body of literature investigating the utility of exercise as an anabolic stimulus for bone has focused mainly on continuous modes of exercise. However, the use of intermittent mechanical loading of varying, unaccustomed loads has been shown to be more osteogenic for

bone, at least in animal trials. Whilst the use of intermittent exercise to enhance the osteogenic response is yet to be fully explored in humans, both cross-sectional and randomised control trials have demonstrated the potential utility for games play, such as soccer, to improve bone health. However, the mechanical loading dose, exercise intensity and exercise-to-rest ratio of the exercise were not well controlled in these studies making it difficult to establish the mechanisms by which intermittent exercise might exert its osteogenic effect in humans. The need to establish the osteogenic effect of fixed ratio, varying exercise-to-rest durations in humans requires multiple and intensive exercise interventions that will be physically demanding and therefore contraindicated in an aging and/or clinical population.

Thus, the overarching aim of the thesis was to investigate the effect of intermittent exercise, varying in the duration of the exercise-to-rest intervals using a fixed ratio, and controlled for external load, on the mechanical loading dose and acute bone turnover marker responses in a cohort of young, healthy active males as a proof-of-concept. In order to achieve this aim the following objectives were set;

1.3 Objectives of the Thesis

1. *To develop intermittent protocols matched for external load (mean speed, distance and duration) on a non-motorised treadmill but which vary proportionally in the frequency of exercise-to-rest intervals and durations.*

2. *To investigate the effect of intermittent exercise of different exercise-to-rest durations on the magnitude and intra-step variability of kinetic and kinematic variables of running gait.*

3. *To assess the utility of the osteogenic index (OI) as a measure of the osteogenic potential of intermittent exercise and establish the use of a novel OI model which incorporates magnitude, frequency and intensity of mechanical loading across multiple loading segments using Fourier analysis.*

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4. *To establish the magnitude of the effect of intermittent exercises of different exercise-to-rest durations on traditional biochemical markers of bone metabolism in a group of young healthy males.*

Thesis structure

Two separate data collection periods were completed for the thesis. The first study is reported in chapter three. The second study contributes to chapters, four, five and six.

Chapter two will provide a detailed review of relevant literature on basic bone biology, bone biomechanics, the response of bone to mechanical loading, and the potential for intermittent exercise to be more osteogenic than continuous exercise. A detailed review on the seminal work that highlights how intermittent exercise can affect our body's systems differently will be reviewed. The final section will be address the use of bone turnover markers to assess bone turnover following an acute bout of exercise.

Chapter three will detail the first study for the thesis entitled 'The validity of intermittent and continuous graded exercise tests for the assessment of peak cardiorespiratory responses on a Woodway Force 3 non-motorised treadmill.' The cardiorespiratory outcomes from the graded exercise test developed from this study will be used to dose the intensity of the intermittent protocols used in subsequent chapters.

Chapter four will detail the second study of the thesis entitled 'The effect of intermittent running on the magnitude, rate and variability of the mechanical loading dose'

Chapter five will detail the third study of the thesis entitled 'quantifying the osteogenic potential of high-intensity intermittent running with varying exercise-to-rest intervals: A re-examination of the osteogenic index'

Chapter six will detail the fourth study entitled 'The effect of intermittent running on biochemical bone turnover markers'

Chapter seven will summarise and synthesise the overall findings of the thesis

CHAPTER 2:
Review of Literature

2.1 Introduction

The objective of this chapter is to review the relevant literature related to bone, and its response to exercise-induced mechanical loading with reference to rest-inserted 'intermittent' exercise. This will provide further information and critique of the concepts addressed in Chapter 1. The review will cover research from both animal and human studies. It seems pertinent to include animal models as much of the pioneering work on bone's response to mechanical load is from various animal models. However, the apparent differences between the micro- and macro-structure of animal bone to human, including ambulatory and other important genetic differences will be discussed in context of the transference of the findings from animal to humans. Whilst the thesis will also include human training studies, the focus will be on acute responses (both intermittent and continuous) to exercise.

The literature review will consist of four main areas: section 2 will discuss basic bone biology, bone cell function and optimising the bone's response to mechanical loads including beneficial effects of rest-inserted loading; section 3 will discuss and review the challenges and complexities of developing intermittent exercise protocols, including a review of the important physiological and biomechanical responses to intermittent protocols; section 4 will discuss techniques for quantifying the osteogenic potential of exercise, focusing on the osteogenic index; and, section 5 will review the use of bone turnover markers to assess short-term bone turnover responses to exercise. This section will conclude with a critique of the most recent studies investigating intermittent exercise and bone remodelling.

2.2 Bone (osseous) tissue background and biology

2.2.1 Bone's structure and multifunctional role

Osseous or bone tissue is the most highly studied of the skeletal-articular system (Robling & Turner, 2009) comprising of bones, tendons and ligaments. Bone plays a multifunctional role in

the body including protection for internal organs, mechanical support for movement by providing levers and pivots driven by the muscular system (Allen & Burr, 2014), provides a reservoir for important minerals (calcium and phosphorus), and is central to hematopoiesis (Robling & Turner, 2009).

Bone structure

The tissue properties of bone are made up of an organic phase (25%), an inorganic phase (70%) and water (5%). The organic phase is made up of predominantly type 1 collagen (~90%), and non- collagenous proteins, glycoproteins, and proteoglycans (Allen & Burr, 2014). Collagen protein provides resistance against tensile forces. The inorganic matrix consists of calcium phosphate, $Ca_3(PO_4)_2$ which interacts with calcium hydroxide $Ca(OH)_2$ to form hydroxyapatite crystals $Ca_{10}(PO_4)_6(OH)_2$, which offers resistance against compressive forces (Burr & Akkus, 2014). At the microstructural level bone tissue is organised based on its functional needs such that bone is denser (cortical or compact bone) or more porous (cancellous, trabecular or spongy bone) (Burr & Akkus, 2014).

Cancellous bone is characterised by a lattice network of fibres, whilst not as strong as the cortical bone, serves to redirect mechanical load to the cortical bone. Cancellous bone is found at the metaphysis and epiphysis of long bones (e.g. proximal femur), flat bones (e.g. the skull) and vertebrae. Its structure provides strength to bone much like the structure of the Eiffel tower (Burr & Akkus, 2014). Cortical bone surrounds the cancellous bone and is located at the shaft or diaphysis of long bones, such as the femur. Cortical bone has small pores associated with the canals of the Haversian system, the osteocyte lacunae, and canaliculi. Together they form the osteon which is a fundamental functional unit of bone (Burr & Akkus, 2014).

2.2.2 Bone modelling and remodelling

In the 1940s Harold Frost began to describe how bone cells (osteocytes, osteoblasts and osteoclasts) work together (Frost, 1999), where osteoclasts are activated allowing for bone resorption to occur. Subsequently, osteoblast cells are activated and a period of bone formation follows (Duncan & Turner, 1995). The two processes by which bone turnover occurs are *modelling* and *remodelling* (Frost, 1997a). With modelling osteoclasts and osteoblasts work independently on the periosteal and endocorticol surface. During modelling, bone is removed from the endocortical surface and added onto the periosteal surface (appositional growth) changing the shape and geometry of the bone. Generally modelling occurs during childhood to reflect the environmental demands of load-bearing and growth of the skeletal system.

Remodelling reflects the continuous resorption and formation of bone where old bone is removed and new bone is replaced (Bellido et al., 2014). The remodelling process occurs through the concerted action of the basic multicellular unit (BMU) which consists of bone resorbing cells (osteoclasts), bone forming cells (osteoblasts), the osteocyte within the cell, and the bone lining cells covering the bone surface (Bellido et al., 2014). The bone remodelling cycle and interaction between osteoclasts, osteoblasts and osteocytes is displayed in Figure 2.1.
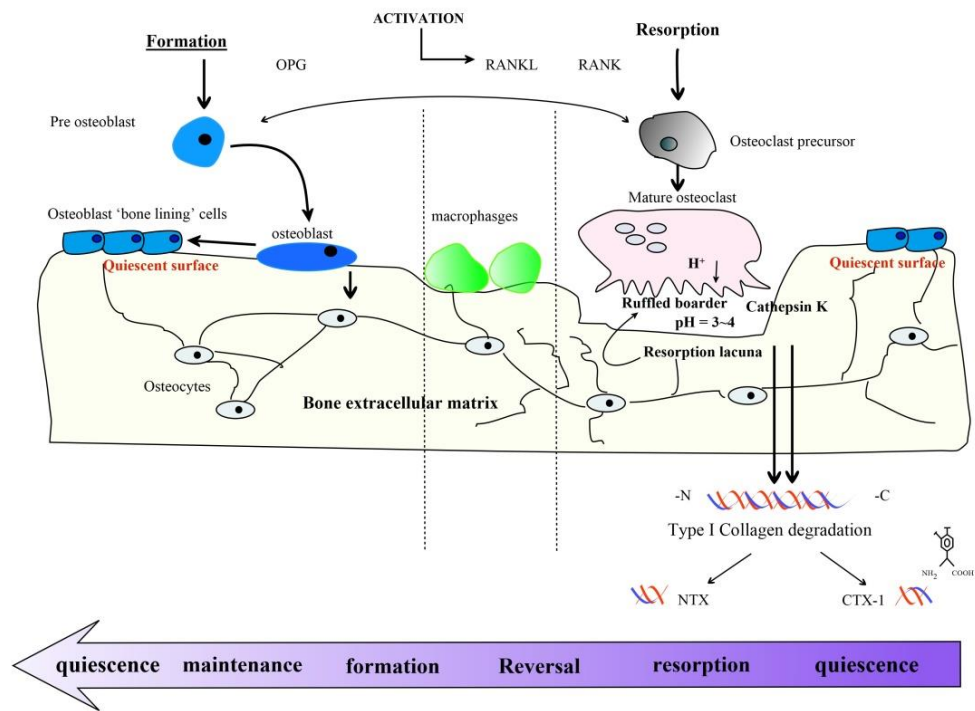
Figure 2.1: The bone remodelling pathway. The remodelling of bone follows an activation (A) →
resorption (R) → formation (F) cycle. This cycle is made up of 5 stages: (1) activation, (2)
resorption, (3) reversal, (4) formation and (5) quiescence. H^+ ions cause extracellular acidification,
which leads to resorption of bone including digestion of type I collagen fragments such as amino-
terminal cross linking propeptide (NTX) and carboxy-terminal propeptide (CTX) (Banfi et al.,
2010). Image adapted from Seibel et al. (2005)

Bone cells

Osteoblasts are mononuclear stromal cells which secrete un-mineralised matrix at the first stages
of bone formation. Osteoblasts become quiescent on the bone surface and become bone lining
cells, or they become encased in the matrix in the lacuna and become osteocytes. There is never
more than one osteocyte that resides in the lacuna and they maintain communication with each
other via dentritic processes that extend from the cell through the canaliculi (Sims & Martin,
2015). Because of this osteocytes have been postulated to be the primary mechanosensory
machinery residing in bone (Bloomfield, 2001). Osteoclasts are giant multinuclear cells of
hematogenic origin which resorb the bone by adhering to the bone surface (Bellido et al., 2014).

The turnover of bone occurs at four envelopes, the periosteal (outer surface), trabecular, endocortical (surrounding medullary cavity) and Haversian (intracorticol).

The remodelling process begins initially with an activation phase (osteoclastogenesis) which occurs when the hematopoietic stem cell is stimulated followed by the generation of mononuclear cells which initiate osteoclast precursors (Bellido et al., 2014). The precursors exit into the peripheral circulation near the resorption site and differentiation of mature osteoclast cells occurs which dissolves the bone. This process requires the presence of macrophage colony stimulating factor (M-CSF) and the receptor activator of nuclear factor kβ ligand (RANK-L) (Robling & Turner, 2009). It is worth mentioning here the importance of the RANK-L / osteoprotegrin (OPG) axis. When OPG is present it acts as a decoy receptor for RANK-L binding to its sites and stopping RANK from binding thus inhibiting osteoclastogenesis (Robling & Turner, 2009). As such, the RANK-L/RANK/OPG axis is an important axis for controlling osteoclastogensis and is activated via a variety of stimuli including parathyroid hormone (PTH), prostaglandins, interleukins, vitamin D3, corticosteroids and mechanical loading (Bellido & Hill Gallant, 2014).

The mature osteoclast, in the presence of β3 integrin, creates a compartment between the ruffled border of the osteoclast and the bone surface. H^+ ions are pumped across the border reducing the pH to dissolve the bone creating resorbed packets of bone called Howships lacunae (Burr & Akkus, 2014). Apoptosis of the osteoclast cells precede the initiation of osteoblasts which fill in the resorption pits depositing un-mineralised matrix centripetally creating concentric lamella layers. At a specific point this depositing ceases leaving the Haversain canal (Allen & Burr, 2014). The initial phase begins with the proliferation of mesenchymal stem cells. Expression of a number of transcription factors including runt-related transcription factor 2 (Runx2), directs the precursor cells towards the osteoblasts lineage and away from the adipocyte, myocyte and chondrocytes. The expression of Runx2 and Wnt signalling components (Wnt/β catenin pathway) are required for further differentiation into the mature osteoblast which produce type 1 collagen and alkaline phosphatase. As the osteoblast moves away from the bone surface some cells get left behind. These cells [osteocytes] become encased in the bone matrix and as the matrix begins to mineralize

the osteocyte matures and expresses other genes including dentin matrix protein 1 (DMP-1) and SOST.

2.2.3 Bone responses to mechanical loading

More than 100 years ago the pioneering work on bone by the German surgeon Julius Wolff resulted in his observation - '*every change in the form and function of a bone or in function of the bone alone, leads to change in its internal architecture and in its internal form*'. The orientation of *Trabecular* bone coincides with the trajectories of direction in which strain is applied to the bone (Frost, 2004), where strain or deformation is the ratio of change in length of the bone from its original length. Wolff proposed that the mechanical load placed on the bone is 'sensed' and responds by changing the architecture of bone (Frost, 2004). In regions of low stress, the trabeculae develop a low density, open-cell, rod like structure. Higher stresses produce a much denser plate-like structure.

Later, Harold Frost proposed that the mechanism that controls the ability of bone to respond to its mechanical environment would behave like a thermostat turning 'on' and 'off' and so was termed the *mechanostat* (Frost, 1987). The biological machinery that determines the strength of bone tissue includes a negative feedback system as depicted below in Figure 2.2.

Mechanical usage → bone → mechanostat → bone mass effect

Feedback loop
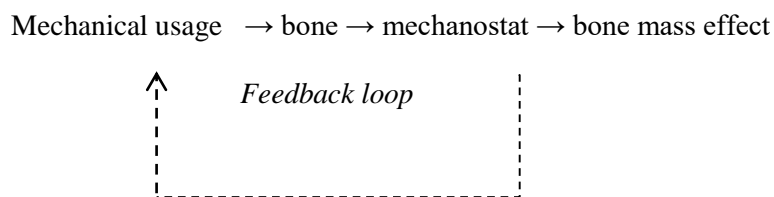
Figure 2.2 The mechanostat (taken from Frost et al. (1987)).

Multiple interacting variables which affect the 'mechanostat' and bone adaption are shown in Figure 2.3.
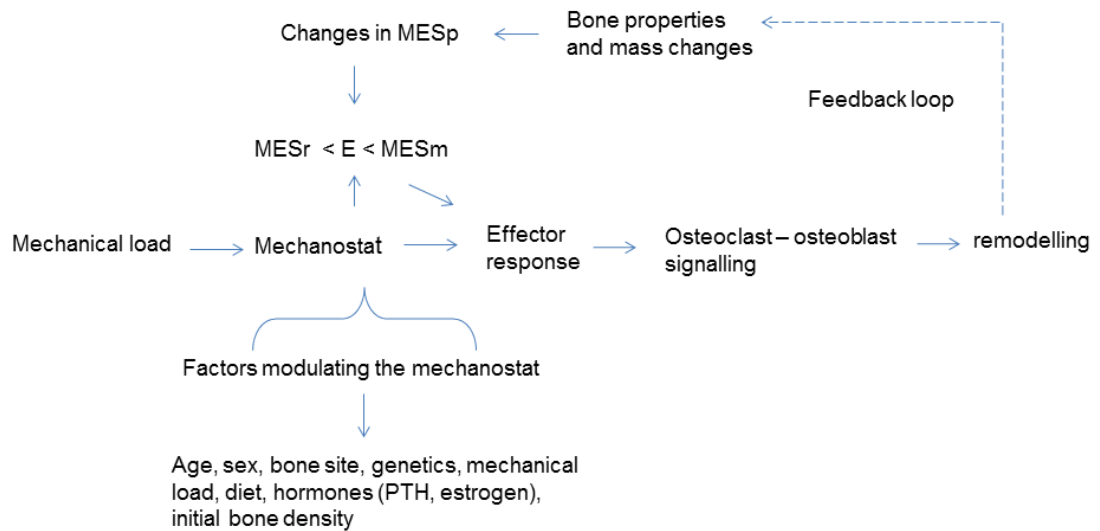
Figure 2.3 The important modulating factors which can influence the mechanostat. E is the typical peak strain, MES is the minimum effective strain, MESr is the strain below MES characterised by disuse resulting in remodelling, MESm is the strain above the MES resulting in modelling.

Intuitively the 'mechanostat' theory proposes that individuals who are more physically active throughout life should have stronger bones than their less active counterparts, provided of course those nutritional, hormonal and ethnic factors are all accounted for. Cross-sectional studies investigating the relationship between BMD and sedentary individuals and athletes from various sporting modalities have provided evidence in support of the 'mechanostat' theory (Hind et al., 2012; Hind et al., 2015). Several components of the mechanical load have been identified that influence the anabolic response including strain rate, magnitude and frequency. The components of the delivered mechanical stimulus likely interact with each other. However, below each of the components are considered in isolation. Within an exercise model it would be challenging to isolate one component and consider it was held constant. However, the mode of exercise has been shown to have important mechanistic features to increase bone properties (Eriksen, 2010). This

has been confirmed in human studies by comparing different groups of athletes from different disciplines (Taaffe et al., 1997).

Magnitude

The magnitude of the mechanical strain is proportional to the adaptation of bone (Cullen et al., 2001). However, one must not consider the relationship of strain magnitude and bone adaptation to be linear (Turner, 1998). According to the 'mechanostat' theory (Frost, 1987) there exists a minimum effective strain (MES) threshold whereby a strain that exceeds the bone's genetically determined threshold will result in (re)modelling of the load bearing bones (MESm). Therefore, provided the mechanical stimulus is large enough bone will change. However, it is likely these strains are close to the fracture index, particularly in healthy young individuals (Frost, 1999). Thus, very high intensity exercise interventions are required to positively stimulate bone adaptation in young individuals (Izard et al., 2016) which might not be appropriate for elderly or clinical populations. Strains below the peak threshold initiate the disuse-mode remodelling (MESr) which enhances remodelling, in favour of resorption. Moreover, repeated mechanical loads have the potential to cause microdamage, thus continuous exercise is likely deleterious to bone. Strains have an operational threshold that lies above the MESm and if high enough can lead to an accumulation of microdamage ultimately leading to stress fractures (SFx) (Frost, 1987). The relationship between mechanical loading and remodelling follows an inverted 'U' shape (Bloomfield, 2001). Between the period of enhanced remodelling during disuse and overuse is a point where remodelling is minimised, which is referred to as the physiologic range (Frost, 2004) likely to be around 1500-2000 microstrain ($\mu\varepsilon$) (Bloomfield, 2001). However, the periosteal surface formation will continue to increase with increased loading (Robling & Turner, 2009).

Accordingly, it has been demonstrated from human studies that high-impact exercise, as measured by the ground reaction force (GRF), (Ebben et al., 2010; Tobias et al., 2014) or peak accelerations (Heikkinen et al., 2007), precipitate the greatest bone mineral density, size and shape. For example longitudinal studies by Taaffe et al. (1997) followed a group of female gymnasts and collegiate

female athletes over 12 months. Lumbar spine, femoral neck and whole body BMD (g/cm^2) measured by dual X-ray absorptiometry (DXA) were greater for gymnasts compared to the female runners and swimmers. Consequently, the authors concluded that sports inducing high skeletal forces in multiple directions are best to improve BMD. These cross-sectional studies make important links between the characteristics of the sporting movements and bone accrual. However, they do not conclusively investigate how and why high, multidirectional forces may be more osteogenic for bone. These studies also fail to control for other important variables such as diet, training load and other daily activities which might be confounding variables. Therefore, mechanistic studies in human models are required

The minimum threshold to increase bone mass in humans has been shown to be in the region of 3.9 g, where g is equal to the acceleration of gravity (9.81 $m·s^{-2}$) (Vainionpää et al., 2007). However, lower accelerations within the region of 1.1 g (such as walking) are associated with a redistribution in bone mass in areas of loading such as the femoral neck in postmenopausal females (Martyn-St James & Carroll, 2008). However, these magnitudes are related to females (postmenopausal) and focus on global magnitudes rather than being skeletal site specific; therefore, more work is required to establish thresholds at different sites across multiple populations.

Modes of exercise such as jumping are considered to be very high impact resulting in peak accelerations in the range of 4-9 g or > 8.5 body weights (Judex & Zernicke, 2000). Conversely, hopping and running produce impacts of slightly lower g's (Milgrom et al., 2000) but still exceeding the osteogenic threshold. Moreover, running exercise has been shown to result in greater strain rates than exercise modes such as drop jumps (Milgrom et al., 2000). Importantly, these exercises apply strain on the bone at particular sites, and it is these sites that will be remodelled in order to maximise the structural integrity of the bone at that site (Lanyon et al., 1975; Burr et al., 1996). Whilst these studies are novel and informative often they are invasive

surgically implanting strain gauges onto the bone (e.g. tibia) and therefore carried out on a limited sample sizes.

Frequency

The frequency of the strain stimulus is also a component of importance whereby the adaptation of bone to a certain magnitude may be enhanced at higher frequency bands (0-30 Hz) (Rubin et al., 2001). Throughout our daily routines our skeleton experiences thousands of very small strains (< 10 µε) such as those from sustained muscular contractions (Fritton et al., 2000). For example, during standing our muscles contract to maintain posture making small adjustments to account for the small deviations in postural sway (de Jong et al., 2010). These muscle contractions subject bone to low magnitude, high frequency (> 30 Hz) vibrations. Animal models have demonstrated that brief exposure to high frequency vibrations produced an osteogenic response (Rubin et al., 2001).

However, human studies utilising whole body vibration (WBV) plates have shown equivocal findings (Bemben et al., 2010; von Stengel et al., 2011), which may be due to the different populations (males vs. females, young vs. old) used. As we have seen from the 'mechanostat' theory, there are multiple variables which affect the response of bone to the various components of mechanical load (Figure 2.5) (Frost, 1987). Moreover, there appears to be a non-linear relationship between the frequency (cycles per second or Hz) of the mechanical stimulus and bone formation (Nagaraja & Jo, 2014) where the greatest response occurred between 5-10 Hz, and beyond 10 Hz (up to 30 Hz) there were no more positive changes (Warden & Turner, 2004). This evidence should be interpreted with caution as data are from animal models and it is likely that the frequency and magnitude of load to which bone responds differs between species. For instance the vibration studies (Rubin et al., 2004; Gusi et al., 2006) which have demonstrated an enhanced osteogenic response have examined frequency ranges between 17-90 Hz. However, they also varied other mechanical strain components such as the magnitude of strain. Therefore, it is difficult to delineate between magnitude, frequency and rate.

Moreover, at a whole body level the vibration studies would induce muscle contractions which would likely be a confounding factor as muscle contraction has been postulated to produce the largest mechanical loads (Frost, 1997b). Due to these limitations it is unclear whether there is an optimal frequency threshold, and the interaction between the magnitude, intensity and frequency requires further investigation, particularly in human studies (Kelley et al., 2014).

Rate

The strain rate, defined as the rate of change in strain (deformation) of bone over a given time has been postulated to be most important to induce an osteogenic response (Burr et al., 2002). This assertion is made from utilising animal models, specifically rat models. This suggests that impact exercise would likely be more osteogenic than non-impact forms of exercise such as cycling (Nagle & Brooks, 2011). The greater strain rates have been shown to result in greater intracortical fluid flow thought to be critical in driving cellular responses (Turner et al., 1995), as such dynamic loading is fundamental for bone adaptation (Lanyon & Rubin, 1984). However, this data are generated from *in-vitro* models and would be difficult to replicate in human models.

In addition to the fundamental components underpinning bone adaptation, a number of 'laws' governing bones osteogenic response to loading have been established (Turner, 1998). These laws have been developed through in-vivo animal models, including the avian ulna model (Turner, 1998). The models are defined by mathematical algorithms. Understanding these phenomena will enable us to optimise exercise programmes to enhance the anabolic effect of bone (Turner & Robling, 2003).

2.2.4 Optimising exercise based on the 3 rules which govern bone formation

Animal studies, specifically rat models, have been influential in improving knowledge on how the type, intensity, duration and frequency of mechanical loading can contribute to increased bone

mass (Turner & Robling, 2005a). Animal models have revealed that the adaptation of bone is governed by three rules or laws, presented in Figure 2.4.
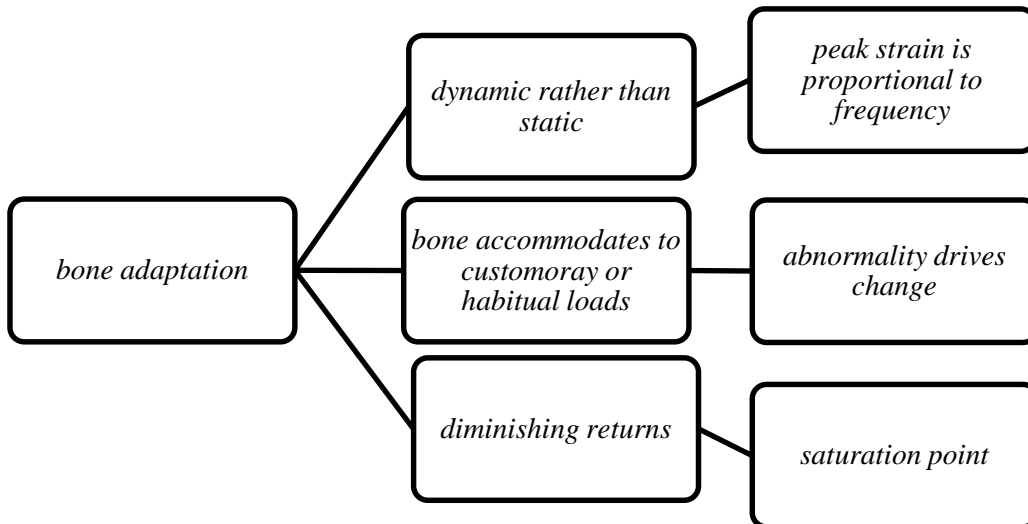
Figure 2.4 The proposed rules which govern bone adaptation (Turner, 1998).

The first rule states that dynamic loading is required for bones to adapt. Furthermore, the frequency (number of cycles per second) of the periodic loading waveform and the magnitude of the strain are proportional, which can be represented mathematically by equation 1.

$$k \cdot \sum_{i-1}^{n} \varepsilon_i f_i \qquad Eq\ 1$$

Where k is a proportional constant such as the number of steps or jumps, ε is the peak to peak strain and f is the frequency of the loading waveform. This equation demonstrates that static loading would not result in adaptation because $f = 0$ (Turner 1998).

The importance of dynamic loading is elegantly demonstrated in *in-vitro* models assessing the shear stress on osteocytes created by interstitial fluid flow (Batra et al., 2005). The dynamic loading increases in bone fluid flow through the lacuno-canalicular network creating hydrostatic

pressure gradients. As these pressure gradients are equilibrated via movement of extra cellular fluid, shear stress increases on the plasma membrane of osteocyctes, bone lining cells and osteoblasts (Robling et al., 2002) triggering a cascade of signalling events. The magnitude of the fluid flow is proportional to the mechanical loading rate and hence bone is more sensitive to dynamic rather than static loading. Whilst this work is crucial for understanding the cellular signalling responsible for bone modelling and remodelling, often these studies induce artificial scenarios in the bone cells milieu which makes it difficult to apply to 'in vivo' research.

The second rule discusses the importance of mechanical loads being unusual and diverse, as it has been shown that bones become accustomed to habitual loads, and therefore do not lead to any further adaptation (Skerry, 1997). Typically, models measuring bone strain have been investigated under steady-state conditions. However, non-steady state events are important to assess as these types of loading patterns occur in natural habitats of animals (Weinstein, 2001). Studies investigating steady-state loading have shown that bone strains increase over a range of gait speeds (Biewener & Taylor, 1986; Lieberman et al., 2003) measured at the midshaft of long bones. However, with non-steady state loading, it is likely the pattern of strain distribution in terms of axial bending would be non-uniform (Moreno et al., 2008).

The final rule of bone adaptation reveals that the sensitivity of bone cells diminishes after a certain number of repetitions of the loading cycle (Umemura et al., 1997). Rat models have provided evidence for a sensitivity threshold which when reached and exceeded can be deleterious to bone formation (Turner & Robling, 2003). It has been demonstrated that the 'mechanostat' desensitises to mechanical loading that continues past a saturation point at ~50-100 cycles (Robling et al., 2002). Interestingly the increase in loading cycles and decrease in mechanosensitivity fitted an exponential decay rate (Umemura et al., 2002) whereby bone lost almost 95% of its sensitivity after only 20 loading cycles. The desensitization of bone to multiple loading cycles fits an exponential model which is expressed in equation 2.

$$Recovery = 1 - e^{-t/\tau} \qquad Eq\ 2$$

Where *e* is the naperian log, *-t* is the time between bouts, and τ is the time constant.

Implicit in the mechanosensory saturation model is the importance of inserting a period of unloading or rest to allow the bone cells to re-sensitise. Turner and Robling (2005) demonstrated that when multiple repetitions of axial loading of a rat ulna were performed in separate discrete bouts of 6 sets of 60 cycles, bone formation exceeded that of the continuous bout of 360 cycles. Currently the cellular and molecular mechanisms involved in loss and restoration of mechanosensitivity are unclear. Potentially the cellular mechanism for rest-inserted loading maybe an increase in the intracellular calcium magnitude, frequency and percentage of cells responding to strain induced by oscillatory fluid flow (Batra et al., 2005). Nevertheless, the phenomenon provides further evidence for a 'mechanostat' given the apparent switching 'on' and 'off' of cellular and molecular mechanisms involved in mechantransduction (Frost, 2004).

What is clear is that there are several timescales to which the mechanosensory saturation and restoration occurs (Gross et al., 2004). These timescales range from seconds to weeks and by manipulating these timescales the magnitude of the osteogenic response changes (Srinivasan et al., 2015). The minimum recovery period seems to be somewhere between 7-14 seconds (Skerry, 2006). However, this has not been established in human studies and therefore these timescales are likely to be different between different species.

A further benefit of the inserted-rest phenomenon is that lower strain magnitudes and frequencies are required to induce an osteogenic response (Srinivasan et al., 2002). This is an important finding as it has been demonstrated that as we age the signals delivered to our bone cells need to be 'louder', potentially caused by the apoptosis of osteocytes in the lucuno-caniculi system (Kohrt, 2001). However, providing larger strain magnitudes to the aging bone would be counterintuitive as the threshold point before a fracture would be lower for a given peak strain and therefore there would be an increase in fracture risk with higher loads. Therefore, if lower strains that would otherwise not be osteogenic could be beneficial with the addition of rest phases this would offer significant clinical implications (Srinivasan et al., 2015).

Section summary

From these pioneering animal models it is apparent that loading which is unpredictable (unusual), predominantly load-bearing (impact), and of high magnitude and rate of force development is most osteogenic for bone (Turner & Robling, 2005b). Furthermore, due to the mechanosensory saturation/ re-sensitisation phenomenon, loading cycles must be of short duration and interspersed with periods of rest (Turner & Robling, 2005a). The use of a rest-inserted loading component in an exercise condition may provide an additional component to enhance the anabolic effects of exercise. The transferability of the animal models to human studies should be made with caution, and this will be discussed in the following sections.

2.3 Intermittent exercise and bone

The insertion of rest phases between periods of loading has high internal validity in movement patterns reflective of both animal and humans in the real world (Biewener, 1990). This type of movement is referred to as intermittent (Weinstein, 2001). Few studies have addressed the use of intermittent exercise as a potential mode to enhance bone remodelling (Helge et al., 2010; Allison et al., 2015), despite recommendations for the use of intermittent exercise in clinical guidelines (Kohrt et al., 2004). In a recent opinion piece on the effects of running on bone (Boudenot et al., 2015) the authors discussed the deleterious effects of continuous running despite it being an impact-mode of exercise. The fatigue on bone caused by the multiple loading cycles was proposed as a potential mechanism for the deleterious effects. To overcome bone fatigue Boudenot et al. (2015) proposed that interval running might offer a more osteogenic environment. Gross et al. (2004) demonstrated that ~10 seconds of rest between periods of exercise has profound effects on bone adaptation increasing periosteal activation more than 15-fold (21.9%) beyond that of the control limb. However, much of the research comparing continuous and intermittent running has been performed in rodents (Umemura et al., 2002; Huang et al., 2008).

An important consideration is to recognise the limitations of the animal models, particularly the ulnar avian models, which brings into question the transference of the findings into human studies. The obvious differences between rodents and humans include; ambulation of four limbs for rats vs. two for humans, the smaller stature of rodents, and the shorter life span of rodents. Moreover, animal bone is thicker relative to human with human bone being ¼ the thickness of the diameter of long bone compared to ½ for animal bone. Further macrostructure differences include more porous cortical bone in humans (Lelovas et al., 2008). This brings into question whether similar rest phases would elicit similar responses in humans. If not, would greater magnitudes, and more loading cycles be required to fully saturate human bone? Moreover, would human models require longer rest periods in the form of hours, days or weeks rather than seconds? The paucity of evidence in humans on the utility of inserted-rest interventions requires attention.

In a recent cross-sectional study (Nilsson et al., 2013) soccer players were reported to have higher peak BMD compared to age-matched individuals who participated in resistance exercise. Whilst association does not prove causality the value of the cross-sectional study has identified a possible relationship between bone health and exercise that is high-intensity, intermittent and includes high-impacts, which somewhat supports the findings of previous rat studies. There is an accumulating body of evidence (Table 2.1) utilising small-sided soccer games (SSGs), as a beneficial exercise model for the prevention and treatment of non-communicable diseases (Krustrup & Bangsbo, 2015) and improvements in bone formation. A number of randomised and non-randomised controlled trials using SSGs have demonstrated improved leg bone mineral content in untrained males (Krustrup et al., 2010b), increased BMD of the tibia in premenopausal females after a 14 week intervention (Helge et al., 2010), and increases in bone turnover markers from baseline levels, in favour of formation, in a group of homeless males (Helge et al., 2014a).

Table 2.1 Relevant studies from the use of small-sided soccer games and bone health.

Author/Year	Study title	Purpose	Design/Method/ participant no. (n=)	Results/Discussion
Helge et al. (2014b)	Street football is a feasible health-enhancing activity for homeless men: Biochemical bone marker profile and balance improved	To investigate use of soccer as intervention for homeless men	32 homeless men, 22= FG, 10 = CG.TRAP 5b, leptin, OC, postural balance, BMD. 4 vs. 4, 16 X 22 m pitch. 1 h ~ 2 x per week	FG = 27% ↑ OC NS change in TRAP5b. NS change in CG. FG aBMD ↑ 1%, NS change CG
Helge et al. (2014a)	Recreational football improves bone mineral density and bone turnover marker profile in elderly men	To investigate the use of recreational soccer on BMD and BTMs in elderly men	26 healthy, sedentary men ~68 years FG = 9, RG = 9, CG = 8. 4, 12-months BMD of femur and whole body. OC, CTX-I, P1NP	FG=BMD in femur ↑ 1.8% (0-4 month) ↑ 5.4% (0-12 months) ↑ in OC and P1NP > 40%. In RG and CG no changes
Helge et al. (2010)	Recreational football training decreases risk factors for bone fractures in untrained pre-menopausal women	To investigate if recreational soccer evoked gains in BMD in premenopausal women.	65 healthy untrained women. RG, FG, CG. pQCT used to assess bone geometry, BMD Postural balance assessed through flamingo balance test. Also measured CMJ, leg extension power. 14-week intervention	BMD increased with recreational football. As did hamstring strength and maximal power. Proximal bone sites from the impact point less change. Results in agreement with cross-sectional studies. pQCT better than DXA

Krustrup et al. (2010b)	Long-term musculoskeletal and cardiac health effects of recreational football and running in premenopausal women	Investigate musculoskeletal and cardiac adaptations elicited by 4 & 16 months of recreational football	28-healthy untrained females. FG, RG, CG. 1 h, 2 x per week for 16-months. 82% HR max for intensity. DXA, echo, maximal dynamic and isometric quadriceps and hamstring muscle strength. Postural balance test, flamingo test. Sudden trunk loading, sprint and intermittent testing.	Greater more favourable long-term adaptations were found in the FG
Mohr et al. (2015)	Effects of soccer vs. swim training on bone formation in sedentary middle-ages women	15 weeks soccer vs 2 x swim intervention (sub-max and HIIE swimming)	83 premenopausal women randomised into soccer (FG), moderate intensity (MS) swim and HIIE swimming (HS), control (CG). DXA, P1NP, CTX-I	P1NP, CTX-I ↑, no change b/w HS, MS, CG. FG increased leg bone mass and blood markers

Notes: small sided soccer games (SSGs) on health and performance parameters including; dual X-ray absorptiometry (DXA), Bone mineral density (BMD), areal bone mineral density (aBMD), football group, (FG), run group (RG), control group (CG), moderate intensity (MS), high intensity intermittent exercise (HIIE), high intensity swimming (HS), peripheral quantitative computer tomography (pQCT), procollagen type 1 amino terminal propeptide (P1NP), carboxyterminal cross-link telopeptide (CTX-I), osteocalcin (OC), Tartrate resistant acid phosphatase (TRAP 5b), no significant difference (NS), heart rate (HR).

Soccer is considered highly intermittent combining movement patterns over the exercise period that include running, jogging, walking, sprinting, tackles, jumps and rapid changes-of-direction (Bangsbo, 1994). A common protocol implemented by the authors (Krustrup et al., 2009; Krustrup et al., 2010a) matches an intensity of ~85% $\dot{V}O_{2max}$ (estimated from monitoring heart rate during games) to a similar intensity during a continuous running protocol. The small-sided soccer games used typically follow a five-a-side or four-a-side format lasting 1 h (Helge et al., 2010; Krustrup et al., 2010b). The duration of the interventions has lasted from 12 weeks to 16 months. The use of duel X-ray absorptiometry (DXA) (Krustrup et al., 2010b) and peripheral quantitative computer tomography (pQCT) (Helge et al., 2010) has been used to quantify BMD and bone strength. The soccer-specific movements during the SSG were assessed using video recordings (Helge et al., 2010), and HR was assessed continuously using HR monitors (Krustrup et al., 2010b; Helge et al., 2014b).

Whilst these are well designed studies including control groups (Krustrup et al., 2009) and randomisation methods (Helge et al., 2010) there are some limitations to note. Firstly, it is unclear how the HR was controlled at a mean of ~85% $\dot{V}O_{2max}$ during the SSGs. The highly intermittent nature of soccer games will result in HR reaching > 90% maximal HR (HR_max) during periods of high-speed running, and then reducing to lower levels in the active rest periods, thus making it difficult to control HR in a field testing environment (Buchheit & Laursen, 2013b). Moreover, the higher intensity exercise will likely introduce cardiovascular drift therefore it is unclear whether exercisers are at the desired intensity (Morton, 2007). Furthermore, the lag in HR at the onset of exercise is slower than the rise in $\dot{V}O_2$, together with the inherent inertia of HR at the cessation of exercise (e.g. recovery), it is possible there was an overestimation of the physiological/ internal training load (Buchheit & Laursen, 2013b). This brings into question the validity of the studies when comparing SSGs to continuous exercise. The difficulties of matching this type of exercise for internal training load and the unpredictable and variable exercise-to-rest ratios introduce a large inter- and intra-individual variability (Buchheit & Laursen, 2013b), and

therefore a more controlled setting is required in order to establish the role of intermittent exercise in bone adaptation.

It is also unlikely that matching the SSGs for intensity and duration will adequately match the overall external training load placed on the exerciser. The multiple changes of direction, and faster running speeds interspersed with walking periods will likely result in a difference in total distance covered and number of loading cycles. This is evident from the relatively short distances covered in football over a 90 minute period (10-12 km) (Bangsbo, 1994). Given the phenotypic response of bone to multiple loading cycles and the laws of diminishing returns (Turner, 1998), it is possible that soccer introduces a favourable mechanical loading environment to enhance osteogenesis. However, due to the lack of control of these variables it is difficult to make any firm conclusions on whether the health improvements were due to the nature of the SSGs *per se* or whether the soccer group simply performed a greater volume of training compared to the continuous run training group, or even *vice versa*.

Moreover, the unpredictable exercise-to-rest ratios of soccer make it difficult to establish any dose-response relationship between the levels of intermittency (frequency in changes of speed) of the exercise bout or comparison between very short (5-15 s), short (30 s) and long (1-3 min) exercise and rest periods. The phenomenon of rest-inserted loading in rodent models has been well controlled delivering a controlled load to the isolated rat bone followed by a controlled rest period (Umemura et al., 1997). Whilst this is difficult to achieve in human protocols, the intermittent protocols and set exercise-to-rest intervals can be made proportional so that one can delineate between the effects of short vs. long intervals and a greater frequency of changes in speed. This might provide important data to support the positive effects of intermittent exercise on bone tissue in human studies and is a focus of the current research question for this thesis.

The mechanical loading pattern between the two forms of exercise (SSG vs. continuous) also should be measured to provide information on the changing mechanical environment, and establish the nuances between intermittent and more continuous loading cycles; however, this

was not performed in the SSG studies. This is understandable because from a practical point of view it would be very difficult to quantify every impact during a soccer simulation. However, the use of accelerometers to quantify the loading dose (Ahola et al., 2010; Chahal et al., 2014) could be implemented to quantify the osteogenic potential. Importantly, it was not the objective of the authors to determine the mechanisms of action and quantify the mechanical strain, and therefore this area requires further investigation.

Section summary

The insights from cross-sectional studies (Breban et al., 2010; Nilsson et al., 2013) and training studies (Helge et al., 2010; Krustrup et al., 2010b) reveal that intermittent exercise, such as soccer, comprises of important attributes that may be advantageous to bone turnover. Although no studies, as yet, have attempted to understand why activities, such as soccer, are anabolic for bone tissue in humans, intuitively, soccer contains all the right characteristics which prove beneficial to bone mass, according to Turner's three governing rules (Turner, 1998). The movement patterns in soccer include periods of high intensity sprinting (high strain magnitudes), interspersed with periods of low intensity movement (jogging, walking and pauses), and includes unanticipated rapid changes-of-direction and jumping resulting in high rates of force development (Bangsbo, 1994). Thus the attributes of this mode of exercise obey the necessary rules considered fundamental for bone adaptation. The periods of inserted rest may contribute to the osteogenic response by allowing the osteocyte network to re-sensitise.

The limitations outlined related to the use of SSGs highlight a need to assess the effect of intermittent exercise on bone in a more controlled setting to provide insight into the potential mechanisms. In doing so, we may be able to utilise these attributes effectively in other forms of exercise to enhance the osteogenic response to exercise. The following questions require investigation:

1. Would changing the duration and frequency of the exercise-to-rest interval have an effect on bone remodelling in humans using short and long intervals, and what is the magnitude of the effect?

2. Would the high-forces occurring during soccer and/or intermittent running interspersed with low-forces counteract each other when controlled for external load?

3. How does the physiological strain induced by intermittent exercise affect bone tissue compared to more continuous exercise?

4. How can the mechanical loading environment be quantified to establish why intermittent running might be more osteogenic?

2.4 Intermittent exercise: History, methodological complexities and acute responses

2.4.1 Intermittent exercise

The use of high-intensity intermittent exercise (HIIE) and high-intensity interval training (HIT), is now common for athletes and general exercisers alike. Research investigating HIIE and HIT is currently very topical (Gibala & Little, 2010; Gibala et al., 2015) particularly due to the reported potent health benefits of HIT and HIIT (Gibala & McGee, 2008) often beyond more continuous exercise. Whilst these two forms of intermittent exercise are synonymous with each other we should be cautious not to use the definitions interchangeably as they will likely elicit different physiological responses (Buchheit & Laursen, 2013b). For the benefit of this review HIIE will characterise intermittent exercise with exercise-to-rest intervals of less than one minute whilst HIT will characterise intermittent protocols with exercise-to-rest intervals greater than one minute (Boutcher, 2011; Buchheit & Laursen, 2013b).

One attributing factor to the popularity of intermittent exercise protocols is that the overall volume (duration + intensity) of training performed to accrue health and performance benefits is lower compared to continuous exercise (Gibala et al., 2012), thus making it a more time efficient strategy for exercisers to implement in their training programmes (Boutcher et al., 2010). The aim of the next section of the review is to discuss the acute physiological responses to intermittent exercise, and link how these responses may affect changes in bone turnover acutely. Methodological complexities related to intermittent exercise will also be discussed in order to establish appropriate intermittent exercise models for the particular population being measured. The review will focus on the responses of healthy male adults as this is where the majority of the literature is directed. Therefore, the transference of the findings to a female cohort should be made with caution.

2.4.2 Intermittent exercise and methodological considerations

The protocols implemented for investigating intermittent exercise vary considerably in their design. Contrasting HIIE and HIT formats can have similar cardiorespiratory responses but be associated with distinctly different anaerobic, neuromuscular and musculoskeletal contributions. According to Buccheit and Larson (2013a) there are at least nine variables which can be manipulated to modify the intermittent protocol (see Figure 2.5). These include; (1) the duration of the exercise and rest phases, (2) the exercise-to-rest ratio (e.g. 2:1, 1:1, 3:1), (3) the exercise mode (e.g. cycling vs. running), (4) the implementation of changes-of-direction or inclines vs. declines, (5) the intensity (i.e. % $\dot{V}O_{2max}$ or HR_{max}) of the exercise and rest phases, (6) the delta change between the intensity of the rest and exercise phases, (7) the frequency of the number of exercise and rest phases within a series, (8) the acceleration and deceleration phases to reach the peaks and troughs, and (9) the total exercise duration.
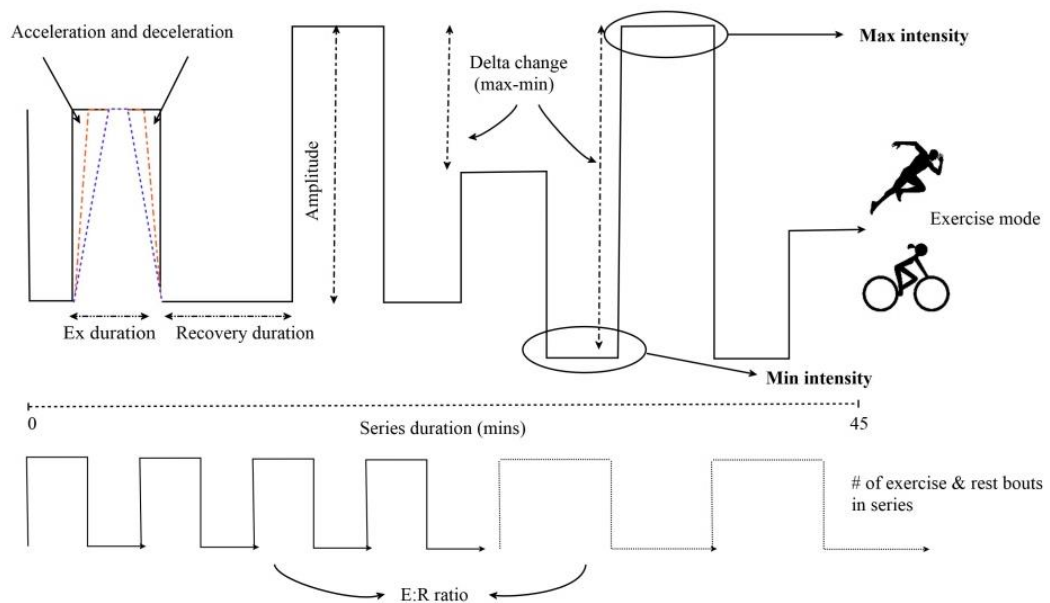
Figure 2.5 A representation of the multiple integrative variables which can be manipulated when programming intermittent exercise protocols (adapted from Buccheit and Laursen 2013a).

2.4.3 The duration of the exercise-to-rest interval

The duration of exercise-to-rest intervals have ranged from 6 seconds (Christmass et al., 1999a) to 4 minutes (Burgomaster et al., 2008). The choice in manipulation of the exercise-to-rest duration depends on the training aims and objectives (Billat, 2001). For example, in order for athletes to maximise gains in their maximal oxygen consumption ($\dot{V}O_{2max}$) time spent at or above their $\dot{V}O_{2max}$ is an important factor to consider (Midgley & McNaughton, 2006). Adapting the exercise-to-rest duration has been shown to affect the physiological strain imposed on the body with long duration exercise-to-rest durations increasing the physiological strain over short durations (Belfry et al., 2012a) (key studies summarised in Table 2.2).

The proportion of the research investigating acute responses to intermittent exercise has been reported in healthy male populations (see Table 2.2). In fact there is an underrepresentation of females in sports science (Costello et al., 2014). The rationale for the use of male populations in

studies is predominantly attributed to the additional challenge to ensure females are tested at specific times during their menstrual cycle. We acknowledge the importance of progressing research to include this population in future research. However, given the studies involved in the thesis will also feature only male participants then these studies are appropriate to include.

When short (6:9 s), medium (12:18 s) and long (24:36 s) exercise-to-rest intervals were compared, higher heart rates, blood lactate, $\dot{V}O_2$, and carbohydrate utilization were observed for the long-duration exercise-to-rest intervals (Price & Halabi, 2005). The results support the notion that longer intervals induce a higher physiological strain (Price & Moss, 2007a). The use of shorter intervals for intermittent exercise may be worthwhile to reduce the anaerobic glycolytic load and the shorter intervals may provide the capacity to exercise at a high-intensity yet with relatively low concentrations of blood lactate (Gosselin et al., 2012).

The results are also supported by early studies using the same exercise-to-rest intervals (Christmass et al., 1999b). Both studies conclude that the longer duration intervals results in a greater contribution of the aerobic system and re-synthesis of PCr. However, Price and Halabi (2005) only speculate this as the authors did not directly measure muscle metabolites. Furthermore, the latter study (Price and Halabi, 2005) uses lower intensity exercise (120% $v\dot{V}O_{2max}$) compared to all-out sprints used in the earlier study (Christmass et al 1999b) which will likely result in the PCr contribution being much lower for lower intensity exercise. Furthermore, whilst not a limitation per se the exercise-to-rest intervals could all be considered as short duration (< 60 s). However, it is interesting that there are still nuances in the physiological responses to exercise-to-rest durations despite all the durations being < 60 s. Thus, these are poignant and important studies investigating the varied physiological responses of different interval durations.

Table 2.2 Physiological and neuromuscular responses to intermittent exercise compared to continuous exercise or intermittent exercise of different exercise-to-rest ratios.

Author	n	Protocol	response	remarks
Akubat and Abt (2011)	12 males	CONT vs. intermittent trial 4 minutes at 25, 50, 75 & 100 $v\dot{V}O_{2max}$	Higher Bla- for intermittent compared to CONT. change in Bla- HR relationship	Greater physiological strain and higher internal training load for intermittent compared to continuous
Balsom et al. (1992)	7 males	X3-intermittent. 30 s rest. 40x15 m (S15), 20x30 (S30), 15x40 m (S40)	↑ Bla- from baseline. Higher for longer sprints. S15=6.8mmol Peak vs. S40=16 mM·L^{-1}	Greater Bla- response with longer sprints
Bucheitt et al., (2007)	9 males	HI vs RS vs. CONT	93±4, 87±2, 80±3% HR$_{max}$ (HI, RS, MC respect) Bla- 11.6±05, 10.9±0.6, 3.5±0.2 (HI,RS, MC respect) % aerobic= 97.3±0.4,85.2±0.7, 92.4±0.3 (HI, RS,MC) %anaerobic=2.7±0.4,14.8±0.7, 7.6±0.3 ANS Sig ↓ for RS and HI	Greater physiological strain with the repeated sprints than CONT. ↑ intermittency ↑ physiol;ogical strain. Change in ANS, ↑ parasympathetic withdrawal Well controlled (not matched for duration though)
Christmass et al., (1999)	7 males	ST (6:9 s) vs. LT (24:36 s) INT. 40 min	>LT (5 mM·L^{-1}) vs. ST (3 mM·L^{-1}) Constant @10 min >ST 40.7±0.8 vs. 36.8±0.5 mL·kg^{-1}min^{-1} >ST 0.84±0.02 vs. 0.77±0.01 KJ·min^{-1}kg^{-1}	Greater physiological strain, higher $\dot{V}O_2$ with long vs. short E:R, greater energetic cost, increase in Bla-

Gaitanos et al., (1993)	8 males	6:30 s work:recovery @ PPO *10 sprints	Sig ↑ Bla- from 0.6±0.1-12.6±1.5 Sig ↑ GLUC 3.9±0.2 to 4.6±0.5. peak@ recovery 5.6±0.6 mM·L^{-1}	_
MacDougall et al. (1977)	6 males	60 s:3 min to exhaustion	↑ 12.8mmol/l GLUC 46% ↑ remained elevated by 12% in recovery	
Price and Halabi (2005)	8 males	E:R ratio 1:1.5120% $\dot{V}O_{2max}$6:9 s, 12:18 s, 24:36 s	↑ RER, lactate, GLUC for long compared to short E:R	Greater physiological strain with long E:R ratio over short
Trapp et al. (2008)	16 males	8*12 s vs. 24*36 s sprint on cycle	↑ catecholamines	↑ in catecholamine response with shorter E:R which might explain greater EPOC and ↓ HRV following more intermittent exercise seen in other studies
Tschakert et al. (2015)	5 males 1 female	CONT, long and short interval sessions	Mean Bla- 5.22 ± 1.41 mM·L^{-1} short vs 9.83 ± 2.78 mM·L^{-1} long	Well-controlled, matched for mean load and exercise duration Short interval similar responses to CONT exercise
Vincent et al. (2004)	8 males	6 s: 5 min wingate. To exhaustion	↑ 14 mM·L^{-1} peak 5-min recovery. 8 mM·L^{-1} for test.↑ AD, ND GLUC: ↑, Peak 5 min post	

Notes: glucose (GLUC), blood lactate (Bla-), heart rate (HR), maximal oxygen uptake ($\dot{V}O_{2max}$), respiratory exchange ratio (RER), autonomic nervous system (ANS), repeated sprint (RS) high intensity (HI), continuous exercise (CONT), short term (ST), long term (LT) exercise-to-rest ratio (E:R), ↑ denotes increase, excess post oxygen consumption (EPOC), electromyography (EMG).

Moreover, these findings are in line with earlier work that reported increases in the exercise and duration of recovery (from 30 s to 3 min with an exercise-to-rest ratio of 1:1) resulted in higher blood lactate accumulation and perceived exertion (Astrand et al., 1960). It was hypothesised that the liberation of energy during the initial phase of short bouts of exercise is practically aerobic (Astrand et al., 1960). Myoglobin was postulated to play an important role as an O_2 store in the muscle that supplies O_2 for aerobic metabolism during the exercise period and is then replenished in the rest periods (Astrand et al., 1960). It was asserted that myoglobin stores reduce if the exercise durations are prolonged (Astrand et al., 1960) thus resulting in reduced O_2 availability. This is supported by greater glycogen depletion observed with longer durations (>30 s) (Essen et al., 1978). Furthermore, the necessity of O_2 availability for performance of high-intensity intermittent exercise has been confirmed by research into intermittent exercise in hypoxic conditions (Balsom et al., 1994).

The seminal research into intermittent exercise (ÅStrand et al., 1960; Christensen et al., 1960) and related work physiology is notable for sparking interest into investigating the use of intermittent exercise as a new research model. A positive of this early work is the control of the mean workload for both continuous vs. intermittent work and accuracy of the method for oxygen intake (SD ± 0.0381 l/min and error of mean of ±0.006 l/min). However data provided by ÅStrand et al. (1960) is reported on one participant, from one experiment, making it difficult to apply the findings to a wider cross-sectional population.

Nevertheless, metabolic and energetic changes seem to be dependent on the duration of the exercise and rest intervals with intermittent exercise. Fat oxidation is lower, there is a higher rate of carbohydrate metabolism, and energy efficiency reduces significantly with longer exercise- and rest- intervals. With longer exercise intervals greater lactate accumulation occurs, and this has been reported to coincide with higher HR and a reduced O_2 availability in the muscle (Christmass et al., 1999b). With increases in blood lactate accumulation there is also reductions in blood pH and changes in acid base balance (Belfry et al., 2012b).

The changes in acid base balance may have deleterious effects on bone. During episodes of acidic load, systemic bicarbonate (HCO_3^-) is consumed to buffer the blood pH, with the deficiency in HCO_3^- then offset by carbonate and phosphate ions which are present in the bone's mineral reservoir (Allen & Burr, 2014). This will result in the bone matrix being dissolved to release the minerals. With chronic acidosis the bone continues in a state of resorption. The hypothesis that lactacidosis induced by exercise may cause greater bone remodelling has been previously postulated (Herrmann et al., 2007).

Data are not clear whether this exercise response may be involved in bone resorption. It has been demonstrated that lactacidosis induced by exercise does not contribute to increased osteoclastogenesis (Herrmann et al., 2007) as shown by the lack of relationship between CTX-I and blood lactate following exercise. However, the time points when the bone resorption markers were taken (3 h and 24 h) have been shown to be the point at which markers return to their normal circadian rhythm as the effects of exercise are transient and moderate in the recovery hours. It is not clear from the study at what times of day the exercise was performed and when the bloods were taken; therefore, it is not clear how well standardised the data are between or within participants. Moreover, limitations in using relationships to determine cause and effect should be considered here.

Higher physiological strain, as assessed by the indirect measurement of the autonomic system, has been reported for HIIE mode and repeated sprint (RS) compared to moderate intensity continuous (MC) exercise (Buchheit et al., 2007). It was concluded that RS resulted in significantly impaired parasympathetic reactivation compared to a MC trial of matched net energy expenditure, and that the impairment related to the level of anaerobic contribution rather than total energetic cost or muscular work. The higher physiological strain reported confirms the wider view that intermittent exercise is more stressful on our physiological system when matched with steady-state exercise of same mean workload (Edwards et al., 1973; Bangsbo 1994). The novel use of recovery kinetics of heart rate variability (HRV) to quantify physiological strain placed on the cardiovascular system, and well matched workloads adds strength to the findings. However, as

duration of exercise can affect the post exercise recovery, this could have been a confounding variable in this study. Recently Cipryan et al. (2016) demonstrated that when the duration of the exercise-to-rest ratio was increased (15, 30 and 60 s) using a fixed exercise to rest ratio (1:1), the shorter duration (15 s) had a greater magnitude of effect of the parasympathetic withdrawal. Given the same amount of time was spent at rest and during exercise phases, and the overall intensity (speed thresholds) were controlled, the prolonged parasympathetic withdrawal with the shorter duration (15 s) protocol may have been affected by the lack of rest between exercise. This is an interesting, well controlled study, further demonstrating that short interval exercise-to-rest durations using a fixed ratio can affect the stress placed on the physiological system differently despite causing similar mean cardiorespiratory responses. Whether this greater disruption to HRV with the very short (15 s) interval would transpire in changes to bone tissue turnover is unclear. A further important aspect of this study is it is one of the few studies to have compared specific short interval exercise durations using a fixed ratio on the same participants.

Experimental models support the role for the sympathetic nervous system (SNS) in bone metabolism, such as the use of propranolol which acts as blockade for the SNS increasing bone mass (Elefteriou et al., 2013). Therefore modulating the SNS and autonomic system might modulate bone mass. However, it is unclear whether there is a link between measures of HRV and bone mass (Maser et al., 2009). Nevertheless, the use of HRV as a measure of cardiovascular strain provides insight into the potential differences in different intermittent movement patterns which might affect changes in bone remodelling either directly or indirectly.

The manipulation of the duration of the exercise-to-rest intervals has important implications for this thesis. As discussed in Section 2.2.4 mechanical loads that are interspersed with rest phases or low intensity exercise increase the osteogenic response of the overall mechanical loading environment. In animal studies the timescale of these changes ranges from seconds to weeks. At the lowest end of the scale rest periods of between 7-14 s seem to be sufficient to re-sensitise the osteocyte network to the proceeding mechanical loads. In human studies it is unclear what the

magnitude of the effect of the inserted rest on osteogenic responses might be as this phenomenon has not been adequately investigated. Furthermore, it is unclear whether rest phases of similar duration to those identified in animal studies would be transferable to humans. Whilst it would not be feasible to directly measure the re-sensitisation of the network using an in-vivo model such as intermittent exercise, measuring the acute bone remodelling responses might offer insight into how intermittent models of different exercise-to-rest intervals might influence bone turnover, and this will be discussed in latter sections of the review.

From a biomechanical point of view, acute neuromuscular and musculoskeletal 'strain' exerted by intermittent exercise can effect tendons, tension on locomotor muscles, joints, muscle fibre recruitment and bones (Buchheit & Laursen, 2013a). Limited evidence exists on the effect of intermittent exercise on the loading of the musculoskeletal system (Krustrup et al., 2010a). The use of surface electromyography has demonstrated different intra-muscular variations, and failure to fully activate musculature by the intermittent protocol (Billaut et al., 2006). Moreover, Brocherie et al. (2015) reported alterations in running mechanics with reductions in vertical stiffness, stride frequency and muscle activity (root mean square of EMG) of the *rectus femoris* and *bicep femoris* over repeated sprinting bouts. These different neuromuscular responses may contribute to different mechanical loading environments on the bone effecting the magnitude, rate and frequency of loading on the bone cells. The variable loading environment through different stride patterns and changes in neural drive could then impact on the different compressive and tensile stresses on the bone. This may ultimately lead to changes in fluid flow, shear stress and piezoelectricity (Duncan & Turner, 1995) effecting the transduction mechanisms within the bone cells (Batra et al., 2005).

Section summary

In summary, when compared to continuous exercise of the same external workload (kJ) or intensity, intermittent exercise appears to place greater physiological and neuromuscular strain on our physiological systems. This is seen with higher submaximal heart rates, greater blood lactate

accumulation, higher $\dot{V}_E$, RPE and inhibition of parasympathetic reactivation in recovery. The specific exercise-to-rest ratios used and duration of the exercise and recovery intervals of the intermittent exercise are however confounding variables and ones that can effect both physiological and mechanical responses. Investigation of the proportional or disproportional change of different exercise-to-rest intervals on our physiological systems requires further work to aid in understanding more about intermittent exercise. It is not clear how these different acute responses might affect bone remodelling.

2.4.4 Methods to prescribe the intensity of intermittent exercise

Another variable which affects the physiological responses to acute intermittent exercise is the intensity of the exercise dose (e.g. percentage of $\dot{V}O_{2max}$) (Kraemer et al., 2002). The overall exercise intensity of the session might be to maintain a mean $\dot{V}O_2$ of 70% for example. However, the individual exercise bouts might be at intensities above $\dot{V}O_{2max}$. The decision on the intensity is made again based on the training aim, the population training, and the desired duration of the exercise session. For example, in a well- controlled study by Billat et al. (2001) running at a mean critical velocity (equal to 85% $v\dot{V}O_{2max}$) allowed middle distance runners to reach and sustain $\dot{V}O_{2max}$ for a prolonged period of time, ultimately stressing the cardiovascular system inducing training benefits. In this study a 15 x 15 s short interval was used. The study however also manipulated the amplitude of the exercise and rest interval intensities. The population used were well trained and therefore the findings are not necessarily transferable to a wider population.

Ultimately the intensity of the exercise interval will dictate how long the duration of the proceeding rest phase will be and the intensity of the rest phase (Wakefield & Glaister, 2009). The intensity of the exercise also has important implications for this thesis as exercise intensity must be high enough above the minimum strain stimulus to shift changes in bone tissue (Scott et al., 2011). The choice in intensity will depend on the type of population being investigated. Lower

volume intermittent exercise has received recent attention in clinical populations. However, a discussion on this is beyond the scope of this review.

There are multiple methods used to control the intensity of intermittent exercise in order to standardise and/or individualise the exercise intensity. These include: percentages (%) of maximum heart rate or heart rate reserve (% $\dot{V}O_2$ or $\dot{V}O_2$ reserve), RPE, % velocities at $\dot{V}O_{2max}$, and other speed threshold based approaches (Buchheit & Laursen, 2013b). The choice of method will likely be dictated by the availability of equipment to assess the athlete and/or patient, and the practicality of each approach. However, the various methods can introduce large inter-individual variability (Bernard et al., 2000). Using incremental exercise tests to exhaustion have been suggested to offer the most objective and accurate approaches. As mentioned in Section 2.3 the use of heart rate based methods are not appropriate for dosing intermittent exercise of short duration intervals (< 60 s) which precludes steady state due to the lag in HR increase and the inertia of HR at the cessation of exercise (recovery phase) (Buchheit & Laursen, 2013b).

The use of velocity or power associated with $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$), defined as the lowest speed/power required to elicit $\dot{V}O_{2max}$ (Billat & Koralsztein, 1996), is a popular method to dose intermittent protocols (Midgley & Mc Naughton, 2006). The $v\dot{V}O_{2max}$ can be determined from the linear relationship between $\dot{V}O_2$ and running speed obtained from a number of sub-maximal running speeds (Billat & Koralsztein, 1996; Hill & Rowell, 1996). Alternatively, the $v\dot{V}O_{2max}$ can be obtained from the performance of an incremental graded exercise test (GXT) to volitional exhaustion (Harling et al., 2003; Midgley & Mc Naughton, 2006).

The method used to determine the $v\dot{V}O_{2max}$ is an important aspect to consider as longer stage durations tend to elicit lower speed values. Furthermore, $v\dot{V}O_{2max}$ has been shown to be inversely related to the terrain or slope on the treadmill (Midgley et al., 2007b; Buchheit & Laursen, 2013b) The ramp rate has also been shown to effect cardiorespiratory responses to the exercise test identifying the need to individualise ramp rates (Myers et al., 1992).

The use of ergometer can also result in small but important changes in cardiorespiratory performance (Bassett & Howley, 2000) which effect the prescription of the exercise dose. Numerous modalities, such as, rowing ergometry (Bouckaert et al., 1983), elliptical cross-trainer (Dalleck et al., 2004), step tests (Keren et al., 1980) and arm ergometry (Reybrouck et al., 1975) have been utilised to determine $\dot{V}O_{2max}$. The manipulation of the exercise mode results in small variations in maximal oxygen uptake. For example, when treadmill exercise is compared to cycle ergometry, the $\dot{V}O_{2max}$ obtained varies between 8-12% higher for treadmill running (McKay & Banister, 1976). However, when well-trained cyclists are used, a higher $\dot{V}O_{2max}$ is achieved on the cycle ergometer (Hagberg et al., 1978). Variability of the $\dot{V}O_{2max}$ can in part be explained by the quantity and type of muscle fibres recruited during the exercise mode (Reybrouck et al., 1975) and the familiarity of the particular form of work to the exerciser (Karen et al., 1980). The limiting factors for the achievement of $\dot{V}O_{2max}$ during unfamiliar forms of exercise have been attributed to local metabolic and circulatory factors (Magel et al., 1975) rather than central oxygen delivery and peripheral oxygen extraction (Bassett & Howley, 2000). Thus, peripheral muscular fatigue may occur before the central circulatory system has engaged maximally (McKay and Bannister 1976). It is therefore crucial that the choice of mode and protocol to assess aerobic fitness reflects the exerciser's training environment in order to obtain a valid and objective measure of $\dot{V}O_{2max}$ (Davies et al., 1984).

2.4.5 Exercise modality for intermittent exercise

The modality of the exercise has also varied considerably across acute intermittent studies including running (Gosselin et al., 2012), stationary cycling (Gibala & Little, 2010), swimming (Mohr et al., 2014) and soccer (Krustrup & Bangsbo, 2015). A common model used is the Wingate test (Gibala et al., 2012; Gibala et al., 2006; Burgomaster et al., 2006). This involves 20-30 seconds of all-out cycling effort repeated 4-6 times with 4 minute rest periods. This type of model has been questioned for its appropriateness for clinical populations, the elderly and the overweight (Gibala et al., 2012), as it requires considerable commitment from the exerciser and

can cause discomfort, particularly for exercisers that are unaccustomed to this type of high-intensity exercise (Bayati et al., 2011). Advantages of using cycle ergometers include a cheaper more accessible piece of equipment with which to perform exercise on (Gibala & McGee, 2008), it has a lower impact on the body's skeletal system reducing the risk of injury and also falling at higher intensity. It is also safer and easier to perform maximal sprints interspersed with rest-pauses and lower intensity exercise on a cycle ergometer. Also the increased energy expenditure required for the acceleration phase can be reduced on a cycle by performing load-less pedalling in between sprints (Edwards et al., 1973). However, evidence from repeated sprints on a cycle ergometer is not transferable to athletes and exercisers who participate in upright bi-pedal sports such as endurance running or soccer (Boutcher et al., 2010). Moreover, cycling is a non-impact mode of exercise and therefore may not be the optimal mode of exercise to enhance bone formation (Nagle & Brooks, 2011).

Utilising laboratory soccer simulations such as the SAFT90 (Small et al., 2009) might offer insight into the mechanisms underlying the reported bone adaptations with soccer compared to continuous exercise. The benefits of these protocols include; the ability to include multiple changes of direction, matching the intensity of soccer performance, and including similar movement patterns to those performed during soccer whilst being performed in a controlled laboratory environment. However, as with the limitation of the SSGs; these protocols involve alternating exercise-to-rest intervals making it difficult to determine whether shorter or longer durations offer a more osteogenic loading environment for bone adaptation. Whilst these protocols can be run over-ground, mimicking soccer more closely, the assessment of loading on the bone is difficult as static force plates would be used. Therefore, not all the loading cycles will be quantified during the protocol which might lead to a reduction in data and an unclear picture of the overall loading environment provided by the exercise.

Simulating soccer performance on a treadmill in a controlled laboratory-based setting may help to determine why SSGs resulted in improved health and fitness beyond that of continuous exercise (Drust et al., 2000). The use of soccer-specific laboratory-based simulations have been developed

from the use of advanced cinematography and analysis of soccer player movement patterns (Reilly, 1997). These simulations offer a way to effectively control the workload, mean speed and control total distance covered during soccer-specific movement patterns compared with a steady-state continuous exercise (Drust et al., 2000). However these sprint-to-rest periods have varied between researchers. Drust et al. (2000) used movement patterns consisting of walk, jog, cruise and sprinting at pre-defined speeds (6, 12, 15 and 21 km·h^{-1} respectively). These speeds were based on observations from earlier research (Bangsbo 1994). The simulation was performed on a MT and all the speeds were standardised rather than individualised for each exerciser. The soccer simulation was compared to an intensity matched continuous steady-state protocol. The authors (Drust et al., 2000) reported that the simulation matched the physiological stresses experienced by actual match-play where the mean (SD) HR for the first and second half of the simulation was reported as 161 ± 8 and 174 ± 7 beats·min^{-1} which equated to approximately 80 and 89% of HR$_{max}$. The aerobic demands between the continuous and intermittent protocol were also similar for mean $\dot{V}O_2$ and HR. However, there was a greater contribution from the anaerobic system for the soccer simulation.

Whilst this was a well-controlled study by Drust et al, (2000) it is impossible for researchers to appropriately replicate soccer-type movements including jumps and cutting movements due to the nature of the treadmill. The use of changes- of- direction (COD) have been shown to enhance the energy demand of intermittent running (Hader et al., 2014). The inclusion of COD in an intermittent protocol would also likely increase the mechanical demands on the musculoskeletal system which would be important for bone adaptation. The unidirectional protocol by Drust et al (2000) may also be strengthened if the speeds were individualised for the exercisers as it is possible that for some of the exercisers top speeds were much higher than they would achieve during over-ground running. As discussed in the previous section the use of percentages of $v\dot{V}O_{2max}$ would be more appropriate (Buchheit & Laursen, 2013b). Furthermore, the acceleration and deceleration is determined by the confines of the MT and these are likely to be

much slower than accelerations performed during soccer match play and the accelerations and decelerations would not be self -paced.

Moreover, soccer simulations involve highly variable exercise-to-rest ratios (Bangsbo, 1994) that make it difficult to establish a dose-response relationships. An interesting question would be to establish whether different exercise-to-rest durations have a similar or different effect on bone remodelling. For example, more accelerations and decelerations incorporated may induce a more variable mechanical loading environment which might be more favourable to maximise bone adaptation.

The study by Drust et al. (2000) failed to obtain information on the mechanical stimulus introduced by the exercise. This is likely due to the requirement of a fully instrumented MT which is inaccessible due to cost of the equipment. Obtaining a measure of the magnitude and rate of strain on the bone elicited by the intermittent exercise would be useful to establish how the changing mechanical environment affects bone. To establish the nuances of the changing loading environment, with multiple higher intensity loading cycles interspersed with lower intensity loading cycles, all the loading cycles during the exercise session must be measured. A further disadvantage of the MT is that it does not replicate the stresses of over-ground running (Schache et al., 2001; Sinclair et al., 2013), and may artificially reduce the intra-stride variability (Schache et al., 2001) improving running economy. Changing the mechanical and physiological environment then may impact on the magnitude of the effect on bone adaptation reducing the effectiveness of the intervention.

An alternative to overcome some of the limitations noted above is the use of a non-motorised treadmill (NMT) (Belli et al., 2001). The NMT allows replication of instantaneous changes of speed and monitoring of power output and workload which are important for games play (Sirotic & Coutts, 2008). Additionally NMTs are utilised by many athletes (track and games players), and have been shown to improve anaerobic power and leg strength, amongst other training variables (Highton et al., 2012). The reliability, validity and accuracy of NMTs has been extensively

examined (Heighton et al., 2012), both for repeated sprints (Brown et al., 2007) and for soccer simulations (Sirotic & Coutts, 2008; Aldous et al., 2014). These studies have reported that NMTs are reliable and valid for both kinetic (vGRF, impulse, load rate) and kinematic (step length, step frequency) parameters (Cronin & Rumpf, 2014) when compared to over-ground running (Highton et al., 2012). However no studies have utilised NMTs to assess the kinematic and kinetic responses during a controlled intermittent running protocols. This may be due to the difficulty in ensuring that individuals can maintain high speeds for a controlled period of time (> 6 s) (Cronin & Rumpf, 2014).

The NMT requires the participant to power the treadmill belt. Thus there is a greater emphasis on the lower limb muscles, resulting in lower peak speeds reached compared to motorised treadmills and increased peripheral fatigue (Davies et al., 1984). Therefore, different physiological stresses occur when performing exercise on the NMTs compared to MTs (Davies et al., 1984), such as greater submaximal $\dot{V}0_2$, ratings of perceived exertion (RPE) (De Witt et al., 2009), and heart rates being elicited by the NMT (Everett et al., 2010). Furthermore, the nature of running on the NMT dictates a greater forward lean, bringing the centre of gravity over the base of support (Belli et al., 2001). The change in posture coupled with shorter step lengths results in an attenuated vertical impact transient (Hagan et al., 2006).

Nevertheless, the NMT is fully instrumented with force transducers (strain gauges) (Figure 2.6) which provide measures of both horizontal (hGRF) and vertical (vGRF) ground reaction forces. A limitation of the horizontal strain gauge is that it only allows a measure of anterior force. However, the measures of hGRF and vGRF enables researchers to obtain measures of external training load such as work (kJ), and assess the exercise-induced mechanical loads imparted on the musculoskeletal system (Hagan et al., 2006). The GRF from running on the NMT can be used to provide indirect information about the internal joint loading (hip, knee and ankle) because the peak of the vGRF coincides with the timing of peak loads on load bearing bones occurring during the stance phase (Novacheck, 1998). The continuous measurement of the vGRF will allow all of the steps during the exercise to be accounted for. Collecting multiple steps on the NMT will allow

a more repeatable pattern to be established with more steps accounted for in comparison to more static based force plate approached (Fellin et al., 2010). Whilst the foot strike pattern will unlikely change, it is likely that an intermittent protocol consisting of multiple accelerations and decelerations will change the magnitude and loading rate of the vGRF components.
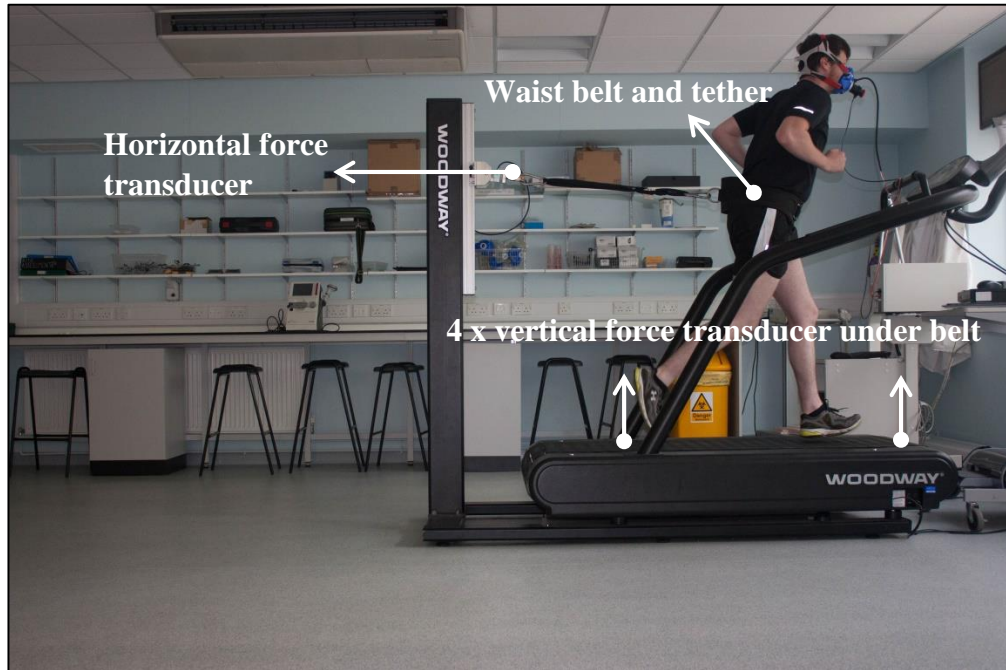
Figure 2.6 The Woodway® Force 3.0 non-motorised treadmill.

Table 2.3 displays the advantages and disadvantages of 3 modalities to simulate intermittent exercise

Soccer simulation (e.g. SAFT90)	Motorised treadmill (MT)	Non-motorised treadmill (NMT)
✓ Simulates soccer movement patterns which make it a more realistic protocol ✓ Can be performed over- ground which is likely more osteogenic (higher impacts) and allows researchers to use static force plates to get a better measure of GRF ✓ Allows assessment of multidirectional movements which are likely more osteogenic	✓ Allows for control of internal and external loads	✓ Simulates intermittent exercise better than MTs ✓ Simulates over-ground running ✓ Allows control of both internal and external loads ✓ Is equipped with capability of measuring vGRF on every step ✓ NMTs are used on the international space station and so information from the NMT is transferable ✓ NMTs are also used in rodent studies and so these protocols could be utilised by animal models to evaluate more invasive measures ✓ Popularity of NMTs is increasing and they are generally cheaper and more accessible to the average user

✖ Difficult to control the internal and external load and match to continuous exercise or other intermittent protocols ✖ Can only obtain GRF on a select number of loading cycles limiting the ability to assess the variability in steps	✖ Does not have force plates under belt to allow assessment of GRF ✖ Might not replicate the physiological stresses of over-ground running ✖ The treadmill reduces the intra-step variability due to the control of the treadmill belt ✖ Does not allow the exerciser to accelerate and decelerate at their natural rate ✖ Only allows unilateral movement patterns	✖ Only allows unilateral movement patterns ✖ Whilst reflective of over-ground running there is a higher submaximal energy expenditure ✖ Requires a change in posture with a greater forward lean similar to that running uphill ✖ Changes the foot strike pattern causing participants to run with less natural foot strike ✖ Is equipped with strain gauges with limited capacity to measure at high sampling rates ✖ The horizontal GRF is limited to the propulsive hGRF

Section summary

The use of soccer-simulation protocols have enabled a deeper understanding of the effect soccer-type movement patterns has on our physiology. The development of NMT may make it easier to replicate the instantaneous changes of speed seen in soccer games and other forms of intermittent running whilst maintaining the ability to control other important variables such as the exercise-to-rest ratio. The use of NMTs have successfully been used for soccer simulations but have not been used as a tool for understanding the mechanisms behind why intermittent exercise may be positive for bone adaptation. However, the use of NMTs may change the physiological stress experienced by the exerciser when compared to a MT. This aspect therefore requires further investigation in order to appropriately control individual intensity and load on the NMT, and identify the physiological differences between the NMT and MT for comparison of studies investigating bone responses to treadmill running. Given the NMT is fully instrumented with force transducers, measures of GRF in both the vertical and anterior horizontal components can be obtained with which to reflect the osteogenic potential of the different exercise conditions.

2.5 Quantifying the osteogenic potential of exercise: the osteogenic index

2.5.1 Assessing the mechanical strain via ground reaction forces

It is well established that not all exercises elicit the same osteogenic response to bone (Skerry, 1997). In order to optimise the osteogenic potential of an exercise session one must be able to objectively quantify the exercise-induced mechanical stimulus. From the evidence discussed so far (Section 2.2.3) dynamic, high-impact intermittent exercise delivered at high strain rates in unusual and diverse ways are considered most osteogenic (Turner & Robling, 2003). Furthermore, the strain must exceed the minimum strain. Human studies using physical activity support animal studies in that the exercise needs to exceed a minimum strain threshold in order to be osteogenic (Vainionpää et al., 2007; Rantalainen et al., 2011b).

Strain is defined as the relative change in length of the bone and is represented mathematically as:

$$\varepsilon = \frac{\Delta L}{Lo} \qquad Eq\ 3$$

Where ε is strain, ΔL is the change in length (L- Lo) and Lo is the initial length.

The change in strain over time or strain rate is expressed as:

$$\dot{\varepsilon} = \frac{d\varepsilon}{dt} \qquad Eq\ 4$$

Where $\dot{\varepsilon}$ is the strain rate, $d\varepsilon$ is the change in strain and dt is change in time

The strain will ultimately lead to stress on the bone:

$$\sigma = \frac{F}{A} \qquad Eq\ 5$$

Where σ is stress, F is force and A is the cross sectional area of bone.

The strain created by mechanical loading is directly proportional to the load applied to bone (Heise & Martin, 2001). Strain has been measured directly from the tibia in human subjects (Milgrom et al., 2000), however this invasive technique is not always viable, especially for long-term intervention studies. Moreover, the invasive nature limits the amount of participants and so information is limited to a small population. We can infer strain from forces and impacts that the individual is exposed to. Strain can be indirectly quantified using the surrogate measure of the vGRF or measurement of peak accelerations using accelerometers (McKay et al., 2005; Vainionpää et al., 2007).

The daily stress stimulus (S) was developed to consider all daily impacts experienced. The premise is that loading cycles can be decomposed into histograms such that:

$$S = \left(\sum_{j=1}^{k} N_j \, E_j^m \right)^{1/m} \qquad Eq \ 6$$

Where; k = the number of different loading magnitudes, E_j = is the *jth* stress magnitude, N_j = the daily count of the stress magnitude, and the exponent m is a weighting factor for the importance of the stress magnitude (typically m = 4) (Ahola et al., 2010).

Whilst the daily stress stimulus offers a unique way to combine all loading cycles into one value, the data only considers the magnitude of the loading cycle. Thus, It fails to consider other important aspects of mechanical load including the frequency at which the load is delivered (Hsieh & Turner, 2001), the intensity (rate) that the load is applied and fails to adjust for the diminishing returns of multiple loading cycles (Turner, 1998). To address these issues, and enable clinicians to obtain an objective measure of the osteogenic potential Turner and Robling (2005) developed a mathematical algorithm termed the osteogenic index (OI).

2.5.2 The osteogenic index

The OI combines the intensity of the mechanical load, such as the peak vertical ground reaction force, with the number of cycles/repetitions performed (Turner and Robling 2005a). The natural logarithm of the number of loading cycles is included to adjust for the mechanical desensitisation of the osteocyte network. The OI is expressed mathematically as:

$$OI = I * Ln(N + 1) \qquad Eq \ 7$$

Where OI is the osteogenic index, I is the intensity of exercise (e.g. the peak ground reaction force or peak acceleration), Ln is the natural log, and N is the number of cycles.

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Given the frequency (Hz) of the loading cycle is also an important component to consider in bone adaptation, the oscillating components of the signal can be isolated using Fourier analysis, where the OI is calculated as:

$$OI \; = \; Ln(1 \; + \; \mathrm{N}) \cdot \sum_{i=1}^{n} \varepsilon_i \, f_i \quad\quad Eq \; 8$$

Where, n = the maximum frequency, ε_i = the ith strain magnitude, f_i = the ith frequency

The OI was developed based on data from animal models which as discussed in previous sections might not be transferable to human studies. However, the OI has been used to assess the mechanical dose of a number of exercise modes, in humans including; bilateral hopping (Rantalainen et al., 2011a), stepping (Santos-Rocha et al., 2006), running (Weeks & Beck, 2008), jumping (Rantalainen et al., 2009) and more recently to assess the loading dose of the sport of Cyclocross (Tolly et al., 2014). The OI has been used to assess the loading dose of both acute exercise sessions (Rantalainen et al., 2009) and utilised over longer training interventions (Rantalainen et al., 2011a). The OI calculated from these different modes is presented in Figure 2.7.
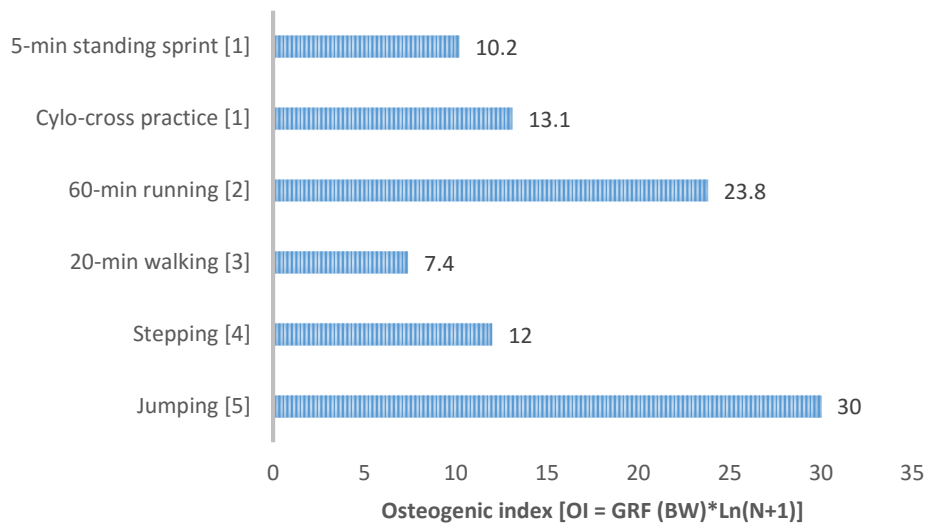
Figure 2.7 The OI calculated from different modes of exercise from previous research using impact exercise. N.B. [1] (Tolly et al., 2014), [2] (Weeks & Beck, 2008), [3] (Turner & Robling, 2003), [4] (Santos-Rocha et al., 2006), [5] (Rantalainen et al., 2009).

Whilst the OI seems capable of distinguishing between discrete bouts of different loading characteristics (e.g. jumping vs. stepping) it is not clear if the OI would be useful to distinguish between similar modes of loading such as intermittent vs. continuous running. Recently the OI has been developed to assess the changing loading environment during different running speeds (Kelley et al., 2014). This involves segmenting the loading cycle into multiple fast Fourier transforms (FFTs) over a number of different frequency bands. The new method was developed for the use of accelerometers and was capable of distinguishing between the nuances of different loading pattern during walking, jogging and running. There was an interaction between the frequency band, magnitude and loading environment where fast running resulted in a 43%, 82% and 118% increase in loading intensity in the 0.1-2 Hz, 2-4 Hz and 4-6 Hz frequency bands respectively when compared to walking. When slow jogging was compared to walking there was an increase in loading intensity by 10%, 35% and 55% in the same frequency bands. This study supports the notion that different physical activities induce different loading magnitudes across different frequency bands at the same time (Cappozzo, 1982). These differences were postulated to be due to the higher speeds causing higher GRFs (Kelley et al., 2014).

As yet this method has not been utilised for assessing the loading dose over an entire exercise session on an instrumented treadmill capable of providing measures of vGRFs on multiple steps. The use of accelerometry is novel and useful for providing measures of load when force plates are not available (Kelley et al., 2014). However, the acceleromters used were only capable of sampling at very low frequencies. Therefore, the loading dose may have been substantially underestimated. Moreover, accelerometers are inherently noisy and difficult to distinguish where peak loads are detected and more noise may compound the estimation of the loading dose. Therefore, further work is required utilising this novel calculation of the osteogenic index.

Section summary

The osteogenic potential of the mechanical loading environment should be assessed in order to optimise the training load and provide the greatest osteogenic stimulus. The use of the vGRF can be assessed in multiple ways (both time and frequency domains) to assess the magnitude, rate and frequency of the stimulus. To date, there is limited attention on quantifying the mechanical loading environment induced by intermittent exercise. Ultimately, to understand how the mechanical loading environment might affect bone, we need to obtain an objective assessment of the turnover of bone tissue. This can be achieved in a variety of ways using radiological and densiometric techniques and also through assessing bone changes biochemically.

2.6 Measuring bone remodelling using bone turnover markers

Chronic changes in bone are usually measured by densitometry or radiological methods (Helge et al., 2010). These methods have many advantages including; assessment of changes in BMD, strength index of bone and changes in periosteal apposition. Moreover, measures of pQCT can distinguish between changes in trabecular and/or cortical bone reflecting how exercise might change the properties on bone. However, a key limitation in the use of densitometry and radiological measures of bone mass and quality is the length of time required to detect changes in bone properties. The use of dual X-ray absortomitory (DXA) will require a minimum of 6 months

but more likely more than 12 months (Kemmler & Engelke, 2004). Short term changes (10 weeks) in bone properties have been identified using pQCT (Izard et al., 2016). An important aspect from this study is that the intensity of the exercise must be very high and close to the fracture index to induce changes in bone properties. The data from this well controlled and novel study utilised young military recruits who would be completing exceptionally high volumes of mechanical loading during their training.

Therefore, these aforementioned static measures of bone are not suitable to assess the short-term (hours to days) dynamic changes in bone (Wheater et al., 2013). As such, cellular components of the bone matrix have been identified as markers of bone resorption or formation (Seibel, 2005). The various bone turnover markers (BTMs) are presented in Figure 2.8. Bone turnover markers can be split into two groups, resorption and formation markers, and further broken down into collagenous and non-collagenous proteins (Seibel, 2005).
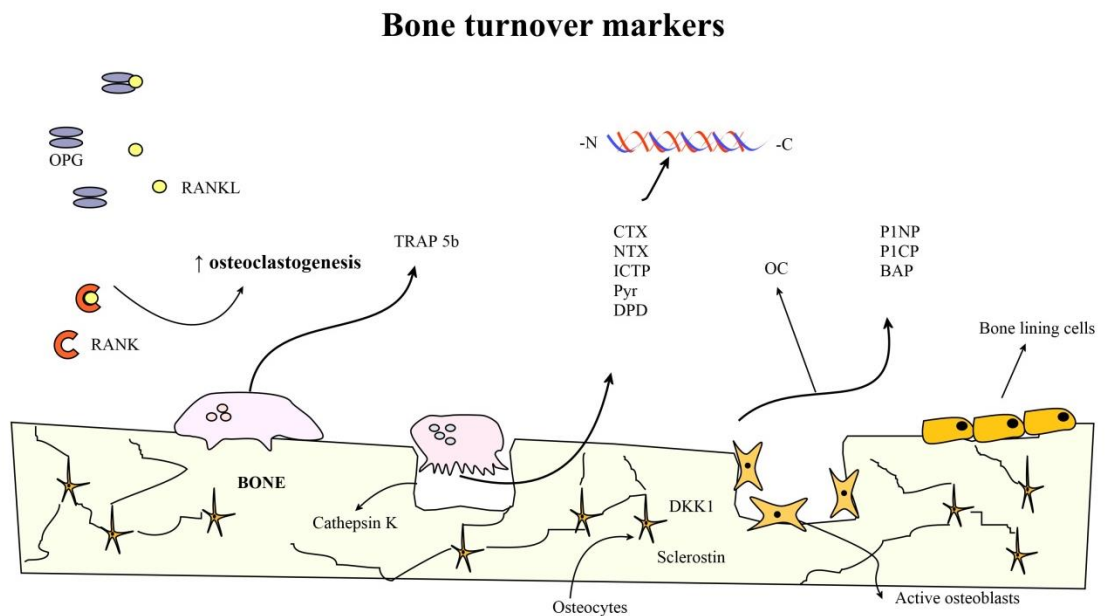
Figure 2.8 Bone turnover markers released during remodelling of bone in the basic multicellular unit. Adapted from Seibel (2005)

Table 2.4 Bone turnover markers

Bone turnover marker	Tissue	Specimen	Notes	Used in response to exercise	Variability
Formation					
Total alkaline phosphatase (AP)	Bone	Serum	Specific product of osteoblasts, 20% cross activity with liver. AP activity makes up approximately 50% from bone and 50% from the liver, in children AP activity makes up ~ 90% owing to the greater skeletal maturation. In healthy adults, once liver disease is ruled out, most techniques for measuring AP show good correlation with bone mineralization	yes	Low intra-indiv variability < 10%. Food has little effect
Osteocalcin (OC)	Bone, platelets	Serum	Specific product of osteoblasts, some immunoreactive forms in blood; may be derived from osteoclasts (can reflect both formation and resorption). is evidence to suggest that OC is important for energy metabolism and its use in response to exercise could reflect energy expenditure rather than bone metabolism.	yes	Intact molecule unstable, inter-lab variability, released during formation and resorption
Procollagen type I carboxyterminal-terminal propeptides (P1CP)	Bone, soft tissue, skin	Serum	Specific product of proliferating osteoblasts	yes	Quantitative measure of newly formed type 1 collagen
*Procollagen type I amino- terminal propeptides (P1NP)	Bone, soft tissue, skin	Serum/Plasma	Specific product of proliferating osteoblasts and fibroblasts; partly incorporated into ECM. procollagen type I propeptides are released by other tissues the turnover rate of these are much slower than that of bone therefore they would contribute minimally to the circulating pool.	yes	Low intra-indiv variability, small circadian variation, responsive to anti-resorptive meds
Osteprotegrin (OPG)		Serum	Decoy receptor for RANKL.	yes	Novel marker. Needs more investigation
Resorption					
Hydroxyproline total and dialyzable (Hyp)	Bone, cartilage, soft tissue, skin	Serum/Urine	Fibrillary collagens and partly collagenous proteins. Present in newly synthesised & mature collagen	yes	Degradation of mature collagen specific to bone, urine so hard to collect second measures, circadian variability

Deoxypyridinoline (DPD)	Bone, dentin	Serum/Urine	Collagens, highest concentration in bone; absent from cartilage or skin	yes	Degradation of mature collagen specific to bone, urine so hard to collect second measures, circadian variability
*Carboxyterminal cross-link telopeptide (CTX)	All tissues containing collagen	Serum/Urine/plasma	Collagen type I, highest concentration probably bone	yes	Variability ↓ fasting, sample stability good especially EDTA. However large circadian variation 2 X nadir 11:1500
Aminoterminal cross-link telopeptide (NTX)	All tissues containing collagen	Serum/Urine/plasma	Collagen type I, highest concentration probably bone	yes	Urine stable, predictor of fracture in postmenopausal, dietary influence, large circadian variability
Cathepsin K	Primarily osteoclasts	Serum	Essential role in osteoclast-mediated bone matrix degradation by cleaving helical and telopetide regions of collagen type I	yes	Clinical validity needs more investigation
tartrate resistance acid phosphatase (TRAP 5b)	Bone and blood	Serum	Predominant in bone (TRAcP osteoclasts, platelets, erythrocytes)	yes	Circadian variability immediately ↑ following exercise. Good automated and manual immunoassays
Sclerostin (SCL) from SOST gene	Bone (produced by osteocytes)	Serum	Released from mature osteocyte, inhibits canonical wnt signalling pathway through LRP 5/6 binding. Inverse r/ship with PTH, ↓ with mechanical loading	yes	Novel, few studies investigating acute response to exercise
Dikkopf-related protein 1 (DKK1)	Bone (produced by osteocytes)	Serum	Same as SCL, regulates bone turnover	?	Novel, needs more analysis
RANKL	Bone	Serum	Binds with RANK, inhibits bone formation, regulatory molecule	yes	Novel marker, analytical problems. Circulating levels may not reflect bone microenvironment

Notes: ***** identifies the two markers recommended for study by IOF. Modified and adapted from Wheater et al. (2013)

2.6.4 Acute exercise and bone turnover markers

The use of BTMs have proven useful for the assessment of bone metabolism in short-term exercise training programmes (< 6 months) (see references in Table 2.5) and for investigating the immediate and recovery cellular responses to an acute exercise bout. Changes in bone cell function likely occur up to 24 h (Bloomfield, 2001); therefore, assessing the response of acute exercise on bone cell properties in the hours following exercise is warranted (Banfi et al., 2010). An increase in blood biomarkers of remodelling indicative of bone formation but without concomitant increases in markers of bone resorption show the effect of the exercise-induced mechanical strain on bone metabolism (Lester et al., 2009).

However, the accumulating body of research investigating the effects of acute bouts of exercise on bone metabolism is equivocal (Table 2.4). For example several authors (Scott et al., 2011; 2012; Haakonssen et al., 2015) have demonstrated that exercise might be deleterious to bone due to an increase in resorption markers with no changes in formation markers. These studies have typically used high-intensity (~65-75% $\dot{V}O_{2max}$) long duration (> 60 min) bouts of endurance exercise characterised by either non-impact exercise such as cycling (Haakonssen et al., 2015) or moderate impact protocols such as running (Scott et al., 2011).

Table 2.5 Studies investigating responses of a variety of bone turnover markers.

Author	Biomarkers	Time variance of bloods	Method	Standardised time of day & nutritional status	Results
Ashizawa et al. (1998)	OC, bLa, B-ALP, TRAP, DPYR,		14 males. 6*10reps*7EX @60-80%1RM.	Y/Y (9 d)	P1CP↓, B-ALP ↓ 2-3 days post EX, OC no effect, ↓TRAP ↑ calcium excretion.
Banfi et al. (2012)	OPG, RANK, RANL	Pre-post training	Rugby training session. 30 elite players	Y/N	No change in OPG/RANK/RANKL
Guillemant et al. (2004)	Plasma, hematocrit, BAP CTX		12-male cyclists, 80% $\dot{V}O_{2max}$, 60 min	N/Y	Acute bone resorption post exercise ↑CTX ICTP
Herrmann et al. (2007)	OC, PINP- formation TRAP, CTX -resoption	Before, 3 and 24 h post	32 healthy, young males/females. Performed 60-min cycle @ 75, 95 and 110% IAT.	N/N	75% IAT OC, PINP↓. pH ↓(7.32)@ 110% NOT 75 or 95% IAT. ↑CTX @ 95 and 110%, lactacidosis does not stimulate resorption in vivo.
Lin et al. (2012)	OC, TRAP, calcium, phosphorus, Bla	5-,15 min post EX, 1,3,6,24,48,72 h post EX	24-young healthy males (Not-reg active).PL, IR, CONT. PL=15*5*40 s rest. IR=10*200 m*60 s rest	Y/Set breakfast. 4 d diet provided	Phosphorus, higher both up to 72 h. PL=sig↑ OC 5 min, 1 h- post vs. CONT. Bla and calcium ↑ with EX, baseline 15 min. OC in IR-may be circadian rhythm. PL- OC↑. TRAP no effect EX unlikely cause bone cellular changes.
Maimoun et al. (2006)	Ca, P, ALP, ALB, PTH, 25- OH D, 1,25 OH D, OC, CTX.	0, 30 & 50 min EX 15 min post EX	7-male competitive road cyclists. Performed 50 min cycle (-15%VT vs. +15%VT).	0800-0830 post 12 h fast.	↑PTH for +VT. Peaked in recovery. 25- OH D, 1,25 OH$_2$D no change CTX↑ for +VT. Formation, resorption return to basal level post EX.

Rogers et al. (2011)	Formation: BAP Resorption: CTX		Repeated- measures, cross-over. RT vs. PLY (fed & fasted). PLY: 1*10, 10 s rest. RT: 3*10 reps, 6 exercises	Y/Y	2 h post, transient ↓ CTX. RT & PLY ↓natural decline in BAP
Rong et al. (1997)	Calcotonin, PTH, OC, ICTP	1, 4, 24 hr post	8 healthy males (23±3) regular activity. E55%-45 min, E85%_15 min and strength (4*10reps 85%1RM)	Calcium intake limited. Time b/w 07:00-08:00	Calcitonin-No effect PTH ↑ strength only ICTP ↓ All exercise.
Scott et al. (2011)	B-CTX, P1NP, OC, OPG, PTH, CORT BAP, 25-OH D.	0800 h, 20,40 & 60 min of EX. 0.5,1,2,& 3 h post-EX.	10 active men. Controlled diet 8 d. 60 min tread (55, 65 & 75% $\dot{V}O_{2max}$).	Y/Y	No effect of EX int on GLU, La, CORT. ↓ β-CTX 2-3hr post EX. ↑ βCX 1 h post for 75% $\dot{V}O_{2max}$, not 55, 65% No effect on bone formation. BAP increased 3-4 days post.
Tosun et al. (2006)	PTH, OC, CTX, P1NP, ALP, ICTP,	30th, 45th minute. ALP 24 h	9 healthy active premenopausal females. 30 min brisk walk + 30 min with 5kg.	0900-1100 h/No	No effect for OC, CTX, P1NP, ICTP ALP, PTH significant. ALP ↑ 24 h for weights+EX, ↓ALP EX
Whipple et al. (2004)	Formation: BAP, PICP Resoption: NTX		3*10 reps, 7 exercises (55-75% 1RM). Blood taken, immediate after, 1,8,24 & 48-h. 15% Pro, 55% CHO, 30% Fat.		No change in serum measure. BAP higher post-ex compared to CON. Resorption lower @1-& 8 h post compared to CON
Welsh et al. (1997)	Osteocalcin BAP		30 min brisk walk		No measureable effect on formation, resorption

Notes: Osteocalcin (OC), Procollagen type I carboxyterminal- terminal propeptides (P1CP), Carboxyterminal cross-link telopeptide (CTX), Aminoterminal cross-link telopeptide (NTX), Bone alkaline phosphatase (BAP), Osteoprotegrin (OPG), Parathyroid hormone (PTH), Alkaline phosphatase (ALP), receptor activator of nuclear factor kappa-beta (RANK), receptor activator of nuclear factor kappa-beta ligand (RANKL), exercise (EX), carbohydrate (CHO), vitamin D (25-OH D), Glucose (GLUC), lactate (La), resistance (RT), plyometric (PLY), Ventilatory threshold (VT), cortisol (CORT)

Others (Whipple et al., 2004) have demonstrated that the overall net effect of exercise on the bone cell activity was increased in favour of formation markers although not statistically different from a control group. These authors investigated the effect of moderate intensity resistance exercise on bone alkaline phosphatase (formation) and type 1 collagen N-terminal telopeptide (resorption). The authors also mention that given the ratio of BAP and NTX was not statistically different from the control group that the net effect on the skeleton would be similar. However, there are a number of issues with this interpretation; (1) whilst the ratio is a useful measure to assess the direction of the bone cell activity (resorption or formation) it is important to note that whilst the ratio may be similar to a non-exercising group the absolute changes in the bone markers must be interpreted alongside the ratio. Here, an increase in bone cell activity, in response to exercise, might occur suggesting a greater bone turnover even if the ratio is the same as the control (non-exercising group). Therefore, there could be an effect on the skeleton. (2) It is important to compare two biomarkers that reflect a similar aspect of bone, for example CTX-I and P1NP reflect collagen degradation and synthesis respectively (Garnero et al., 2003). It would be inappropriate to look at a ratio between BAP and CTX-I as BAP is released directly from osteoblasts and reflects osteoblast activity, whereas CTX-I generated by Cathepsin K and reflects the degradation of type 1 collagen (3) it is unlikely that BAP is appropriate to measure acute BTM changes BAP reflects osteoblast activity and this is unlikely to change in such a short time period and then be detectable in serum. Therefore, there is an issue with the potency of change and the matching of the time periods.

Moreover, the study by Whipple et al. (2004) conflicts with an earlier study investigating the effects of resistance exercise on bone turnover (Ashizawa et al., 1998) showing a decrease in bone formation markers. This difference could in-part be explained by the higher intensity resistance exercise (~80 % 1 repetition maximum) used by Ashizawa et al. (1998). This would suggest that moderate intensity exercise offers a more favourable environment for bone compared to higher intensity loading. This would seem counterintuitive given bone responds to strain of higher magnitudes and rates (Turner, 1998) which would be offered by resistance exercise. However, it is possible that given the diminishing returns caused by mechanosensory saturation (Turner, 1998)

that there lies a threshold where bone may become more fatigued with higher loads quicker. Therefore, a lower intensity exercise of a similar duration to a higher intensity exercise could be more favourable, although this requires further investigation. This bone fatigue might explain the prolonged increase in bone resorption markers seen with ultra-endurance events (Kerschan-Schindl et al., 2009). This would also suggest that when participating in higher-intensity impact exercise that the duration does and should not be performed over long continuous bouts. However, it is not clear how well controlled the follow up time points were standardised within and between participants by these authors which would lead to high variability in the bone turnover marker responses.

The conflicting responses demonstrated in the multitude of studies investigating bone turnover markers can be explained by a myriad of factors including; the experimental population used, such as trained (Banfi et al., 2012) vs. untrained athletes (Rantalainen et al., 2009), the use of impact (Rogers et al., 2011) vs. non-impact exercise (Haakonssen et al., 2015), the duration (Kristoffersson et al., 1995)and intensity of exercise session (Maimoun et al., 2006; Scott et al., 2011), and the choice of BTMs used (Banfi et al., 2010). Furthermore, few studies have included a non-exercising control condition which may result in reductions in the magnitude of observed effect of exercise (Rogers et al., 2011) and therefore future studies should include a control condition to enhance our understanding of the acute responses to exercise.

Moreover, important confounding factors which can affect the variability of the measures are poorly accounted for such as diet (Clowes et al., 2002), pre-activity levels (Seibel, 2005), whether the participants were in a fasted or fed state (Clowes et al., 2002), and control for diurnal variation (Bjarnason et al., 2002). There is also evidence to suggest that BTM must be adjusted for the effects of haemoconcentration changes which may artificially augment BTM responses (Brahm et al., 1997b). Therefore, plasma volume (PV) changes in response to exercise should ideally be measured and used to adjust responses.

Currently recommended bone turnover markers

Currently, the Bone Marker Standards Working Group (Vasikaran et al., 2011b) recommends the use of C-terminal telopeptide of type I collagen (CTX) and amino terminal propetide of type I collagen (P1NP) as traditional markers of bone resorption and formation respectively. There are three major macro-molecular products of collagen degradation available as commercially available assays. The amino-terminal cross linked telopeptide of type I collagen (NTX) and the carboxyterminal cross-linked telepeptides (CTX and ICTP). Collagen type I is the major organic component of the extra cellular matrix (ECM) and is present as a triple helix. The cross-links covalently link individual collagen fibres into a triple helix. The folding of the three alpha chains create procollagen molecule. The major sites of the collagen cross link is the N and C –terminals telopeptides linked via pyridinium and pyrole componds. During breakdown these N and C-terminal telopeptides are released into the circulation. They are relatively small and can be removed via the kidney. The osteoclasts dissolve the bone matrix and lysosomal enzymes Cathepsin K and TRAP digest the exposed type I collagen releasing the degradation products (CTX and NTX).

These markers have been successfully used to assess the effect of continuous load bearing exercise on bone remodelling (Scott et al., 2011; 2013). These are well designed and well controlled studies, controlling dietary intake and importantly sampling time of the biomarkers. However, as running at higher intensities and speeds would evoke greater mechanical strain, it is unclear whether it was the intensity (% $\dot{V}O_{2\mathrm{max}}$) of the exercise which caused greater bone turnover alone or higher mechanical strain in the aforementioned study by Scott et al., (2011). Moreover, whilst endurance running is considered a moderate impact activity (Weeks & Beck, 2008; Kelley et al., 2014) and has been shown to improve cortical and trabecular bone density (Li et al., 2014; Sumida et al., 2014), the continuous loading at high intensities may be deleterious to bone as evidenced by lower BMD in runners (Barrack et al., 2010; Tenforde et al., 2015) and, increased risk of stress fractures, in endurance runners (Tenforde et al., 2010) and military recruits (Milgrom et al., 1985).

The studies which have used bone turnover markers related to type 1 collagen degradation and synthesis (e.g. CTX and P1NP) in response to acute exercise are included in Tables 2.6 and 2.7 below. All but one study included below has focused on healthy male populations to investigate. This is likely due to the need for repeat high intensity protocols less suitable for sub-clinical populations and the need to control for the menstrual cycle when using female participants which would be a confounding factor if not well controlled. A further important aspect of these studies is the apparent lack of use of control condition used to compare bone turnover maker responses. The majority of the acute studies have also investigated a concept such as the effect of mode on bone turnover marker response.

Table 2.6 Studies investigating the acute effects of impact-exercise in a healthy male population on type I collagen makers of bone turnover.

Author	Participants	Experimental design	Control group	BTMs	Sample collection points	Results
Kerschan-Schindl et al. (2009)	(n = 18, Male =16, Female =2)	Spartathlon (246 km running race)	N	CTX-I, OPG, OC, RANKL	Day before race, immediately after, 3 d after	CTX-I ↑ immediately post remaining elevated 3 d post
Rantalainen et al. (2009)	Male (n =15)	Continuous exhaustive jumping	N	CTX-I, P1NP	Pre, immediately post, 1 h, 2 h, 24 h, 48 h	CTX-I ↑ BASE on day 2. P1NP NS
Rogers et al. (2011)	Male (n = 12) age: 43 (5)	PLY, RE (fed and fasted) Control was fasted	Y	CTX-I, OC, TRAP5b BAP	Pre, immediately post, 15 min, 30 min, 1 h, 2 h, 24 h	CTX-I ↓ up to 2 h with PLY, NS ↓ with RE. NS change with control. With PLY-Fed CTX-I increase transiently over 24 h
Scott et al. (2010)	Male (11- RA, 10-ET, 10-control).	Exhaustive running	Y	CTX-I, P1NP, OC, OPG	Pre, 1-4 d	B-CTX-I ↑ from BASE on all days for ET and RA. P1NP, NS
Scott et al. (2011)	Male (n = 10)	Continuous (60-min) treadmill running. 55, 65, 75% $v\dot{V}O_{2max}$	N	CTX-I, P1NP, OC, OPG	Pre, 20, 40, 60 min Ex, 0.5, 1, 2, 3 h post. 1, 2, 3, 4 d post	55 = CTX-I ↓ 16% from pre during Ex, CTX-I ↓ 39% post Ex. 65 = similar responses. 75 = CTX-I 3% ↑ @ 60 Ex. This maintained up to 1 h post Ex.

Scott et al. (2012)	Male ($n = 10$)	Continuous (60-min) treadmill running. Fed vs. Feeding.	N	CTX-I, P1NP	Pre, during, 1,2,3 h Days 1-4	CTX-I ↑ with EX, not sig diff from pre up to 2 h post
Scott et al. (2013)	Male ($n = 10$)	3 h, 24 h recovery between 60 min running @ 65% $v\dot{V}O_{2max}$	N	CTX-I, P1NP, bone ALP, OPG	Pre, 1-5 d following	NS changes of CTX-I, P1NP following repeated running
Zittermann et al. (2002)	Male athletes (n = 18)	RWxD 60-min running, 70% speed at Bla 4 mM·L^{-1}, participants fed	Y	CTX-I, P1CP, Ca, PTH, calcitriol,	Pre, 3 h post exercise	NS diff in CTX-I

Notes: C-terminal telopeptides of Type I collagen (CTX-I), procollogen type 1 amino terminal propeptide (P1NP), bone alkaline phosphatase (BAP), intact calcium (iCa), parathyroid hormone (PTH), tartrate resistant acid phosphatase 5b (TRAP5b), osteocalcin (OC). Randomised within subject crossover design (RWxD), resistance exercise (RE), plyometric exercise (PLY), whole body vibration (WBV), time trial (TT), endurance trained (ET), recreationally active (RA), no significant change (NS).

Table 2.7 Studies investigating the acute effects of low-impact exercise in a healthy population on type I collagen makers of bone turnover.

Author	Participants	Experimental design	Control group	BTMs	Sample collection points	Results
Barry and Kohrt (2007)	Male ($n = 20$) endurance athletes	RWxD. Three 35 km TT under conditions of calcium supp	N	CTX-I, BAP, iCa, PTH	Pre and immediately post exercise	CTX-I ↑ by 0.24 (0.06) pla, 0.17 (0.005) Ca before, 0.28 (0.06) Ca during (ng·mL^{-1})
Bemben et al. (2015)	Male ($n = 10$)	RWxD. RE, WBV & RE + WBV	N	CTX-I, BAP, TRAP5b	08:00-10:00, pre, immediately post and 30 min post	CTX-I 20.8% ↓ from pre-post with WBV otherwise NS changes in CTX-I-1
Guillemant et al. (2004)	Male ($n = 12$) triathletes	60 min cycle @ 80% $\dot{V}O_{2max}$ w/out calcium load. Pre-feeding	N	BAP, CTX-I, PTH	8:30 – 12:30 every 30 min	Low calcium = 45-50% ↑ in CTX-I up to 2 h post suppressed by calcium intake. NS BAP
Haakonssen et al. (2015)	Females ($n = 32$) cyclists	90 min cycling 60% MAP and 10-min TT	Y	CTX-I, CTX-II, PTH, P1NP	Pre, immediately-, 40, 100, 190 min post	CTX-I ↑, PTH ↑, P1NP NS
Maimoun et al. (2006)	Male cyclists ($n = 7$)	15% below VT, 15% above VT (+VT). 50 min cycle	N	CTX-I, BAP, PTH	Pre, 30, 50 min during and 15 min post	16.8% ↑ CTX-I with +VT only BAP 14 %↑

Notes: C-terminal telopeptides of Type I collagen (CTX-I), procollogen type 1 amino terminal propeptide (P1NP), bone alkaline phosphatase (BAP), intact calcium (iCa), parathyroid hormone (PTH), tartrate resistant acid phosphatase 5b (TRAP5b), osteocalcin (OC). Randomised within subject crossover design (RWxD), resistance exercise (RE), plyometric exercise (PLY), whole body vibration (WBV), time trial (TT), endurance trained (ET), recreationally active (RA), no significant change (NS), ventilatory threshold (VT).

2.6.3 Bone turnover markers and the osteogenic index

Bone turnover markers have also been used to assess the effects of training interventions. Lester et al., (2009) used a short-term (8-weeks) exercise programme to assess changes in bone density, geometry and bone tissue turnover. The study included the use of DXA, pQTC, serum biomarker for formation including bone alkaline phosphatise (BAP), OC, and serum markers of resorption including TRAP, serum C-terminal telopeptide fragment of type I collagen (CTX-I). The osteogenic index was calculated for an aerobic running programme completed 3x per week, alternating between a steady state jog (20-30 min at 75% HR_{max}), an interval training sessions (20-30 min 1x800, 1x400, 2x200 with 5 min rest), a steady-state threshold run (20-30 min 80-85% HR_{max}), a resistance programme (periodisation programmes following a light, moderate and heavy programme 3x per week), and a combined exercise group (both resistance programme and running programme performed 3x per week). The OI was calculated based on previous research estimating the vGRF for similar types of exercise. The intensity of the running sessions were based on percentages of HR maximum (HR_{max}). It was not articulated what equation was used to establish HR_{max}. The intensity of the internal and external loads between the groups were not well matched making it difficult to establish whether a greater volume of work was performed between training interventions.

The programme did not induce changes observed through densitometry techniques (DXA, pQTC). However, an increase in BAP and OC were reported for both the combined (15.8 vs. 8.5% respectively) and resistance group (16.6% vs. 19.8% respectively). It was expected that the combined group would result in higher markers of bone formation than both the aerobic and resistance groups and that the overall OI would be higher for the combined programme. The results partially supported this hypothesis in that the combined group did result in increased markers of bone formation. However, these were not greater than the resistance exercise group despite the lower OI compared with the combined group (OI = 16±1.9 vs. 36.9±5.2 respectively). The OI calculated in this particular study only estimated the GRF from published data, and considered only the vertical GRF. As forces would continuously change as the body's centre of

gravity changes throughout movements it is possible that the OI was underestimated in certain movements, particularly for the resistance group exercise. Moreover serum biomarkers of bone turnover are also affected by diet. Nutrient intake of all participants was monitored at the pre- mid- and post-training periods using a 5 d food diary. Results of these diaries reported that nutrient intake was similar for all participants. Aside from inherent issues associated with diet assessment methods, it may have been more appropriate to have controlled nutrient intake better during the programme by providing them with a standardised diet; although this may have been impractical throughout the 8-week period. Finally there was no mention of the time that the blood samples were taken and whether these times were standardised for all participants. Considering the high inter- and intra- subject variability for blood markers (particularly CTX-I) this is a major limitation of the research. Despite this the successful use of blood biomarkers to determine positive improvements in short-term exercise programmes is encouraging.

A recent study (Erickson & Vukovich, 2010) adds further support for the use of the OI as a quantitative tool to determine the osteogenic effects of different modes of exercise. Erickson and Vukovich (2010) used a plyometric jumping protocol either completing one session of jumps (J1 x group) or two sessions of jumps (J2 x group) 3 d per week for 8-weeks. Repetitions and load increased over the 8-week period for both groups but the same number was completed by each group (e.g. week-1 = J1 2x10 jumps @ 80% body mass, vs J2 = 2x5 jumps per session @ 80% body mass repeated with 6 h rest between). The results showed a reduction (12%) in CTX-I from baseline to week 8, and an increase in BAP (40%) from baseline to week 8 in J2 compared to J1 which showed a 4% increase in CTX-I and a 23% increase in BAP. This was accompanied by an increase in the OI of 33% for J2 group suggesting that there was a more favourable response on the skeleton when jumps are segmented. However, this was a pilot study and it is likely that the sample size was too small to show statistically significant results. *Post hoc* analysis by the authors revealed that ~26 participants would be required per group compared to the 7 used in the study. The 12% reduction in CTX-I is likely well within the variability of the measure and is therefore unlikely to be a clinically meaningful change. Further work is needed to confirm the effects of exercise mode and rest intervals using the OI and bone turnover markers. Also further work is

needed to understand what constitutes a clinically meaningful OI in order to direct future exercise training doses.

The relationship between the OI and bone turnover markers has also been assessed acutely in the recovery hours following a bilateral hoping protocol (Rantalainen et al., 2009). The study demonstrated that the hoping protocol induced bone turnover response, in favour of resorption, as demonstrated by a statistically significant increase (31%) in CTX-I at 48 h post exercise. Interestingly, there was a positive relationship between the loading parameters and bone formation. This supports the role strain rate and magnitude play in bone remodelling. However, whilst the blood data were corrected for plasma volume changes the authors failed to control for diet which can strongly affect bone markers, specifically CTX-I (Clowes et al., 2002). It is also not clear how well the pre- and post- exercise plasma collection methods were controlled. CTX-I is very variable being substantially affected by circadian variation and diet (Seibel, 2005). Therefore, the strength of evidence must be interpreted with caution.

2.6.3 Bone turnover markers and intermittent exercise

Few studies have acknowledged the importance of rest-inserted or intermittent exercise on acute responses on bone turnover markers (Lin et al., 2012; Mezil et al., 2015). In a recent study (Lin et al., 2012) investigated the acute effects of plyometric jumping and intermittent running on biomarkers of bone turnover.. Twenty fount participants were randomised into three groups; a plyometric jumping group (n =8), and intermittent running group (n=8) and a control (non-exercising) group (n=8). The intermittent running protocol was only included as a lower impact exercise to compare to the plyometric jumping protocol. The plyometric jumping group resulted in a greater increase in osteocalcin (a specific biomarker of bone formation) than the control group at 5 min and 1 h post exercise. The intermittent running group also reported higher markers of bone formation in comparison to the control group, although this was not statistically different and was concluded that the responses reflected circadian variation.

The results of this study would suggest that exercise involving high-impact jumping does result in immediate increases in bone formation and that the use of serum biomarkers can identify bone changes after an acute bout of exercise. This was a well-controlled study that standardised the diet of the participants over the 4 d period. A further strength was the use of a control group in order to control for the diurnal variation observed with certain biomarkers such as tartrate-resistant acid phospahtase (TRAP5b). The use of parallel groups over a crossover design has some disadvantages. For example; because the participants in a crossover trial receives both interventions a smaller sample size is needed. The intra-subject variability is a major limitation with the use of a parallel group, and a crossover design would have better suited the research question. The use of osteocalcin as a choice in marker should also be questioned as this marker has been shown to potentially reflect both resorption and formation and likely reflect energy expenditure.

Furthermore, this study made no link to the findings from animal studies, and no mention of mechano-sensitivty as a potent mechanism for bone turnover. There was also no direct measurement of the GRFs induced by the exercise. This makes it difficult to comment on the association between mechanical impacts induced by exercise mode and serum marker response of bone turnover. Moreover, the control of the intensity between the exercise conditions was not well controlled. Therefore, it is unclear whether the overall load was higher for the plyometric jumping group. The use of the OI may help in establishing the importance of the overall mechanical load between conditions.

Recently, Mezil et al. (2015) investigated the effect of a high-intensity intermittent exercise protocol performed on a cycle ergometer on BTM responses. The authors demonstrated that BAP (bone formation marker) was significantly elevated above baseline at 1 h post exercise and remained so up to 24 h post-exercise, although the bone resorption marker (NTX) was lower than baseline at 24 h. Data suggests that the intermittent protocol enhanced bone turnover with an increase in bone formation markers and concomitant reduction in resorption markers respectively. However, the study did not provide a comparison with other modes of exercise or variations of

the intermittent exercise. We know that variations in exercise-rest intervals during intermittent exercise can have profound effects on the differences in physiological and biochemical responses (Buchheit & Laursen, 2013b; 2013a). Therefore, it is unclear what the optimal exercise-rest interval might be and therefore further investigation is required to optimise the protocol. The study also failed to include a control condition; therefore it is unclear whether the exercise-induced changes in BTMs were a true response of exercise or as a result of diurnal variation. The mode of exercise used (impact vs non-impact) is also important. Cycle ergometry is a non-impact exercise which as discussed is linked to negative responses to bone in athletes. Whilst muscle forces are important and potentially determines the predominant changes in bone morphology (Judex & Carlson, 2009), cycle ergometry does not create the eccentric loading phase that is important for bone (Hawkins et al., 1999) and therefore may not be the optimal load to induce bone formation.

Section summary

Bone turnover markers are a viable option to assess the acute responses of bone remodelling following exercise. Continuous protocols lasting approximately 60 minutes have shown increases in bone turnover in favour of resorption suggesting an acute deleterious response to bone. However, there is limited information regarding the effect of intermittent exercise on bone turnover. Of the studies that exist, many did not include a comparison to other modes of exercise or variations of intermittent exercise models. Therefore, it remains unclear what role different exercise-to-rest durations might have on the acute responses. Based on previous studies, it is recommended that to ensure valid and reliable responses of BTM to exercise the following guidelines are followed:

- At least 12 h fasted.
- Avoid exercise 24-48 h prior to testing.
- Where possible adjust for plasma volume changes which occur with exercise.

Due to the cost of BTMs it is not feasible to examine multiple immunoassays, however a minimum of two is recommended, one for formation and one for resorption (Banfi et al., 2010). Current recommendations from the IOF (Vasikaran et al., 2011a) favour P1NP and CTX-I (formation and resorption markers respectively).

2.7 Summary and conclusion

Bone responds to mechanical loads of high frequency, magnitude and intensity (rate). Moreover, mechanical loads which are unhabituated, bi-directional and importantly are performed on a limited number of loading cycles interspersed with recovery bouts are most osteogenic. Therefore the use of intermittent mechanical loading is beneficial for bone, however the majority of evidence has been provided by animal models. Currently the role of intermittent exercise and its potential anabolic effects on bone have not been fully investigated in human studies. Evidence from small-sided soccer games and HIT style cycle ergometry has provided positive evidence for bone formation, although mechanisms of action are unclear. It is also unclear whether there is an optimal duration for the exercise-to-rest intervals and what the magnitude of the effect on bone turnover markers would be. The aforementioned studies also failed to quantify the mechanical loading environment, and therefore it is unknown whether the intermittent loading environment offers a more osteogenic stimulus. This can be achieved through the indirect assessment of strain using GRFs and also the OI.

The review also demonstrated the complexity of intermittent exercise models through the multiple interacting variables, and it is important to address this to ascertain the optimal intermittent dose required for bone remodelling. To address this issue it is necessary to develop a number of intermittent weight-bearing protocols which are matched for the external training load but vary in different exercise-to-rest durations.

Whilst motorised treadmills are common, the ability to measure force during running is not accessible as the cost of a fully instrumented motorised treadmill is high. Furthermore, motorised treadmills may not reflect the training environment as discussed in the review. However, non-motorised treadmills may offer a unique and novel ergometer to assess the stress and strain of intermittent exercise. The limitation of these treadmills and limited knowledge on the physiological stress imparted by the treadmill requires further investigation. Moreover, if the non-motorised treadmill is used it is clear that the exercise dose prescribed for running on the treadmill would be different to a motorised treadmill.

In summary:

1. The use of an ergometer which can measure mechanical load via the ground reaction force on every loading cycle is required. Moreover, the use of an ergometer which reflects the movement patterns of intermittent exercise and enables the exerciser to accelerate and decelerate easily at their natural rate is required, hence the NMT will be used throughout the thesis.

2. To overcome some of the limitations related to the SSG studies there is a need to develop well-controlled protocols matched for speed, distance and external work. This can be achieved on the NMT. Protocols of different exercise-to-rest durations which differ in the frequency of changes in speed yet differ proportionally in their intermittency will help to establish a dose-response relationship.

3. The use of velocity at $v\dot{V}O_{2max}$ will be used to dose the speed and intensity of the intermittent protocols.

4. At the time of this thesis there is limited information on the cardiorespiratory responses to running on the NMT, and the effect exercise intensity has on bone turnover. It is therefore pertinent to assess cardiorespiratory responses.

5. Bone turnover markers, specifically P1NP and CTX-I will be used to establish the effect of acute exercise on bone metabolism.

CHAPTER 3:
The validity of intermittent and continuous graded exercise tests for the assessment of peak cardiorespiratory responses on a Woodway Force 3 non-motorised treadmill

3.1 Abstract

Aims: The Woodway Force 3 non-motorised treadmill (NMT) has the capacity to measure both vertical and anterior horizontal ground reaction force (GRF) to quantify the osteogenic potential of exercise. However, the greater energetic requirement of continuous running on the NMT coupled with reduced time to exhaustion (TTE) and reduced peak speeds limits the transferability of reference speeds obtained from a continuous graded exercise test (GXT) performed on the NMT. Therefore the aim of this study was to investigate peak cardiorespiratory responses to intermittent and continuous GXTs on a NMT compared to a motorised treadmill (MT).

Methods: Implementing a randomised post-only crossover design, sixteen healthy males (28±8 years, height 178.0±0.7 cm, body mass 79.7±10.2 kg) completed one continuous GXT on a MT, and three GXTs on a NMT. The continuous MT protocol (Cont-MT) increased by 1 km·hr^{-1}·min^{-1}. One continuous NMT protocol (Cont-NMT) mirrored the MT protocol, with the second and third NMT trials utilising an intermittent step protocol, alternating between higher and lower speeds every 15 s (15-NMT) and 30 s (30-NMT). Data are presented as means (SD) and analysed using a one way repeated measures ANOVA, with peak and submaximal data analysed using Cohen's *d* (95% CI)

Results: There was no statistically significant difference in $\dot{V}O_{2max}$ between Cont-MT vs. Cont-NMT (MD = 0.1 mL·kg^{-1}·min^{-1}; 95% CI = -1.1 to 1.3; P = 1.00, trivial). There was a statistically significant reduction in peak HR (MD = 9 beats·min^{-1}; 95% CI = 4 to 13; P = 0.0001, Moderate), together with large reductions in $v\dot{V}O_{2max}$ (MD = 5 km·hr^{-1}·min^{-1}; 95% CI = 4 to 7; P = 0.0001) and time to exhaustion (TTE) (MD = -287 s; 95% CI = -341 to -234; P = 0.0001) for Cont-MT vs. Cont-NMT. Similar $\dot{V}O_{2max}$ (MD = 1 mL·kg^{-1}·min^{-1}; 95% CI = -1 to 4; P = 0.701, Trivial), and peak HR values (MD = 3 beats·min^{-1}; 95% CI = 1 to 7; P = 0.170, Small), with a greater TTE (MD= 59 s; 95% CI = 17 to 101; P = 0.004, Moderate) and a smaller reduction in $v\dot{V}O_{2max}$ (MD = 1.2 km·hr^{-1}·min^{-1}; 95% CI = 0.3 to 2.0; P = 0.009, Moderate) were demonstrated for 15s-NMT vs. Cont-MT. However, $\dot{V}O_{2max}$ was statistically significantly higher for Cont-MT vs. 30s-NMT (MD = 3 mL·kg^{-1}·min^{-1}; 95% CI = 0 to 6; P = 0.040, Small), with a statistically significant reduction in peak HR (MD = 6 beats·min^{-1}; 95% CI = 3 to 9; P = 0.0001, Small).

Conclusions: Similar $\dot{V}O_{2max}$ is obtained on a NMT using a continuous GXT when compared to a MT. However, $v\dot{V}O_{2max}$ is statistically significantly reduced, with a very large statistically significant reduction in TTE and a moderate reduction in peak HR. When an incremental protocol is performed intermittently (15 x 15 s) on a NMT, a similar $\dot{V}O_{2max}$ can be achieved, together with smaller reductions in $v\dot{V}O_{2max}$, and peak HR. Conversely, longer exercise-to-rest intervals (30 x 30 s) resulted in significant reductions in $\dot{V}O_{2max}$ and peak HR. As such, shorter exercise-to-rest intervals may better reflect peak aerobic performance on a NMT.

3.2 Introduction

In Chapters one and two the need to develop a number of well-controlled intermittent exercise protocols on the non-motorised treadmill were highlighted. The development of the Force 3 non-motorised treadmill (NMT) by Woodway offers a unique opportunity to quantify the osteogenic potential of various intermittent running protocols. An advantage of the NMT is that it enables the exerciser to accelerate and decelerate at their natural rate (Davies et al., 1984), reflecting the nature of intermittent exercise (Aldous et al., 2014) which could not be replicated so easily on a motorised treadmill (MT) (Nigg et al., 1995).

Whilst an NMT may better reflect intermittent movement patterns compared to an MT, there is a greater energetic cost of running at sub-maximal speeds due to the requirement of the exerciser to overcome the inertia of the treadmill belt (De Witt et al., 2009). This leads to greater peripheral fatigue, reduced times to exhaustion (TTE) and lower peak speeds achieved on the treadmill at similar intensities (Davies et al., 1984; De Witt et al., 2009). Therefore, reference speeds such as the velocity at maximal oxygen uptake ($v\dot{V}O_{2max}$) (Billat, 2001), obtained using a continuous graded exercise test (GXT) on an MT might not be transferable to the performance of intermittent exercise protocols on an NMT. As such, the assessment of peak cardiorespiratory performance to obtain reference speeds for the prescription of exercise intensity used in subsequent Chapters (4, 5, and 6) must be performed on the NMT.

To date, limited information exists regarding the use of the NMT as a tool for the assessment of cardiorespiratory fitness (Davies et al., 1984; Mauger et al., 2013; Morgan et al., 2015). Early research (Davies et al., 1985) reported no statistically significant differences for $\dot{V}O_{2max}$ between a continuous MT compared to a continuous NMT protocol (mean difference [MD] = 1.2 mL·kg^{-1}·min^{-1}; 95% CI = -0.4 to 0.4; P = 0.094). However, no information on the type or set-up of the ergometer was included, nor information on peak heart rate. Interestingly, 4 out of the 10 participants achieved their highest $\dot{V}O_{2max}$ values on the NMT compared to other protocols. However, peak velocity achieved during the continuous GXT was reduced by approximately 30%

on the NMT compared to the MT. The findings were further corroborated recently by Morgan et al. (2015) using an modernised NMT with a curved belt to allow a better running style. Therefore, it is unclear whether the performance of a continuous GXT on an NMT provides an appropriate environment to establish a valid measure of peak aerobic performance.

As an alternative, intermittent GXTs have shown to result in greater times to exhaustion (TTE) (Metaxas et al., 2005), higher peak speeds (Mier & Alexander, 2011), and in some cases, higher $\dot{V}O_{2max}$ (Girard et al., 2006; Mier & Alexander, 2011) which might be more reflective of cardiorespiratory performance in some cases. However, few studies (Assadi & Lepers, 2012) exist on the use of intermittent GXTs performed in a laboratory environment. Moreover, given different exercise-to-rest durations and frequency of changes in speed have an effect on cardiorespiratory data (Tschakert & Hofmann, 2013) it is not clear how the duration of the exercise-to-rest interval using a fixed ratio (1:1) might affect the cardiorespiratory performance. Similar cardiorespiratory responses are evident when two short duration intervals (15 s and 30 s) are compared (Cipryan et al., 2016). However, it is not clear whether the NMT might have a more profound effect on performance due to the locomotive difficulties of running for continuous periods (De Witt et al., 2009).

Therefore, the aims of the current study were: (1) to investigate the cardiorespiratory responses of a continuous GXT on a NMT compared to a continuous GXT on a MT, and (2) to investigate the cardiorespiratory responses to intermittent GXTs using very short (15 x 15 s) and short (30 x 30 s) fixed ratio exercise to rest intervals. We hypothesised that; (1) a similar or higher $\dot{V}O_{2max}$ would be achieved on the NMT when a continuous GXT was performed compared to the MT couple with a lower peak speed on the NMT compared to the MT, and (2) reductions in peak speed would be smaller if performed using an intermittent GXT, and that the achieved $\dot{V}O_{2max}$ might be greater when performing intermittent GXTs compared to continuous GXTs.

3.3 Methods

Sample size estimation was performed *a priori* (G-Power software, Franz Faul, Universitat Kiel, Germany) for the primary outcome measure of $\dot{V}O_{2max}$. Based on previously published $\dot{V}O_{2max}$ data using a NMT (Davis et al 1984), the effect size (*f*) for analysis of variance was calculated as 0.63 (Beck, 2013). A sample size of *n*=11 provided 95% power at an alpha level of 0.05. Figure 3.1 shows participant recruitment and randomisation to the protocols. Sixteen males were included in the final analysis (age 28±8 years, height 178.0±0.7 cm, body mass 79.7±10.2 kg, body fat 15.6±5.0%). The inclusion and exclusion criteria were; healthy males, aged between 18-45 years, were all non-smokers and free of any cardiovascular, respiratory and metabolic diseases and were free of any musculoskeletal injuries. We required participants' who were recreationally active but not highly-trained and participated in a both intermittent and continuous sports (Participants include; *n*=4 soccer training 2-3 x per week, *n*=12 mid-distance club runners, 2 x per week). The study was approved by the Departmental Ethics Committee and conformed to the Declaration of Helsinki.
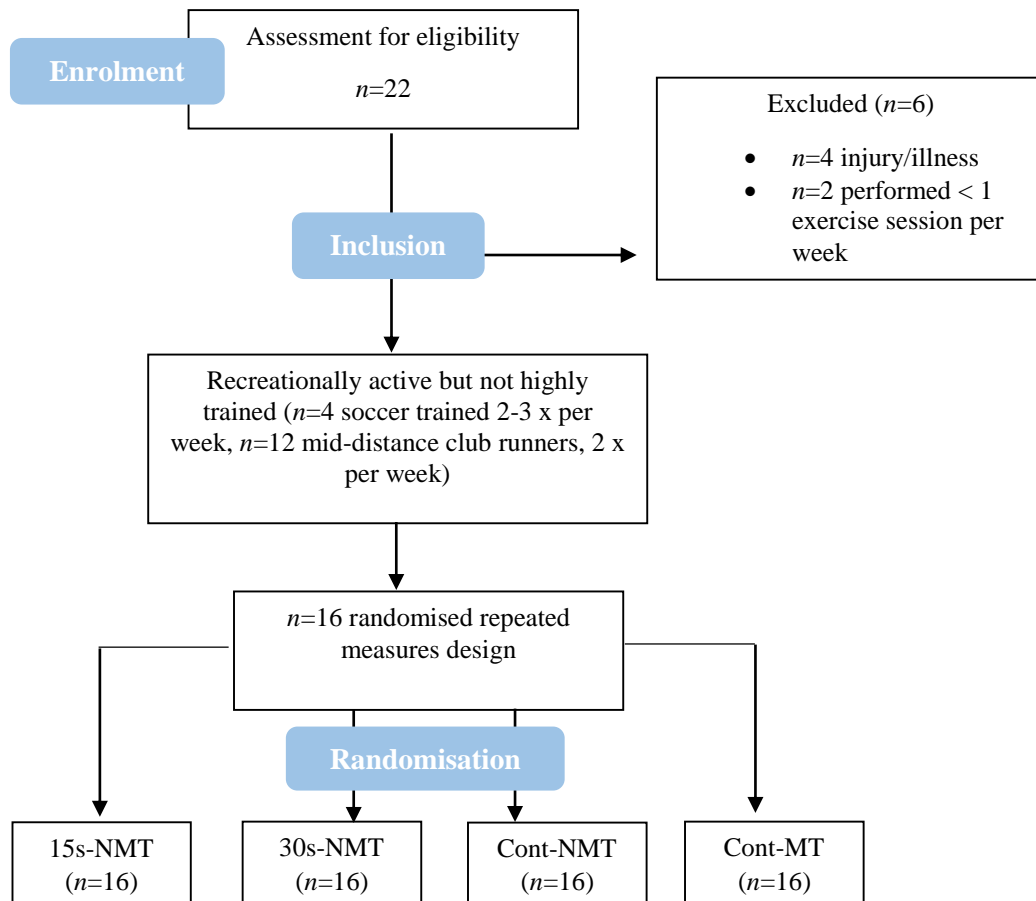
Figure 3.1 The progress of participants through phases of a randomized repeated measures crossover design.

Participants completed four GXTs in a randomised order using a computer based randomiser programme. Trials were separated by a minimum of 24-48 h and completed within a maximum 14 day period. This has been shown to be an insufficient time period for $\dot{V}O_{2max}$ to change (Santos-Silva et al., 2007). In order to improve running performance on the NMT, participants performed two standardised familiarisation sessions prior to the first GXT (Sirotic & Coutts, 2008). The familiarisation sessions consisted of two separate bouts of 15 min, separated by 24 h. Session one included; walking and submaximal running for 30 s intermittent bouts (Hamilton et al., 1991). Session two consisted of walking, jogging and running, followed by a repeated sprint session consisting of five repeated maximal sprints. Participants were deemed to be familiarised to the treadmill when they were capable of achieving and maintaining the desired speed within a

two second period (Highton et al., 2012). Participants were given verbal instruction by the researcher to maintain the appropriate speeds. Participants also obtained visual and auditory feedback on when to change speed via the Pacer performance software. A red line on the screen depicts the target speed and a green line depicts the actual speed (See Figure 3.2) when running on the NMT. A standardised five minute warm-up on a cycle ergometer (Monark 842E, Monark Exercise AB, Varberg, Sweden) at 75 W preceded all GXT protocols to standardise the warm up across ergometers. The following constraints were placed on the participants to improve the strength of the study design:

- Avoid strenuous physical activity for 24 h prior to testing.

- Avoid caffeine consumption for 4 h prior to testing.

- Maintain normal hydration by drinking to thirst prior to testing.

- Consume a high carbohydrate meal 2 h prior to testing.

- All GXTs were performed at approximately the same time of day for each participant ($\pm$ 1 h)

To add to the existing small body of literature comparing physiological responses of MTs and NMTs participants performed a traditional continuous GXT on a motorised treadmill (MT) (Cosmos, h/p/cosmos, quasar lt, Germany) (Cont-MT) and a NMT. The speed on the MT increased every minute by 1 $km \cdot hr^{-1}$ (see Figure 3.3 A), with the gradient of the treadmill set at 1% to represent the energetic cost of outdoor running (Jones & Doust, 1996). Commencing treadmill speed was individualised for each participant (6 or 8 $km \cdot hr^{-1} \cdot min^{-1}$) for the Cont-MT GXT by aiming to elicit a HR response of ~40-50% of age predicted maximum HR using the formula 206.9 – (0.67 x age) (Gellish et al., 2007) and a test duration of approximately 10-12 minutes (Buchfuhrer et al., 1983).

Three modified GXTs were performed on the NMT (Woodway Force 3, Woodway Ltd), integrated with the Pacer performance software (Version 2013, Innervations). The NMT set-up is

shown in Figure 3.2. The continuous incremental NMT protocol (Cont-NMT) mirrored the Cont-MT protocol (see Figure 3.3 A). An intermittent, incremental 30 s NMT protocol (30s-NMT) and 15 s NMT protocol (15s-NMT) also followed a step protocol increasing by 1 $km\cdot hr^{-1}\cdot min^{-1}$. However, within each 1-minute stage the participant ran intermittently between the target speed and a lower speed of 4 $km\cdot h^{-1}$ (refer to Figure 3.3 B and C respectively), with the initial target speed for all individuals being 4 $km\cdot h^{-1}$. An active rest period at 4 $km\cdot h^{-1}$ reflected the walking component of the 30-15 IFT (Buchheit, 2008), with pilot testing suggesting this to be a comfortable walking speed for participants on the NMT.

Pilot testing consisted of a number of trials varying the exercise-to-rest ratio, the duration of, and the intensity of the rest phase. This was performed on an $n = 3$); however, data from these participants is not available to present in the thesis. The use of a 1:1 exercise-to-rest ratio was used in order to fit an equal volume of exercise and rest into each one minute phase. The use of the 15 x 15 short interval protocol follows common short exercise-to-rest intervals used in previous research (Billat et al., 2001) and is a popular intermittent training protocol utilised by coaches. The 30 x 30 exercise-to-rest interval was used as the longer interval approach as used by others (Margaria et al., 1969; Cipryan et al., 2016), consisting of 50% less acceleration and deceleration phases within each one minute period compared to the 15s-NMT protocol. Whilst the 30 s interval might be still considered short the physiological differences on a NMT are unclear, and is therefore worthwhile establishing to aid in the prescription of exercise intensity on an NMT.
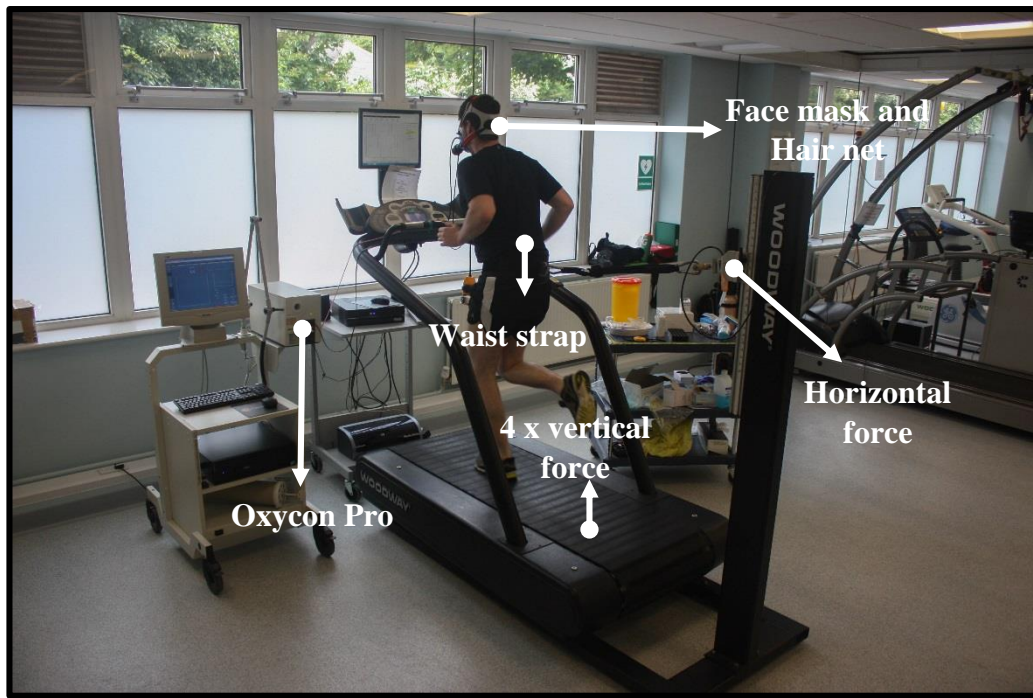
Figure 3.2 The general set-up of the NMT during the graded exercise tests.
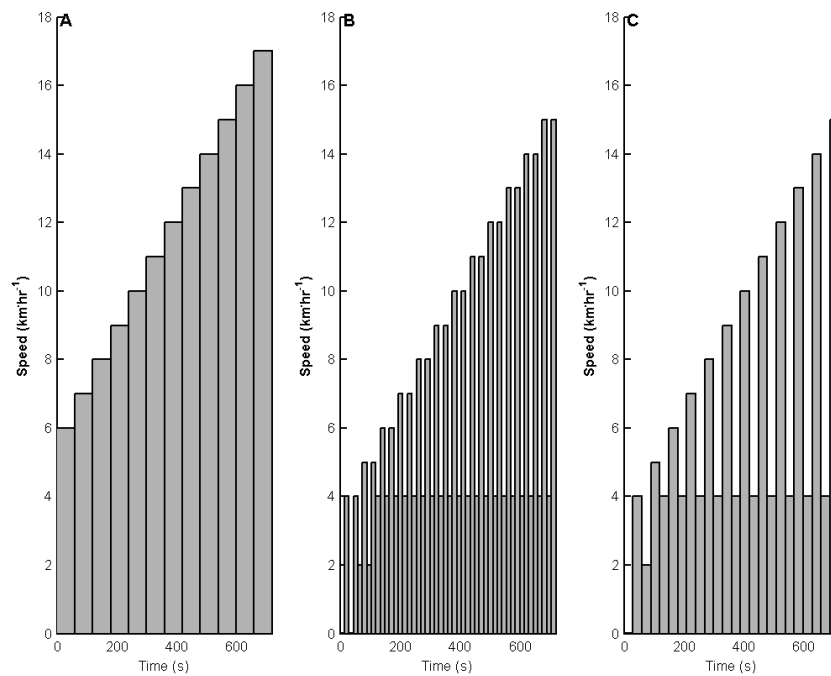
Figure 3.3 (A) continuous motorised treadmill (Cont-MT) and non-motorised treadmill (Cont-NMT) protocols, (B) the intermittent 30 x 30 s NMT protocol (30s-NMT) and (C), the intermittent 15 x 15 s NMT (15s-NMT) protocol.

3.3.1 Peak Cardiorespiratory Variables

Respiratory data were recorded continuously throughout each GXT. Calibration of the Oxycon Pro was performed immediately prior to each exercise test as per manufacturers' guidelines. Certified standard calibration gases of 16.4% O_2 and 4.5% CO_2 were used (Cryoservice Ltd, Worcester, UK). The turbine flow meter, used for the determination of $\dot{V}_E$, was calibrated with a 3 L syringe (Cosmed Srl). Room temperature (°C), relative humidity (%) and barometric pressure (mmHg) were recorded separately from a weather station. Heart rate was recorded continuously throughout each GXT (Polar S801i, Polar electro, Finland). Rating of Perceived Exertion (RPE) (modified category-ratio scale) (Foster et al., 2001) expressed as an arbitrary unit (AU) was obtained in the final 15 s of each stage, and taken just before participants commenced running for the final interval of the 15s-Int condition (thus in the final 15 s of the 1 min stage). An example of the RPE scale is presented in Appendix E.

A verification phase was performed 15 min after the termination of the GXT (Midgley et al., 2006). The verification phase serves as further confirmation that the $\dot{V}O_{2max}$ has been achieved, and is analogous to the concept of a plateau obtained from discontinuous incremental exercise (Midgley et al., 2006). For the Cont-MT, the verification phase consisted of running to volitional exhaustion at one stage (1 km·h^{-1}) higher than that achieved before termination of the $\dot{V}O_{2max}$ test. The verification phase performed for the NMT protocols required the participants to run to volitional exhaustion at an intensity which matched the speed of the final stage of the modified GXT. This was performed to ensure time to exhaustion was sufficient to allow $\dot{V}O_2$ to reach its peak (Midgley & Carroll, 2009; Kirkeberg et al., 2011).

All breath-by-breath data were processed using 30 s retrograde averages (Midgley et al., 2007a) obtained directly from the Oxycon Pro data management software®, with the highest $\dot{V}O_2$ in the final stages deemed to be $\dot{V}O_{2max}$ (Midgley et al., 2007a). $\dot{V}O_{2max}$ was deemed to be achieved if ≥2 of the following criteria were met (Keren et al., 1980): a plateau in $\dot{V}O_2$ defined as a change of less than 0.2 L·min^{-1} despite an increasing workload (Howley et al., 1995), a respiratory

exchange ratio (RER) of >1.15, a maximal heart rate (HR_{peak}) within ±10 beats·min^{-1} of the estimated HR_{peak} (206.9 - [0.67 x age]), and a RPE greater than 8 (Howley et al., 1995). The highest mean $\dot{V}O_{2max}$ obtained in the verification phase was taken as the $\dot{V}O_{2verif}$ (Midgley & Carroll, 2009; Scharhag-Rosenberger et al., 2011). The $\dot{V}O_{2max}$ was deemed to be verified when the $\dot{V}O_{2verif}$ differed by no more than 2% compared to the $\dot{V}O_{2max}$ (Midgley & Carroll, 2009). The 2% criterion was based on the error in $\dot{V}O_2$ determination from the turbine flow meter measurement error reported by the manufacturer, as reported by previous authors (Midgley et al., 2006; Midgley & Carroll, 2009).

3.3.2 Ventilatory Threshold Assessment

Detection of the first and second ventilatory thresholds were performed to assess the differences in energetic requirements and perceived exertion at sub-maximal intensities. Furthermore, it is not clear whether ventilatory thresholds can be accurately measured based on an intermittent GXT. The method for the detection of the first ventilatory threshold (VT_1) and second ventilatory threshold (VT_2) (also termed the respiratory compensation point), were obtained from the deflection of the ventilatory equivalents for oxygen ($\dot{V}_E.\dot{V}O_2^{-1}$) and carbon dioxide ($\dot{V}_E.\dot{V}CO_2^{-1}$) as described by previous authors (Whipp et al., 1989; Lucia et al., 2000). The determination of VTs from intermittent GXTs has been previously reported (Buchhiet et al., 2009) showing good agreement with the continuous GXT. An example VT_1 and VT_2 from a representative participant is presented in Figure 3.4.
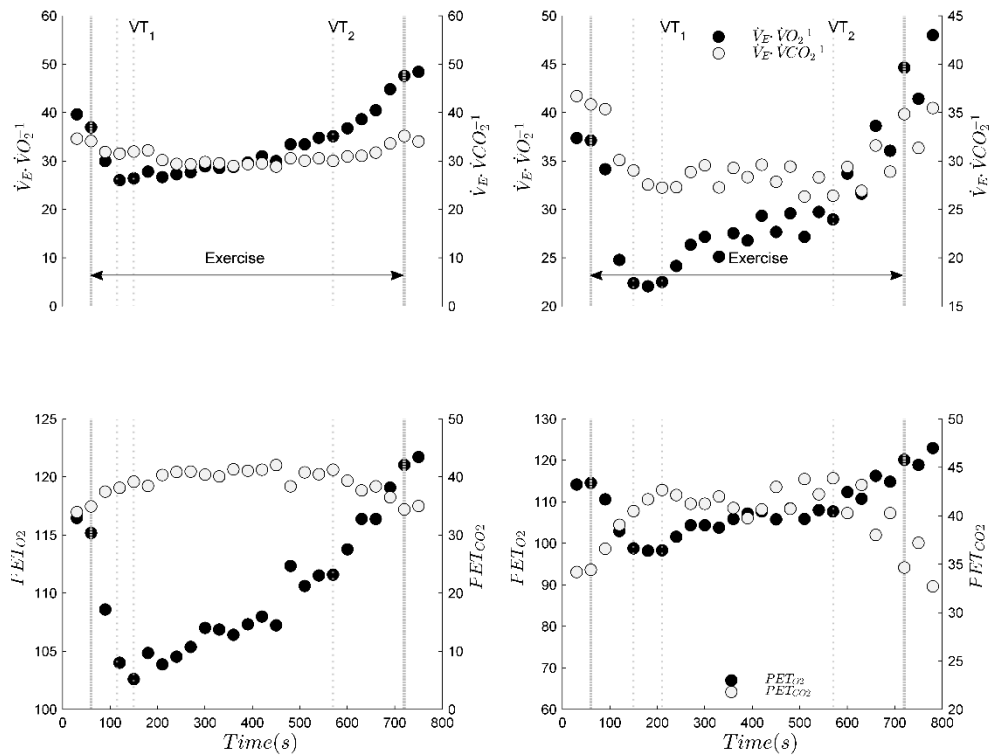
Figure 3.4 Ventilatory equivalents for a representative participant. The top 2 graphs show the gas exchange response from the 15s-NMT protocol. The bottom 2 graphs show the gas exchange response from the 30s-NMT protocol. VT_1 is located where the $\dot{V}_E\dot{V}O_2^{-1}$ curve inflects upwards, with a concomitant upward inflection in PET_{O2}. The nadir of the $\dot{V}_E\dot{V}CO_2^{-1}$ curve occurs at higher intensities and reflects the start of the respiratory compensation point for metabolic acidosis (VT_2) (Whipp et al., 1989).

3.3.3 Statistical Analysis

SPSS Version 19 (IBM, New York, USA), and Microsoft Excel 2010 (Microsoft, Washington, USA) were used for data analysis. Normality of distribution was assessed via the Shapiro-Wilk test. Logarithmic transformations were performed to correct for non-uniform distributions where necessary. The continuous maximum values of $\dot{V}O_2$, HR, TTE, BF, RER, relative O_2 pulse, $\dot{V}CO_2$, $\dot{V}_E$, and sub-maximal values of HR, and RPE at VT_1 and VT_2 are expressed as mean (SD). Non-

normally distributed variables ($v\dot{V}O_{2max}$ and RPE) are presented as medians and interquartile range (ICR). For the comparisons of peak, and sub-maximal cardiorespiratory data, a one-way repeated measures analysis of variance (ANOVA) was used. However, where parametric assumptions could not be met, the non-parametric Friedman test was chosen. Where a significant difference was detected, a post hoc test with Sidak adjustment was performed to further delineated the within subject factors. The mean difference and 95% confidence intervals (MD [95 % CI]) were also calculated to show the precision of the population point estimate.

The magnitude of the effect of condition on the maximum primary cardiorespiratory outcome variables for; $\dot{V}O_{2max}$, $v\dot{V}O_{2max}$,, HR, TTE, and for the primary sub-maximal variables at VT_1 and VT_2 ($\dot{V}O_2$ and HR) were expressed as a standardised effect size. The between group pooled SD (expressed as a coefficient of variance [% CV]) was used to calculate Cohen's *d* statistic and 95% confidence intervals as described by Hopkins (Hopkins et al., 2009).The threshold values for effect size magnitudes were 0.00-0.19 (trivial), 0.20-0.59 (small), 0.60-1.19 (moderate), 1.20-1.99 (large), and ≥ 2.0 (very large). Uncertainty in each effect was expressed as the probability that the effect was substantially positive or negative. If the probability of the true effect being positive or negative were both more than 5% then the effect was reported as unclear. Otherwise the effect was reported as the magnitude of the observed value using qualitative descriptions and their relative percentages as follows; <5% (very unlikely), 5-24% (unlikely), 25-74% (possible), 75-94% (likely), 95-99% (very likely), >99% (almost certain) (Hopkins et al., 2009). Data were not adjusted for multiple comparisons but a 95% CI was chosen to coincide with the 0.05 alpha level used with the P - values.

3.4 Results

Descriptive statistics (mean (SD) and median (ICR)) for peak cardiorespiratory values are displayed in Table 3.1. All participants obtained at least 3 of the specified criteria to verify the achievement of $\dot{V}O_{2max}$ for all of the GXT protocols. See Table A-D in the appendix B for all criteria achieved for *n*=16. The standardised effect sizes are presented in Table 3.2

Table 3.1 Mean (SD) peak values for parametric and median (ICR) peak values for non-parametric cardiorespiratory variables recorded during all four conditions, (n=16).

	Cont-MT	Cont-NMT	15s-NMT	30s-NMT
Parametric data				
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	46 (7)	46 (7)	45 (7)	43 (6)
HR$_{peak}$ (beats·min^{-1})	187 (8)	178 (9)	184 (9)	181 (10)
BF (breaths·min^{-1})	52 (8)	56 (8)	52 (3)	51(6)
$\dot{V}_E$ (L·min^{-1})	151 (20)	151 (21)	151 (18)	146 (22)
$\dot{V}CO_2$ (mL·min^{-1})	4652 (433)	4806 (514)	4820 (428)	4645 (252)
RER	1.3 (0.1)	1.3 (0.1)	1.4 (0.1)	1.4 (0.1)
TTE (s)	640 (74)	352 (44)	698 (53)	711 (45)
O$_2$ pulse (mL·beat^{-1})	25 (4)	26 (4)	24 (5)	24 (4)
$v\dot{V}O_{2max}$ (km·h^{-1})	15.1 (1.5)	10.3 (0.9)	13.8 (1.0)	13.8 (0.8)
Non-parametric				
RPE (AU)	9.0 (8.0-9.5)	8.0 (7.5-9.0)	8.0 (7.0-9.0)	9.0 (7.5-10.0)

Note: Cont-NMT, continuous non-motorised treadmill test; Cont-MT, continuous motorised treadmill test; 15s-NMT, 15 x 15 s intermittent non-motorised treadmill test; 30s-NMT, 30 x 30 s intermittent non- motorised treadmill test.

Peak oxygen consumption, heart rate, velocity at $\dot{V}O_{2max}$, and time to exhaustion (TTE)

$\dot{V}O_{2max}$ was statistically significantly different between conditions; $F_{(1.746,\ 26.194)} = 5.185$, P = .016. Post hoc analysis revealed a statistically significant difference for Cont-MT vs. 30s-NMT only, where Cont-MT $\dot{V}O_{2max}$ was higher (MD = 3 mL·kg^{-1}·min^{-1}; 95% CI = 0 to 6; P = .040, Small). The Cont-MT was not statistically significantly higher than Cont-NMT (MD = 0 mL·kg^{-1}·min^{-1}; 95% CI = -1 to 1; P = 1.000, Trivial) or 15s-NMT (MD = 1 mL·kg^{-1}·min^{-1}; 95% CI = -1 to 4; P = 0.701, Trivial).

Peak HR was statistically significantly different between conditions; $F_{(3, 45)} = 14.747$, P = .0001. Post hoc analysis revealed statistically significantly higher peak HR for Cont-MT versus Cont-NMT (MD = 9 beats·min^{-1}; 95% CI = 4 to 13; P = 0.0001, Moderate), Cont-MT versus 30s-NMT (MD= 6 beats·min^{-1}; 95% CI = 3 to 9; P = 0.0001, Small). The 15s-NMT vs. Cont-NMT was not statistically different. However the effect size was small (MD= 3 beats·min^{-1}; 95% CI = 1 to 7; P = 0.170, Small).

There was a statistically significant difference in $v\dot{V}O_{2max}$ between conditions; $F_{(3, 45)}= 118.3$, P = 0.0001. $v\dot{V}O_{2max}$ was higher for Cont-MT versus Cont-NMT (MD = 5.0 km·h^{-1}; 95% CI = 4.0 to 7.0; P = 0.0001, Very large), Cont-MT vs. 15s-NMT (MD = 1.2 km·h^{-1}; 95% CI = 0.3 to 2.0; P = 0.009, Moderate) and Cont-MT vs. 30s-NMT (MD = 1.0 km·h^{-1}; 95% CI = 0.1 to 1.7; P = 0.027, Moderate).

Time to exhaustion was statistically significantly different between conditions; $F_{(3, 45)} = 249.313$, P = 0.0001. Post hoc analysis revealed a statistically significant reduction in TTE for Cont-MT versus Cont-NMT (MD = -287 s; 95% CI = -341 to -234; P = 0.0001, Very Large). The intermittent GXT increased TTE and were greater than the Cont-NMT for the 15s-NMT. (MD = -346 s; 95% CI= -396 to -296; P = 0.0001, Very Large) and 30s-NMT (MD = -358 s; 95% CI = 410 to 307; P = 0.0001, Very Large). A statistically significantly higher TTE was found for 15s-

NMT versus Cont-MT (MD= 59 s; 95% CI = 17 to 101; P = 0.004, Moderate) and 30s-NMT

versus Cont-MT (MD = 71 s; 95% CI = 25 to 117; P = 0.002, Moderate).

Table 3.2 displays Cohens *d* and 95% confidence intervals for primary cardiorespiratory data

$\dot{V}O_{2max}$	Effect size	lo	hi	interpretation	percent	Interpretation
MT vs NMT	0.01	-0.1	0.12	Trivial	0,100,0	Most Likely
MT vs 15s-NMT	0.16	-0.08	0.39	Trivial	35,65,0	Possibly
MT vs 30s-NMT	0.37	0.12	0.62	Small	91,6,0	Likely
NMT vs 15s-NMT	0.15	-0.12	0.42	Trivial	35,64,1	Possibly
NMT vs 30s-NMT	0.36	0.1	0.62	Small	90,10,0	Likely
15s-NMT vs 30s-NMT	0.2	0.03	0.38	Trivial	52,48,0	Possibly

HR	Effect size	lo	hi	interpretation	percent	Interpretation
MT vs NMT	0.9	0.59	1.2	Moderate	100,0,0	Most likely
MT vs 15s-NMT	0.34	0.04	0.64	Small	83,17,0	Likely
MT vs 30s-NMT	0.57	0.35	0.79	Small	100,0,0	Most likely
NMT vs 15s-NMT	-0.56	-0.84	-0.27	Small	0,1,99	Very likely
NMT vs 30s-NMT	-0.28	-0.61	0.05	Small	0.30,69	Possibly
15s-NMT vs. 30s-NMT	0.25	-0.04	0.54	Small	63,36,0	Possibly

$v\dot{V}O_{2max}$	Effect size	lo	hi	interpretation	percent	Interpretation
MT vs NMT	3.29	2.87	3.7	Very large	100,0,0	Most likely
MT vs 15s-NMT	0.8	0.36	1.23	Moderate	99,1,0	Very likely
MT vs 30s-NMT	0.66	0.24	1.08	Moderate	98,2,0	very likely
NMT vs 15s-NMT	-2.94	-3.37	-2.52	Very large	0,0,100	Most likely
NMT vs 30s-NMT	-3.38	-3.87	-2.88	Very large	0,0,100	Most likely
15s-NMT vs. 30s-NMT	-0.21	-0.66	0.24	Small	4,45,52	Unclear

TTE	Effect size	lo	hi	interpretation	percent	Interpretation
MT vs NMT	4.2	3.65	4.75	Very large	100,0,0	Most Likely
MT vs 15s-NMT	-0.81	-1.22	0.4	Moderate	0,0,100	Most Likely
MT vs 30s-NMT	-1.03	-1.5	-0.56	Moderate	0,0,100	Most Likely
NMT vs 15s-NMT	-6.37	-7.01	-5.72	Very large	0,0,100	Most Likely
NMT vs 30s-NMT	-7.2	-7.92	-6.48	Very large	0,0,100	Most Likely
15s-NMT vs. 30s-NMT	-0.23	-0.56	0.1	Small	1,42,57	Possibly

Note: NMT = Non-motorised treadmill, MT = Motorised treadmill, 15s-NMT = 15 x 15 NMT, 30s-NMT = 30 x 30 NMT, Time to exhaustion (TTE), heart rate (HR), maximal oxygen uptake ($\dot{V}O_{2max}$), speed that elicits maximal oxygen uptake ($v\dot{V}O_{2max}$), lower bound (lo) upper bound (hi) for 95% CI.

Peak RPE, peak RER and peak relative O_2-Pulse

The peak RER values were statistically significantly different between conditions; $F_{(3, 45)} = 7.363$, $P = 0.0001$. Post hoc analysis revealed lower RER values for Cont-MT compared to 15s-NMT (MD = -0.087; 95% CI = -0.14 to -0.03; P = 0.001) and 30s-NMT (MD = -0.061; 95% CI = -0.103 to -0.020; P = 0.003). Ratings of perceived exertion could not be normally distributed. The Fridman test revealed no statistically significant differences in RPE between conditions; $X^2(3) = 4.707$, $P = 0.195$.

There was a statistically significant difference in peak relative O_2- pulse between conditions; $F_{(1.655, 24.818)} = 6.916$, $P = 0.006$. Post hoc analysis revealed a statistically significantly lower peak relative O_2-pulse for Cont-MT compared to Cont-NMT (MD = 1.1 mL·kg^{-1}·beat^{-1}; 95% CI = 0.5 to 1.8; P = 0.001) and 30s-NMT compared to Cont-NMT (MD = 1.9 mL·kg^{-1}·beat^{-1}; 95% CI = 0.6 to 3.1; P = 0.003).

Peak $\dot{V}_E$, BF, $\dot{V}CO_2$

There was no statistically significant main effects between conditions for peak $\dot{V}CO_2$: $F_{(3, 45)} = 1.167$, $P = 0.333$, peak $\dot{V}_E$: $F_{(3, 45)} = 0.549$, $P = 0.652$, and peak BF: $F_{(1.942, 29.137)} = 0.156$, $P = 1.00$.

Cardiorespiratory measures at Ventilatory Thresholds (VT$_1$ and VT$_2$)

Descriptive statistics for the submaximal cardiorespiratory measures are displayed below in Table 3.2. All data were normally distributed except the RPE at VT$_1$ and VT$_2$. Cohen's *d* and 95% confidence intervals for submaximal $\dot{V}O_2$ and HR are displayed in Table 3.3.

Table 3.3 The mean (SD) for parametric and the median (ICR) for non-parametric submaximal cardiorespiratory data ($n =16$).

	Cont-MT	Cont-NMT	15s-NMT	30s-NMT
$\underline{VT_1}$				
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	26 (4)	28 (6)	23 (5)	22 (5)
HR$_{peak}$ (beats·min^{-1})	137 (9)	147 (9)	126 (15)	128 (14)
RPE (AU)	2 (2-3)	3 (2-3)	2 (1-2)	2 (1-2.5)
$\underline{VT_2}$				
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	38 (6)	39 (9)	38 (6)	36 (7)
HR$_{peak}$ (beats·min^{-1})	169 (12)	167 (12)	168 (13)	163 (9)
RPE (AU)	5 (4.5-7)	5 (3.5-7)	5 (4.5-7)	5 (4-5)

Oxygen consumption at VT_1 ($\dot{V}O_2$-VT_1) revealed a statistically significant main effect between conditions; $F_{(3, 45)}$ = 10.740, P = 0.0001. Post hoc analysis revealed a higher $\dot{V}O_2$-VT_1 for Cont-MT compared to 15s-NMT (MD = 3 mL·kg^{-1}·min^{-1}; 95% CI = 0 to 6; P = 0.023, Moderate) and 30s-NMT (MD = 4 mL·kg^{-1}·min^{-1}; 95% CI = 0 to 8; P = .020, Moderate) and a higher $\dot{V}O_2$-VT_1 for Cont-NMT compared to 15s-NMT (MD = 5 mL·kg^{-1}·min^{-1}; 95% CI =1 to 9; P = .012, Moderate) and 30s-NMT (MD = 6 mL·kg^{-1}·min^{-1}; 95% CI =2 to 10; P = .004, Moderate). $\dot{V}O_2$-VT_1 was not statistically significantly different between Cont-MT vs Cont-NMT (MD = -2 mL·kg^{-1}·min^{-1}; 95% CI = -4 to 0; P = 0.239, Small). There was no statistically significant main effect for $\dot{V}O_2$ at VT_2 ($\dot{V}O_2$-VT_2) between conditions; $F_{(3, 45)}$ = 2.694, P = 0.052.

Heart rate at VT_1 (HR-VT_1) revealed a statistically significant main effect between conditions; $F_{(1.898, 28.476)}$ = 12.861, P = 0.0001. Post hoc analysis revealed that HR-VT_1 for Cont-NMT was higher compared to Cont-MT (MD = 10 beats·min^{-1}; 95% CI = 6 to 14; P = .0001, Moderate), 15s-NMT (MD = 21 beats·min^{-1}; 9 to 34; P = 0.001, Large), and 30s-NMT (MD = 19 beats·min^{-1}; 95% CI = 8 to 29; P = 0.0001, Large). There were no other statistically significant interactions. Heart rate at VT_2 (HR-VT_2) revealed no statistically significant main effect for condition; $F_{(3, 45)}$ = 1.305, P = 0.285.

Ratings of perceived exertion at VT_1 (RPE-VT_1) and VT_2 (RPE-VT_2) were not normally distributed, despite log transformations and so the Friedmans non-parametric test was used. A significant main effect was found for RPE-VT_1 between conditions ($X^2(3)$ = 11.890, P = 0.008. However, pairwise comparison did not show any statistically significant differences. No statistically significant main effects was found for RPE-VT_2 ($X^2(3)$ = 2.619, P = 0.454).

Table 3.4 Cohen's *d* and 95% confidence intervals (CI) for submaximal $\dot{V}O_2$ and HR data (n = 16)

		Effect size	Upper (CI)	Lower (CI)	probability	Qualitative Interpretation
$\dot{V}O_2$-VT1	Cont-MT vs. Cont-NMT	-0.34	0.02	-0. 70	80,20, 0	Likely
	Cont-MT vs. 15s-NMT	0.66	0.24	1.08	99,1,0	Most likely
	Cont-MT vs. 30s-NMT	0.86	0.33	1.40	99,1,0	Most likely
	Cont-NMT vs. 15s-NMT	0.91	0.34	1.48	99,1,0	Very likely
	Cont-NMT vs. 30s-NMT	1.08	0.61	1.56	100,0,0	Most likely
	15s-NMT vs. 30s-NMT	0.23	-0.32	0.76	55,36,6	Unclear
$\dot{V}O_2$-VT2	Cont-MT vs. Cont-NMT	-0.07	-0.35	0.20	3,80,17	Unclear
	Cont-MT vs. 15s-NMT	0.05	-0.16	0.26	7,92,1	Likely Trivial
	Cont-MT vs. 30s-NMT	0.37	-0.02	0.75	81,18,0	Likely
	Cont-NMT vs. 15s-NMT	0.12	-0.27	0.50	32,63,5	Unclear
	Cont-NMT vs. 30s-NMT	0.39	-0.01	0.79	84,16,0	Likely
	15s-NMT vs. 30s-NMT	0.32	-0.04	0.68	76,24,0	Likely
HR-VT1	Cont-MT vs. Cont-NMT	-1.07	-1.36	-0.78	0,0,100	Most likely
	Cont-MT vs. 15s-NMT	0.84	0.19	1.5	92,2,0	Very likely
	Cont-MT vs. 30s-NMT	0.74	0.14	1.34	96,3,0	Very likely
	Cont-NMT vs. 15s-NMT	1.63	0.97	2.3	100,0,0	Most likely
	Cont-NMT vs. 30s-NMT	1.59	1.00	2.18	100,0,0	Most likely
	15s-NMT vs. 30s-NMT	-0.13	-0.86	0.61	18,40,42	Unclear
HR-VT2	Cont-MT vs. Cont-NMT	0.09	-0.30	0.47	27,66,7	Unclear
	Cont-MT vs. 15s-NMT	-0.04	-0.42	0.35	11,70,19	Unclear
	Cont-MT vs. 30s-NMT	0.37	-0.18	0.91	74,24,2	Possibly
	Cont-NMT vs. 15s-NMT	-0.12	-0.73	0.48	14,47,40	Unclear
	Cont-NMT vs. 30s-NMT	0.30	-0.19	0.80	67,31,2	Possibly
	15s-NMT vs. 30s-NMT	0.41	-0.31	1.14	73,22,5	Unclear

The individual differences in $\dot{V}O_{2max}$ and peak HR between the criterion measure and the NMT trials are shown below in Figure 3.5.
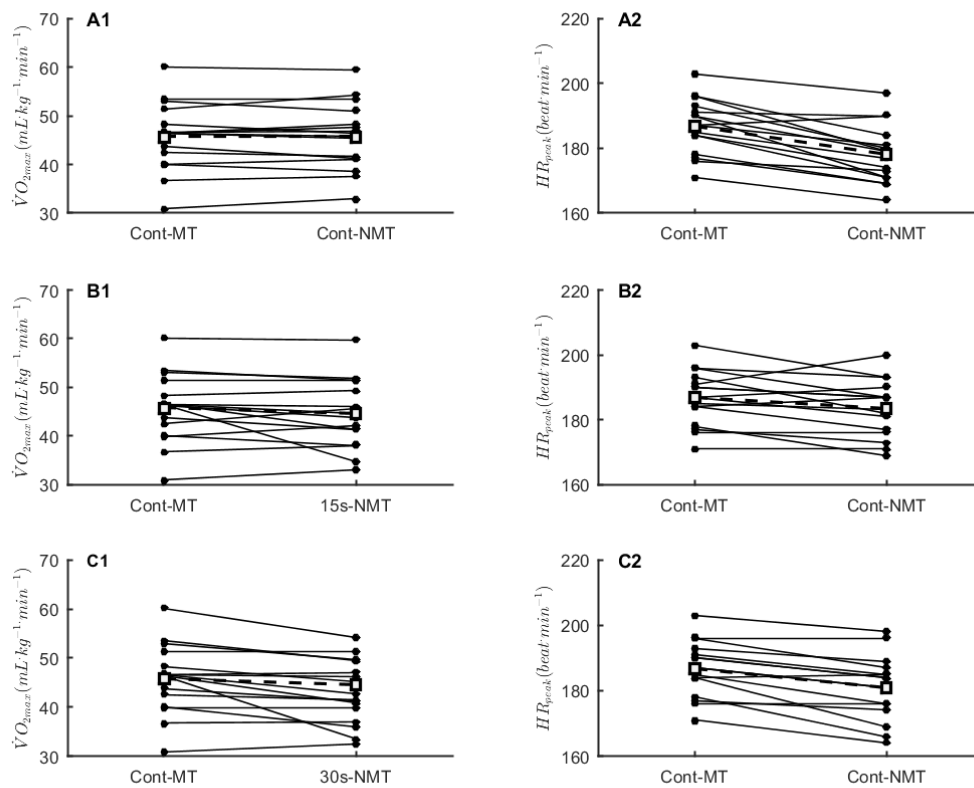
Figure 3.5 A1-C1 Individual participant differences in $\dot{V}O_{2max}$ (mL·kg⁻¹·min⁻¹) and peak HR (A2-C2) for (A) Cont-MT vs. Cont-NMT (B) Cont-MT vs. 15s-NMT (C) Cont-MT vs. 30s-NMT. The dashed line with square markers at either end reflects the population mean

3.5 Discussion

The purpose of this study was to develop an appropriate GXT on the NMT to obtain peak cardiorespiratory data to use to prescribe the exercise dose for subsequent protocols used throughout the thesis. Furthermore, this study served to provide important information on both sub-maximal and maximal cardiorespiratory performance on the NMT. Given intensity (e.g. % $\dot{V}O_{2max}$) has an effect on acute bone remodelling (Maimoun et al., 2006; Scott et al., 2011) it is important to establish the physiological stresses induced by the ergometer when compared to other studies using motorised treadmills to aid in comparison of the effects on bone turnover.

3.5.1 Peak cardiorespiratory responses Cont-NMT versus Cont-MT

There was no statistically significant difference in $\dot{V}O_{2max}$ between Cont-MT and Cont-NMT and the difference expressed as a standardised effect size was trivial (Table 3.2) However, the $v\dot{V}O_{2max}$ for the Cont-NMT was reduced by approximately 30% compared to the Cont-MT with a statistically significant moderate reduction in peak HR (MD = 9 beats·min^{-1}; 95 % CI = 4 to 13; P = 0.0001) and a very large reduction in TTE (MD = -287 s; 95% CI = -341 to -234; P = 0.0001).

A similar mean TTE was reported by earlier authors (Davies et al., 1984) (6.0 ± 2.2 minutes) using a continuous incremental test on a NMT, commencing at 10 km·h^{-1}, increasing by 2 km·h^{-1}, every 3 minutes. Whilst peak speed was not reported in the earlier study, based on the protocol, the mean peak speed could be calculated and estimated to be ~14 km·h^{-1}. The higher speeds achieved in the earlier study, compared to the present study, is likely due to the higher trained state of the athletes used. However, it is not clear on the set-up of the NMT used by the previous authors and so small differences in the ergometer may also have contributed to the findings. Importantly, the similarities in $\dot{V}O_{2max}$ between the NMT and MT trials together with reduced peak velocities and TTE reported in the earlier study (Davies et al., 1984) is corroborated by our data. Moreover, in a recent study investigating the differences in graded exercise testing on an MT and an NMT, $\dot{V}O_{2max}$ was also similar. Again the speed achieved on the NMT was lower compared to the MT. The NMT used by the authors was an upgraded version of the NMT utilising a curved treadmill belt negating the need for the waist belt and horizontal pulley. Therefore, improving the running style and posture of the exerciser over the earlier models used in the current study.

The very large reductions in $v\dot{V}O_{2max}$, TTE, and moderate reductions in peak HR (Table 3.2) observed for Cont- NMT, compared to Cont-MT, can be partly attributed to the high degree of inertia and frictional resistances imparted by the treadmill belt (Chelly & Denis, 2001; McKenna & Riches, 2007; Highton et al., 2012). When compared to running velocity over-ground, running

at maximal velocity on a NMT is reduced by approximately 20-25% (McKenna and Riches 2007; Lakomy, 1987). Additionally, the exerciser must continually apply propulsive horizontal forces throughout NMT running (Highton et al., 2012) which increases the energetic cost of maintaining the required speed (Kram & Chang, 1999; De Witt et al., 2009). The greater frictional resistance and retarding forces experienced throughout NMT running requires greater activation and strength of lower limb muscles, typically the quadriceps (Franks et al., 2007; McKenna and Riches, 2007). The higher frictional resistances experienced on the NMT, likely increases peripheral muscular fatigue, leading to increased percieved exertion at submaximal speeds (Smoliga et al., 2014) and reduced TTE, which may prevent the cardiovascular system engaging maximally (McKay & Banister, 1976).

The finding of lower peak HR, despite similar $\dot{V}O_{2max}$ responses is interesting and can be related to previous authors who compared a progressive speed protocol with a progressive incline protocol (St Clair Gibson et al., 1999). As with NMT running, progressive incline running requires greater propulsive horizontal forces, reduced step length, and decreased vertical ground reaction forces (Padulo et al., 2012). The previous authors (St Clair Gibson et al., 1999) did not offer an explanation for the reduction in peak HR despite achieving similar peak $\dot{V}O_2$ between the two modes of exercise. ,Our explanation for acheiveing a simiar peak $\dot{V}O_2$ despite reductions in HR on the Cont-NMT is that thekinematic and kinetic differences may reduce the efficiency of muscle fibre recruitment patterns, potentially increasing oxygen uptake by these muscles and effecting blood flow to the periphery thus attaining higher $\dot{V}O_2$ values for the given workload.

Due tothe requirement to apply greater propulsive horizontal forces, NMT running dictates a greater forward lean compared to MT running (Lakomy, 1987; Highton et al., 2012). Consequently, the centre of mass (CoM) moves over the base of support, reducing the vertical oscilations of the CoM (Brughelli et al., 2011). Whilst a reduction in vertical CoM ossicilations have been shown to improve running economy on a MT (Saunders et al., 2004), an increased trunk lean on an NMT results in greater mechanical work performed at the hip, knee and ankle

joints (Heighton et al., 2012). This would likely increase oxygen consumption at sub-maximal speeds, decreasing running economy (Saunders et al., 2004; Davies et al 1984).

Our results demonstrate that there was no statistically significant difference in $\dot{V}O_2$ at VT_1 for cont-NMT compared to Cont-MT. Therefore, we do not conclusively provide evidence for a higher sub-maximal energy expenditure. However, the standardised effect size and 95% CI suggest a likely small increase in $\dot{V}O_2$ at VT_1 for the NMT compared to the MT with confidence intervals crossing from trivial positive to negative moderate suggesting wide confidence intervals and high variability in data. There was also a moderate increase in HR at VT_1 for the Cont-NMT compared to the Cont-MT suggesting higher physiological strain at sub-maximal intensities which agrees with previous authors (Morgan et al., 2015). The variability in our data at sub-maximal intensities could be related to the limitations of assessing ventilatory equivalents for intermittent protocols, such as the use of longer time averaging segments (e.g. 30 s) to account for the rest phases which ultimately reduces the number of data points, thereby effecting the resolution of the data (Robergs, 2001). Therefore, the sub-maximal data should be interpreted with caution. However, this would not be the case for the Cont-NMT, suggesting that the participants may have adapted to the treadmill differently likely due to the requirement to power the belt. Those participants who had stronger lower limb muscles may have better adapted to continuous running on the NMT. Nevertheless, the demonstration of statistically significantly reduced $v\dot{V}O_{2max}$ despite trivial differences in $\dot{V}O_{2max}$ between protocols provides direct evidence of a greater energetic requirement at lower speeds on the NMT compared to the MT.

We have confirmed that whilst a continuous GXT on an NMT elicits a trivial differences in $\dot{V}O_{2max}$ to a traditional continuous GXT on an MT, other important variables such as the peak HR and $v\dot{V}O_{2max}$ are both substantially reduced (See Table 3.2). This brings into question the validity of using a continuous GXT on a NMT to assess cardiorespiratory performance and the transference of this data to dose subsequent intermittent protocols using percentages of $\dot{V}O_{2max}$ or HR.

3.5.2 Peak cardiorespiratory responses for intermittent versus continuous GXTs

Because of the aforementioned locomotor challenges imparted by the NMT, continuous running on the NMT is difficult and decreases time to exhaustion due to higher energetic demands (De Witt et al., 2009). However, the NMT has been shown to reliably reflect intermittent type movement patterns (Aldous et al., 2014). Thus, our secondary aim was to assess the validity of using an intermittent GXT on the NMT. Intermittent GXTs have been shown to result in greater TTE (17.31 vs. 14.12 min) (Metaxas et al., 2005) and in some cases result in higher $\dot{V}O_{2max}$ (64 vs 59 mL·kg^{-1}·min^{-1}; $P < 0.05$) (Girard et al., 2006) compared to continuous GXTs. Therefore, we hypothesised that the use of an intermittent GXT on the NMT may better reflect the training environment, and result in more appropriate reference speeds for the prescription of intermittent exercise (Bangsbo, 1994; Castagna et al., 2005) which can be used in latter experiments within this thesis.

Indeed, there were trivial differences in $\dot{V}O_{2max}$ for Cont-MT vs 15s-NMT, and a smaller not statistically significant reductions in peak HR, and a statistically significant moderate reduction in $v\dot{V}O_{2max}$. Time to exhaustion was also higher for 15s-NMT compared to Cont-MT. Conversely, there was a small statistically significant reduction in $\dot{V}O_{2max}$ for Cont-MT and 30s-NMT, together with a small statistically significant reduction in peak HR. The similar $\dot{V}O_{2max}$, TTE, and $v\dot{V}O_{2max}$ values, together with smaller statistically non- significant reductions in peak HR for the 15s-NMT potentially warrants the use of the 15s-NMT to assess peak cardiorespiratory performance over the Cont-NMT.

Interestingly, there was a trivial difference in $\dot{V}O_{2max}$ between intermittent protocols. However, there were small reductions in peak HR for the 30s-NMT compared to the 15s-NMT which might be explained by the acute physiological changes that occur when using very short vs. short exercise-to-rest durations of less than 60 s (Price & Halabi, 2005; Price & Moss, 2007b). Longer durations of exercise have been shown to result in greater reliance on anaerobic metabolism, resulting in greater metabolic strain and increased percieved exertion despite similar times to

exhaustion (Price & Moss, 2007b). The 30 s condition is still considered short, and previous comparisons have shown similar cardiorespiratory responses when comparing fixed ratio 15 s and 30 s intervals (Cipryan et al., 2016). However, few studies have compared fixed ratio (1:1) short intervals in the same participant group, and whilst our data show similar $\dot{V}O_{2max}$ values, the small reductions in HR might be related to the locomotive difficulties in running on the NMT for prolonged periods as discussed for the Cont-NMT. Therefore, further work is required to assess acute responses to very short vs short interval intermittent exercise on the NMT.

The active recovery periods may have some advantages for the exerciser on the NMT by allowing a greater exercise capacity. Although not measured in the current study, the use of active recovery periods may have increased blood and muscle lactate clearance, due to increased blood flow to the lower limb muscles, during the active recovery phase (Essen et al., 1977). The advantage would be a reduction in the anaerobic glycolytic load with the shorter rest intervals (Belfry et al., 2012b) which may provide the capacity to work at a higher intensity yet with relatively lower levels of blood lactate (Gosselin et al., 2012) and reduction in perceived exertion, (Seiler & Hetlelid, 2005) increasing TTE.

The higher HR obtained during the intermittent GXT protocols compared to the continuous NMT protocol, could be explained by the shorter time spent at the required speed, interspersed with active rest periods. Thereby, the active rest periods may enhance blood buffering capacity of hydrogen ions (H^{+}) and encourage replenishment of phosphocreatine stores (PCr) through enhanced oxidative phosphorylation (Billat et al., 2001). The lower RPE-VT$_1$ values for the intermittent NMT protocols compared to the continous NMT and MT protocols confirm a reduction in peripheral fatigue and reduced percieved exertion for intermittent NMT running at sub-maximal intensities. The lower RPE, HR and $\dot{V}O_2$ at VT$_1$ for the intermittent protocols may reflect a greater efficiency and economy of movement. Ultimately, this allows the exerciser to run for longer, attain higher speeds, encouraging the central circulatory system to engage maximally and optimise cardiorespiratory performance. However, based on the very wide confidence

intervalsfor the submaximal data it appears there is a large inter-individual variation between participants. This is likely due to the variety of training backgrounds of the exercisers, and also the limitations of assessing ventilatory equivalents for intermittent GXTs.

The present results contrast with those studies (Girad et al., 2005; Girad et al., 2006; Alexander and Mier 2011) that have reported a higher $\dot{V}O_{2max}$ following an intermittent GXT compared to a continuous protocol. However, the higher $\dot{V}O_{2max}$ may have be achieved for the intermittent GXT with athletes who are familiar with intermittent movement patterns, such as soccer players (Metaxas et al., 2005). Moreover, whilst intermittent GXTs may overcome some of the biomechanical and physiological challenges imparted by the NMT, there is greater variability between participants as demonstrated by the wide confidence intervals. This supports the importance of chosing incremental protocols that suit the training environment of the exerciser (Magel et al., 1975; Buchheit & Laursen, 2013b). We included participants who performed both forms of exercise (intermittent and continuous). However, the participants were amateure athletes.

The participants used in the current study had a mixture of experience in both intermittent and continuous exercise both on ergometers and over-ground running. The variability in the data can be attributed to the different experience levels of running on an NMT and performing intermittent movement patterns. A more homogenous group of participants; for example soccer players, might have all obtained higher $\dot{V}O_{2max}$ values on the intermittent protocol as demonstrated by previous authors (Mier & Alexander, 2011) which warrants further study.

3.6 Conclusion

To the authors knowledge, this is the first study to develop an intermittent GXT on a NMT to assess aerobic capacity in a laboratory environment. The results of the present study indicate that the use of a continuous GXT performed on a NMT may not be a valid tool to optimise physiological conditions for oxygen utilisation and assessment of aerobic capacity. Whilst a similar $\dot{V}O_{2max}$ can be achieved with a continuous NMT, the moderate reduction in peak HR and

very large reductions in peak speed suggest low efficacy for establishing appropriate reference speeds and peak heart rates for the prescription of appropriate training intensities. When an incremental intermittent test is used on a NMT, closer peak HR and peak speeds are achieved together with a similar $\dot{V}O_{2max}$, and TTE., The very short duration exercise-to-rest intervals (15 x 15 s) utilised by the 15s-NMT protocol elicits more appropriate peak cardiorespiratory responses, with similar peak $v\dot{V}O_{2max}$ compared to the short duration exercise-to-rest intervals (30 x 30 s) elicited by the 30s-NMT. As such, it is recommended that the use of shorter duration exercise-to-rest intervals be used to obtain appropriate peak cardiorespiratory data for use in prescribing exercise dose when performing intermittent exercise on an NMT.

CHAPTER 4:
The effect of intermittent running on the magnitude, rate and

variability of the mechanical loading dose

4.1 Abstract

Aim: Intermittent running is characterised by brief periods of high intensity exercise interspersed with periods of lower intensity exercise. Thus intermittent running might impart a diverse range of mechanical loads of different magnitudes, frequencies and intensities on the musculoskeletal system that could be beneficial to bone above that of continuous exercise. Our aim was to establish the magnitude and variability of the loading environment on controlled intermittent running protocols with a fixed 1:1 ratio but varied exercise-to-rest durations.

Method: Twelve healthy active males (mean (SD) age 23 (4) years, height 179.6 (4.4) cm, body mass 79.7 (7.0) kg) performed five 45 min intermittent running protocols on a Woodway Force 3 non-motorised treadmill (NMT) following a randomised crossover design. Experiment 1: three of the intermittent protocols differed in their exercise-to-rest durations (5 s intervals [5s-Int], 20 s intervals [20s-Int] and 80 s intervals [80s-Int]), and thus different in the frequency of intermittency. Experiment 2 (randomised): three of the protocols differed in the rate of acceleration and deceleration but matched for the exercise-to-rest duration (2 s by 20 s intervals [20s2s-Int], 4 s by 20 s [20s4s-Int] and 6 s by 20 s [20s6s-Int]). The primary outcome measures for experiment 1 & 2 were peak vGRF, impulse and load rate and the intra-step variability assessed via the coefficient of variance (%CV). All protocols were matched for total distance and mean speed and of similar external work. Data are expressed as mean (SD) and were analysed using a one-way repeated measures ANOVA, with mean difference (MD) and 95% confidence intervals.

Results: The results are split into 2 experiments. **Experiment one -** There was no significant difference between conditions for impulse ($P = 0.175$), maximum load rate (LR) ($P = 0.104$) or average load rate (ALR) ($P = 0.345$). However, peak vGRF were statistically significantly different between conditions ($P = 0.023$) with the 5s-Int being greater than the 80s-Int (MD =0.09; 95% CI= 0.01 to 0.17, $P = 0.022$), and an 8% greater variability (%CV) in vGRF. **Experiment two -** The mean data for impulse, vGRF and load rate were similar between the intermittent conditions as was the % CV.

Conclusion: shorter more frequent exercise-to-rest durations during running does not impact on the mean loading magnitude and rate. However, there is an increase in the step-step variability of the loading characteristics seen in the % CV when the exercise-to-rest interval is manipulated. This is likely due to the changes in high and low speed. Therefore greater changes in the minimum and maximum speeds during running increase the variability in the loading environment which might have advantages for the osteogenic potential of the exercise.

4.2 Introduction

Running is a common mode of exercise used to stimulate bone remodelling in animal (Huang et al., 2008) and human (Scott et al., 2013) models. However, the continuous repetitive nature of the loading cycles may saturate the bone cells, 'desensitising' the osteocyte network (Umemura et al., 1997; Burr et al., 2002) to the applied mechanical stimulus evoking deleterious effects on bone such as increased risk of stress fractures (Warden et al., 2006).

As an alternative to continuous exercise, intermittent exercise characterised by brief high-intensity loading interspersed with periods of low-intensity loading (Bangsbo, 1994) may offer a greater osteogenic stimulus (Helge et al., 2010; Krustrup et al., 2010b). The enhanced osteogenic potential of intermittent exercise could be explained by the diverse range of strain rates and strain magnitudes coupled with the inserted rest phases (Turner & Robling, 2003). However, the different mechanical loading environment induced by intermittent running has not been extensively quantified. Investigating how intermittent running, matched for mean speed and duration, with a similar volume of external work and a fixed 1:1 ratio, might change the mechanical loading environment, will inform and contribute to the intermittent exercise programming puzzle (Buchheit & Laursen, 2013b)

To understand how the mechanical loading environment of different intermittent running modalities might offer a different osteogenic stimuli, the mechanical load induced by the exercise must be quantified (Tobias et al., 2014). The use of vertical (vGRF) and horizontal (hGRF) ground reaction forces have been proposed as surrogate measures to assess the compressive stress and strain imposed on the skeleton during exercise (Cavagne & Lafortune, 1979). The peak vGRF and area under the curve (vertical impulse) can be used to assess the magnitude of the strain (Ebben et al., 2010). Furthermore, strain rate, has been proposed to be more important component of mechanical loading for bone than the strain magnitude (Burr et al., 2002). The maximum and average load rate (ALR) of the GRF curve have been used to estimate the rate of strain of the mechanical load during various activities including running (Heikkinen et al., 2007; Ebben et al., 2010) and intermittent movement patterns (Tolly et al., 2014).

Animal models have demonstrated that the diversity of the mechanical loading environment is important for bone remodelling (Moreno et al., 2008). Therefore, the variability of mechanical loading cycles during non-steady state locomotion is also important to quantify. Intra-individual variability occurs as a result of asymmetry and stride variability (Belli et al., 1995) during running which could be exacerbated by variations in the frequency of the exercise-to-rest ratio and the rate of the acceleration and deceleration required to reach the target speed (Buchheit & Laursen, 2013a; 2013b). To date the variability of kinetic and kinematic parameters of running gait during intermittent exercise of varying exercise-to-rest durations has not been investigated.

To investigate the effect of intra-step variability of the loading characteristics, multiple consecutive steps must be assessed (Belli et al., 1995) during the exercise period. In a laboratory environment this can be achieved using a fully-instrumented treadmill. Typically these ergometers are very expensive making them less accessible for use. Moreover, motorised treadmills (MTs) have been shown to modify the gait cycle during running as opposed to over ground running (Nigg et al., 1995; Baur et al., 2007) potentially decreasing the variability in the gait cycle as the individual is restricted to the confines of the paced motorised system. The use of a fully instrumented non-motorised treadmill (NMT) offers a unique and novel opportunity to assess multiple loading cycles during intermittent exercise. However, to date the mechanical loading environment during non-steady-state running on an NMT has not been fully explored.

Therefore, the aim of this study was to assess the magnitude, rate and variability of the mechanical loading dose, assessed via components of the vGRF when the frequency and duration of the exercise-to-rest interval (fixed 1:1), and rate of acceleration and deceleration are The mean speed, and duration of the exercise sessions should be controlled to ensure similar distance and overall external work was performed. It was hypothesised that the high loading cycles would be cancelled out by the lower loading cycles therefore there would be little effect on the mean kinetic and kinematic mechanical loading dose.

4.3 Methods

Participants

A total of 22 healthy active males were recruited to the study, with ten of these participants not included in the final analysis due to the following:

- $n = 7$ were unable to complete the full 45 min of at least three exercise conditions.

- $n = 1$ was a smoker and so did not meet the inclusion criteria.

- $n = 1$ dropped out of the study in week one due to an unrelated injury obtained outside of the confines of the study.

- $n = 1$ dropped not included in study as deemed to unfit based on GXT performance as did not complete enough exercise per week as specified in inclusion criteria.

Participants were included in the study if they were between the ages of 18-30 years, free of any cardiovascular, respiratory and metabolic diseases and were classed as low risk (< 1 risk factor) according to ACSM coronary artery disease (CAD) risk factors (ACSM, 2009). Participants were excluded if they were a smoker, had a musculoskeletal injury, or did not participate in at least 3-4 sessions of both continuous and intermittent impact modes of exercise per week as per study one. From the remaining twelve participants, one participant was unable to finish the entire 45 min exercise bout for the final two conditions required for experiment two. Therefore, the total participant n for experiment one was 12 and for experiment two, was 11.

Design

Participants attended the exercise physiology laboratory over a seven week period. In week one, participants' completed three preliminary testing sessions, consisting of medical screening, habituation to the NMT, and assessment of maximum oxygen consumption ($\dot{V}O_{2max}$) and to establish the velocity at $\dot{V}O_{2max}$). Participants completed five exercise trials over a six week period. The design followed a randomised within-subject crossover design (Figure 4.1).
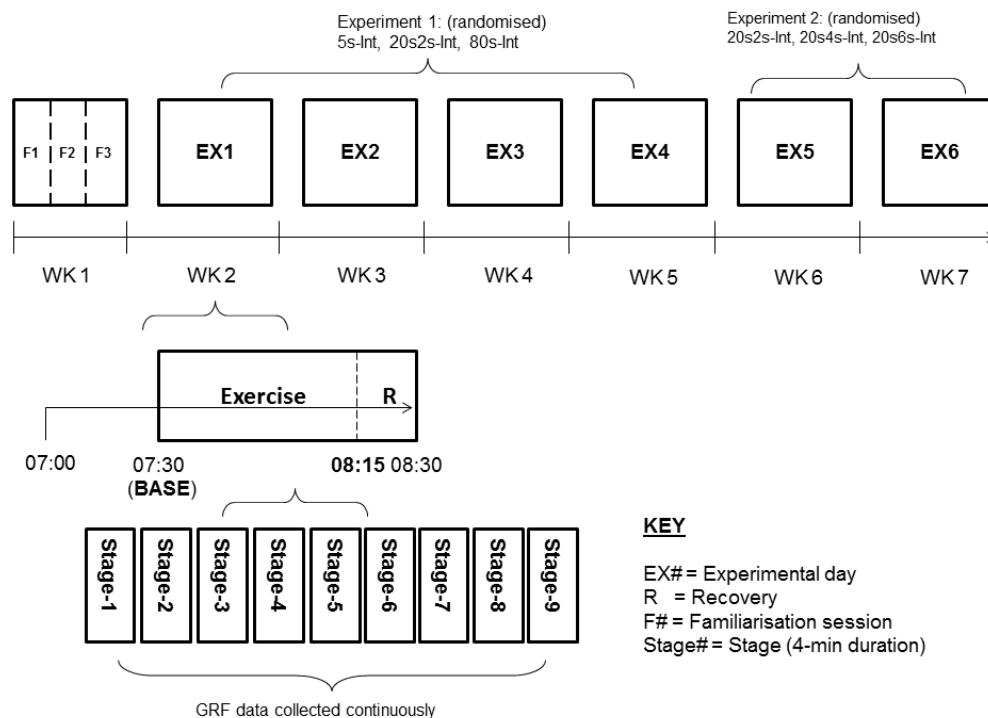
Figure 4.1 study design using randomised within subject crossover design.

For experiment one, the order of the very intermittent (5s-Int), moderately intermittent (20s2s-Int), and less intermittent (80s-int) protocols were fully randomised to avoid learning effects using a computer randomiser software. For experiment two, the 20s2s-Int protocol was used as the highest acceleration/deceleration rate condition, and so was unavoidably always performed before the proceeding moderate acceleration/deceleration rate condition (20s4s-Int) and lowest acceleration/deceleration rate condition (20s6s-Int). The orders of the 20s4s-Int and 20s6s-Int conditions were randomised to overcome some bias of learning/accommodation effects, therefore experiment 2 was still randomised but some bias may exist.

Pre-trial familiarisation and exercise tests

The NMT familiarisation protocol used has been previously outlined in Chapter 3 (Section 3.3). On the second visit to the exercise physiology laboratory, participants performed a 5 min warm

up on the NMT, followed by an intermittent GXT on the NMT, to obtain an estimation of the participants' maximal oxygen consumption ($\dot{V}O_{2max}$), peak heart rate, and primarily to identify the velocity at which $v\dot{V}O_{2max}$ was attained ($v\dot{V}O_{2max}$), defined as the highest velocity achieved at maximal oxygen consumption (Billat & Koralsztein, 1996). The procedure for the intermittent GXT has been fully outlined and discussed in Chapter 3. The findings from Chapter 3 demonstrated that the 15 s non-motorised treadmill graded exercise test (15s-NMT) was the most suitable GXT to assess cardiorespiratory performance and attain submaximal reference speeds. The results of the 15s-NMT test were used to estimate the treadmill velocity corresponding to 55%, 75% and 95% $v\dot{V}O_{2max}$.

Exercise trial procedures

Participants attended the exercise physiology laboratory once per week and adhered to the following pre-testing guidelines:

1. Refrain from <u>any</u> exercise 48 h prior. Light walking was appropriate, but where possible, physical activity was to be avoided.

2. Attend the physiology laboratory following a 12 h fast.

3. Maintain normal hydration through drinking to thirst.

4. No alcohol or other psychoactive substances should be consumed 24 h prior to testing.

All participants attended the exercise physiology lab between the hours of 06:30 and 08:00, and at the same time of day (± 15 min) to control for diurnal variation. Participants declared any changes (if any) to their pre-medical form before commencing. Participants performed a 10 min seated resting heart rate measure prior to commencing the exercise. A five minute warm up on the NMT at 55% $v\dot{V}O_{2max}$ preceded the exercise session. The warm up consisted of running intermittently alternating every 15 s between 55% $v\dot{V}O_{2max}$ and a walking speed of 4 km·h⁻¹. This movement pattern was used to re-familiarise the participants quickly to intermittent running on the NMT.

Depending on randomisation, for experiment one, participants' either completed the very intermittent protocol (5s-Int), the moderately intermittent (20s2s-Int) or the lowest intermittent protocol (80s-Int). The schematic representation of these protocols is displayed in Figure 4.2. For the 5s-Int, the exercise to rest intervals varied every five seconds. Participants were required to run between 95% and 55% $v\dot{V}O_{2max}$ (mean of 75% $v\dot{V}O_{2max}$), interspersed with walking at 4 km·h^{-1} every five seconds. Participants were required to reach the target speed in two seconds. As outlined in the Method section of Chapter 3, participants obtained visual and auditory feedback of the target speed and actual speed. There were a total of 48 changes of speed in each four minute exercise bout, with nine, four-minute bouts interspersed with one minute of passive recovery between each bout. The total exercise bout (not including the warm-up) was 45 min.

The 20s2s-Int protocol was the same as the 5s-Int, however, participants varied every 20 s between the speeds. The protocol had exactly 1/4 fewer changes in speed compared to the 5s-Int protocol, thus was considered less intermittent. The 80s-Int protocol was the most continuous protocol. However, due to the difficulty of running continuously on the NMT for prolonged periods of time (De Witt et al., 2009) a fully continuous protocol could not be used as participants would have fatigued too quickly. Participants ran for 80 s at 75% $v\dot{V}O_{2max}$ interspersed with 80 s of recovery walking. The protocol had 1/4 fewer changes in speed compared to the 20s2s-Int protocol and 1/16 less changes in speed than the 5s-Int.

The protocols were designed to be controlled for mean speed and duration, and consequently distance ($D = S * T$). Where: D = distance, S = Speed, T = time. The protocols were also matched for total rest duration. All protocols followed a 1:1 exercise-to-rest ratio to allow control for mean speed, duration and time spent at rest and exercise intervals. Therefore, we could manipulate the effect of frequency of intermittency, and investigate short vs. long exercise-to-rest durations. The reported mean speed includes the walking, and also displays the mean without the walking element.
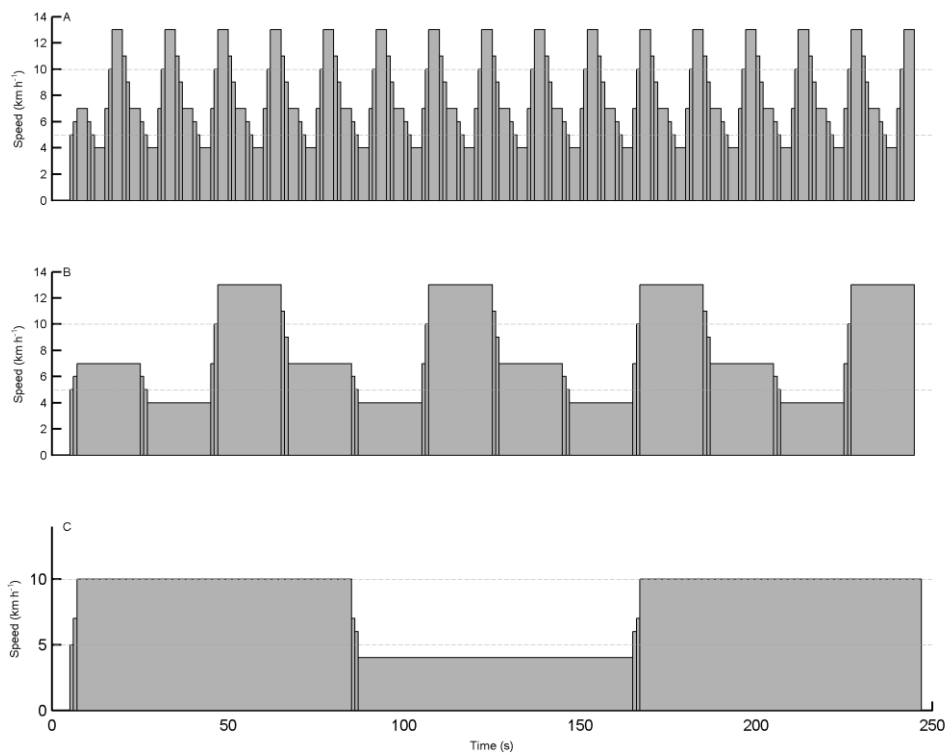
Figure 4.2 The three running protocols for experiment 1. The top panel (A) is the 5s-Int protocol, changing speed every 5 s, the middle panel (B) is the 20s2s-Int protocol, changing speed every 20 s, and the bottom panel (C) is the 80s-Int protocol changing speed every 80 s. The darker bars represent the acceleration and deceleration period.

For experiment two, the exercise-to-rest intervals were controlled. However, the time to reach the target speed was manipulated, thus acceleration and deceleration rates were varied. For 20s2s-Int, participants were required to reach the target speed in 2 s, for 20s4s-Int participants were required to reach the target speed in 4 s and for 20s6s-Int participants were required to reach the target speed in 6 s. The protocols are displayed in Figure 4.3.
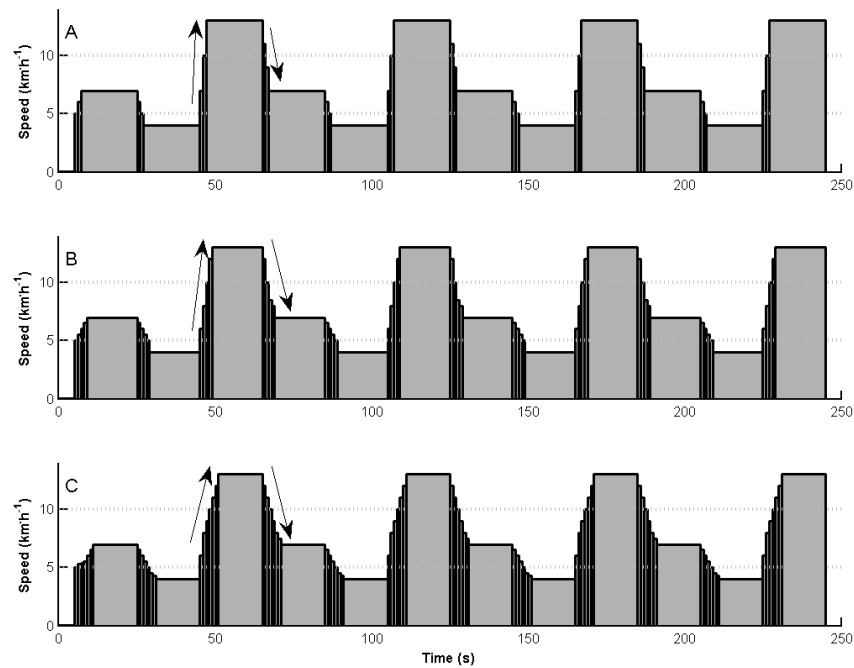
Figure 4.3 The three running protocols for experiment 2. The top panel (A) is the 20s2s-Int protocol, achieving the target speed in 2 s, the middle panel (B) is the 20s4s-Int protocol, varying every 20 s, achieving the target speed in 4 s, and the bottom panel (C) is 20s6s-Int protocol, varying every 20 s, achieving the target speed in 6 s. The arrows depict the acceleration and deceleration phases. The darker bars represent the acceleration and deceleration period.

Running mechanics and NMT set-up

Calibration of the NMT vertical and horizontal load cells for measuring GRF were performed every three months as specified in the manufacturers' guidelines. The horizontal force was calibrated by hanging a known mass (5 kg) from the transducer. The known mass is multiplied by 9.81 and this value was entered into the textbox labelled "h force 1" within the Pacer Performance software. A second, heavier mass is then applied to the transducer and the resulting value entered into the textbox labelled "h force 2" within the Pacer Performance software. When both forces have been calibrated, the horizontal force calibration factor is displayed. The same procedure is followed for the vertical force calibration factor. For "v force 1" the body weight (in

Newton's) of one individual was used as the first calibration weight, and for "v force 2" the body weight of three individuals were used as outlined in the manufacturer's guidelines.

For all trials, the vGRF data were collected via four individual vertical force transducers underneath the treadmill belt (see Figure 4.4), and the anterior hGRF data was collected via a horizontal strain gauge attached to the vertical strut, at the rear of the NMT (Modell BS-500 Class III, Transcell Technology Inc, Buffalo Grave, USA). The GRF data were set to a sampling rate of 200 Hz, with a filter cut off, of zero, utilising the integrated Pacer Performance software (Fitness Technology, Joondalup, WA, Australia). The raw data were exported from the Pacer Performance software into a custom designed MATLAB (MathWorks, Inc., 83 Natick. MA, USA) programme for analysis of kinematic and kinetic variables. A sampling rate of 200 Hz is the optimal capacity of the force transducers. Previous researchers, for similar variables of interest, have also adopted this sampling rate (Brughelli et al., 2011). Prior to each exercise session the vertical and horizontal force transducers were zeroed, as outlined in the user guide, and the new zero offset for the transducers was set.
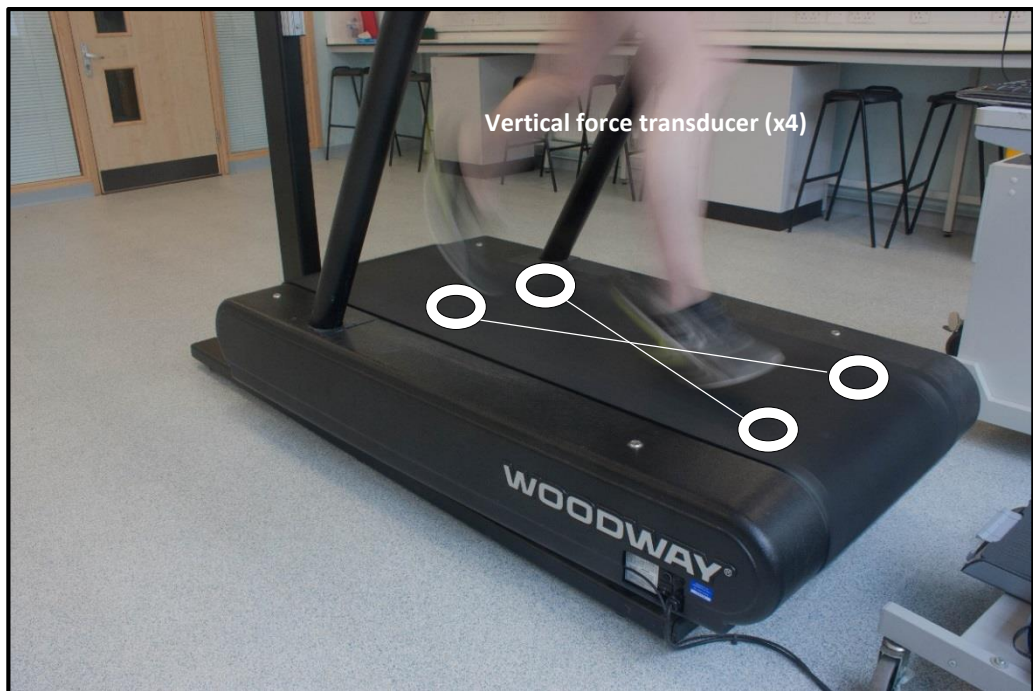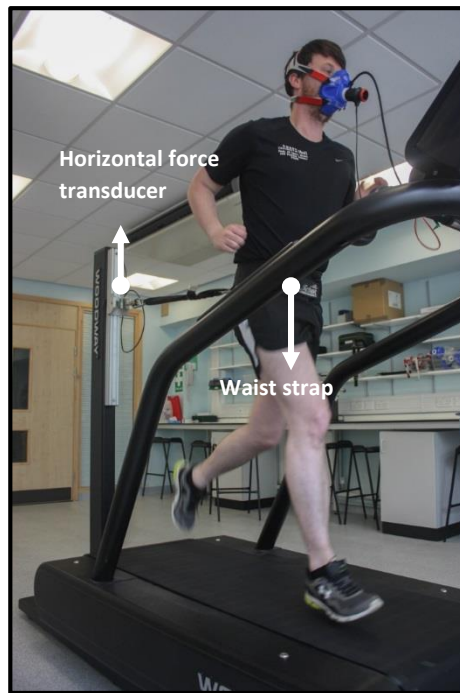
Figure 4.4 The equipment used for data collection including Woodway non-motorised treadmill, and vertical and horizontal strain gauges. The NMT characteristics such as linearity of the strain gauges can be found in Appendix A

Vertical and anterior horizontal GRF data were digitally filtered using a bi-directional, low pass, Butterworth filter (Cronin & Rumpf, 2014). The frequency cut off was optimised using residual

analysis (Winter, 2009) (Figure 4.5). Residual analysis calculates the difference between the filtered and unfiltered signals over a range of frequencies from 0 Hz to the Nyquist frequency (0.5* sampling frequency) (Winter, 2009). The residual at any cut off frequency is calculated as follows:

$$R(f_c) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(X_i - \hat{X}_i)^2}$$

Where X_i = raw data at ith sample and $\hat{X}_i$ = filtered data at the ith sample.
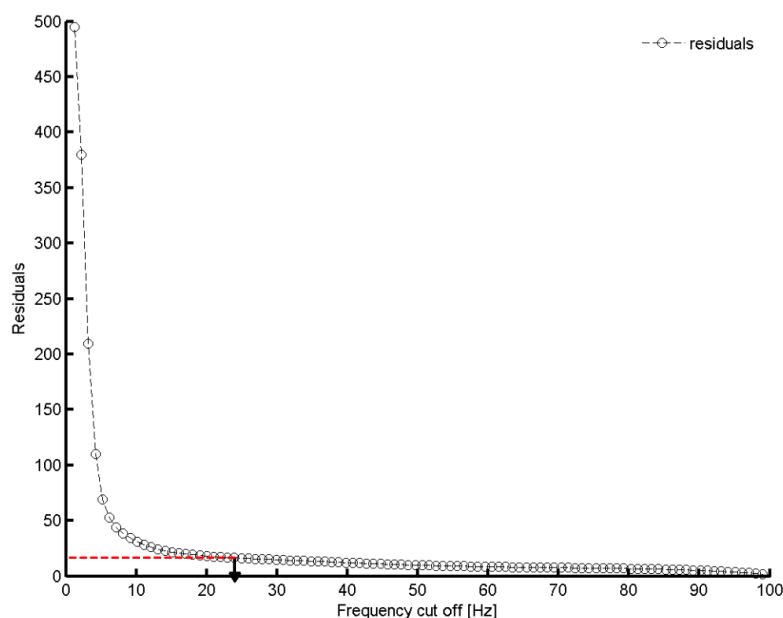
Figure 4.5 Plot of the residuals from vGRF data collected during the 80s-Int running protocol. The frequency cut off was determined as f_c=24 and is displayed by the black arrow.

The frequency cut off (f_c) was determined using an automated algorithm developed in MATLAB. Once the residuals were calculated, the period of the curve containing 95% of the signal, that is most linear, is considered noise. Residual analysis was computed for all trials, across all protocols and for all participants. The mean and standard deviation of the f_c used between conditions were:

5s-Int = 27 (3), 20s2s-Int = 27 (3), 80s-Int = 25 (5), 20s4s-Int = 28 (4) and 20s6s-Int = 28 (4).

Step detection procedure

To determine the start and end of each step, we applied a step detection threshold using the vertical GRF in which data above 15% of the individual's body weight (Newtons) was considered the period of ground/initial contact (Cronin & Rumpf, 2014). An example of the step detection threshold can be seen in Figure 4.6. The data between the start and end of the step was then segmented into individual steps to make processing easier. The step detection threshold was used to calculate the aerial time (AT), contact time (CT), step frequency (SF), step length (SL), vertical impulse, average load rate (ALR), maximum impact load rate (LR), rate of peak force development (RPFD), signal energy, vertical (kVert) and leg stiffness (kLeg).
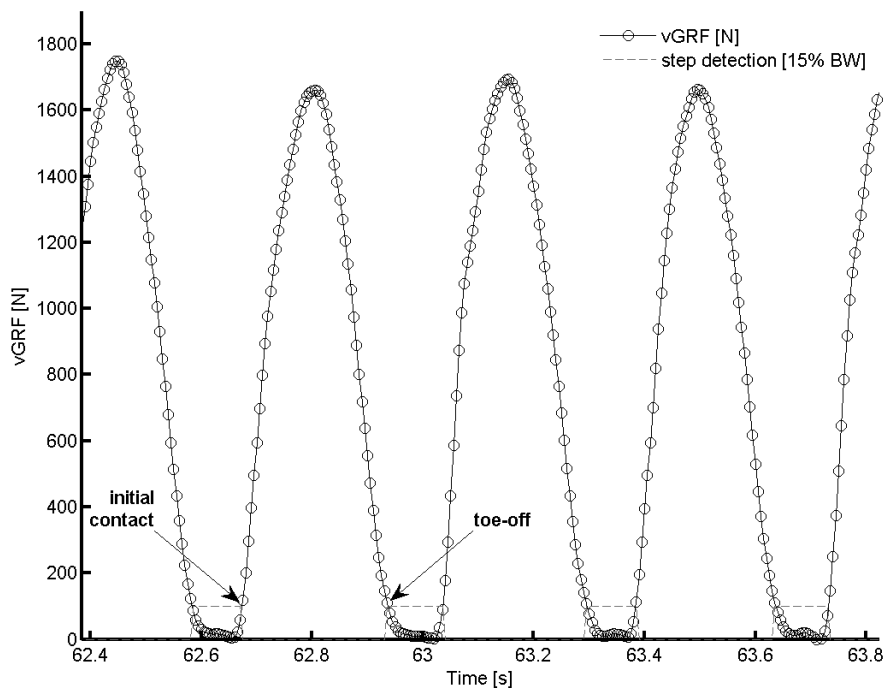
Figure 4.6 The force-time curves over a segment of time during the 80s-Int condition. The dotted line represents 15% BW threshold which was used to distinguish the start (toe-on) and end (toe-off) of a step.

The step detection threshold failed when the force data of the strain gauges did not return to zero. This situation occurred when a participant did not have a flight phase in between steps, which is a characteristic of walking gait. During the lower running speeds some participants adopted a

running style similar to 'plodding'. This meant that they failed at times to remove the trailing foot fully off the belt. Whilst the peak forces were high during these periods, there was no discernible flight phase. In order to include these steps the step detection threshold was modified using a higher detection threshold of 500 N. The running data were over-smoothed using a low frequency cut-off (i.e. 10 Hz). This gave a definitive lowest point to determine the start and end of each step. The positions of the nadirs were overlaid on the normally filtered data. This step detection threshold was used to find all of the peaks during the running stages to calculate the number of steps achieved that were not performed during the walking (rest) phases.

Every step in the four-minute stage was detected using the step detection threshold algorithm (Figure 4.5) and included in the analysis. The mean ($\bar{x}$), standard deviation (σ) and coefficient of variation (% CV) was calculated for all the pooled steps from the nine bouts of 4-min exercise data, for all the variables. The 'peak' vGRF was the maximum peak vGRF detected out of all the steps detected. The number of steps with a peak above the following four arbitrary thresholds was also calculated to estimate which (if any) condition produced more steps of a higher peak. The thresholds were; 2.0, 2.2, 2.4 and > 2.6 BW. We also calculated the percentage of steps (for each participant, across each condition) which demonstrated a vertical impact transient.

Mechanical step variability using the percentage coefficient of variance (%CV) as an outcome variable has been previously reported (Belli et al., 1995). The %CV was used to assess the intra-step variability of all steps in each condition. The % CV was calculated for vertical impulse, the vGRF, load rate, Leg and vertical stiffness, step length and aerial time.

The % CV was calculated as follows;

$$\sigma = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_i - \mu)^2}$$

$$\% \, CV = \frac{\sigma}{\mu}$$

The following variables were derived from the force transducers, and photomicrosensor (measuring the speed and revolutions of the treadmill belt):

Kinematic variables

Contact time (CT) (s): Time between the start of step detection threshold to the end of the step detection threshold.

Aerial time (AT) (s): Time outside the step detection threshold.

Step rate (#steps/s): Number of steps per second, was calculated as 1/(CT + AT) (Brughelli et al., 2011).

Stride length (m): velocity divided by the stride frequency.

Vertical displacement of the CoM (m): The vertical displacement of the CoM was determined by double integration of the vertical acceleration (Cavagna et al., 1976). The vertical acceleration was obtained via vGRF (Newtons) minus the body mass after accounting for gravitational acceleration (9.81 m·s^{-2}). The trapezoidal rule was used for the integration of velocity and displacement. Performing integration on the acceleration data results in a continuous linear (drift) trend, with the drift being greater when more data were used. To overcome the linear drift in the integration of acceleration to velocity, and velocity to displacement, we calculated the vertical CoM displacement on individual steps. This resulted in a reduction of data points for which the integration was performed, thus resulting in less noise being compounded during the integration. As discussed previously, once the start and end of the steps were determined the data points between the step detection thresholds were segmented into individual cell arrays. This made

processing more efficient and reduced the amount of data to process on each pass of the loop. An example of the CoM displacement in relation to the vGRF can be seen in Figure 4.7.
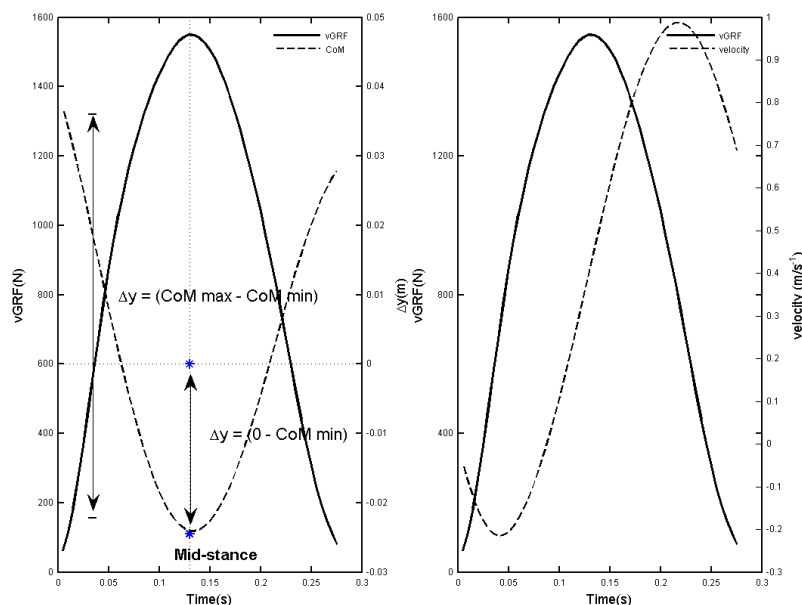
Figure 4.7 The change or delta vertical centre of mass displacement (Δ CoM) calculated over a specified segment. The blue star markers represent the position of the peaks and nadirs of the CoM. The difference between the peak and the corresponding nadir is the Δ CoM.

Kinetic variables

Vertical impulse, signal energy and load rate were calculated on individual GRF curves normalised to body weight (mass x 9.81) (Figure 4.8).

Vertical impulse (BWs): The Impulse of each step was calculated from the acceleration curve as follows;

$$I = \int_{t_1}^{t_3} a(t)dt \qquad\qquad (\mathbf{1})$$

Signal Energy (BWs): The signal energy was calculated from the acceleration curve as follows;

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$$E = \int_{t_1}^{t_3} |a(t)|^2 dt \qquad\qquad (2)$$
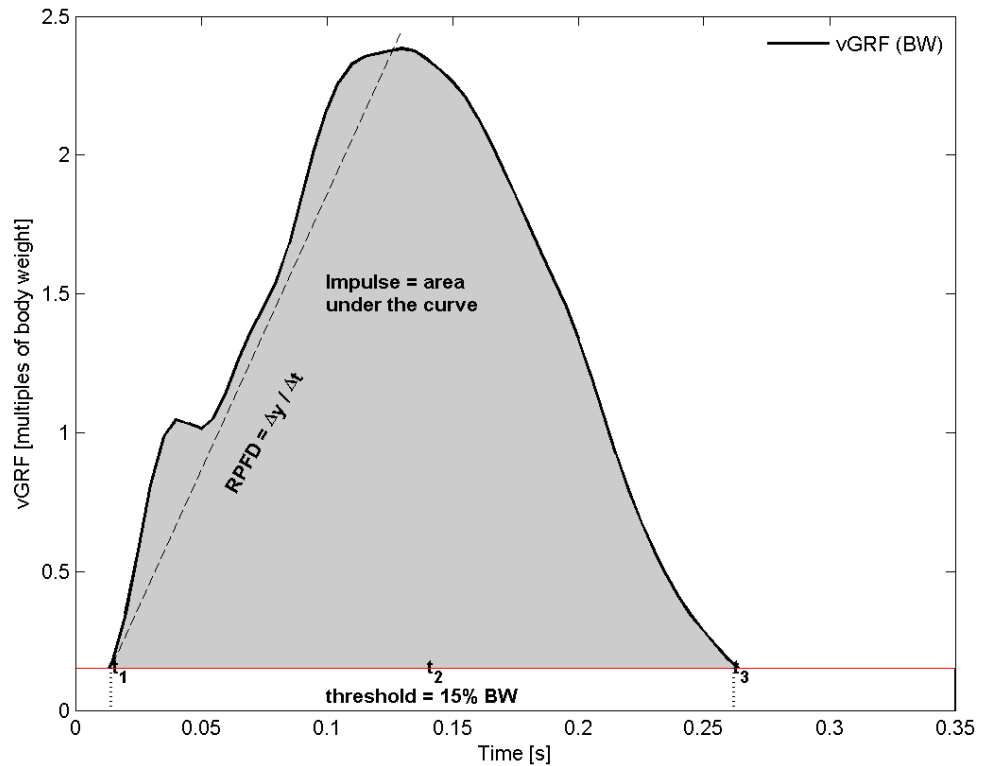
Figure 4.8 The normalised GRF curve and calculation for impulse, signal energy and rate of peak force development (RPFD). Where; t_1 = time @ initial contact, t_2 = time at peak vGRF, t_3 = time @ toe off. The RPFD = (force at t_2- force at t_1) /(t_2-t_1).

A graphical representation for calculating the maximum loading rate (LR) and average load rate (ALR) of the GRF is presented in Figures 4.9 and 4.10.

The average load rate (ALR (BW·s^{-1})) was calculated as follows:

$$slope = \frac{y_2 - y_1}{x_2 - x_1} \qquad\qquad (3)$$

Where; $y_2 = 80\%$ of the VIT or active peak of the GRF curve, $y_1 = 20\%$ of the VIT of active peak, $x_2 = $ time at 80%, $x_1 = $ time at 20%

The maximum loading rate was calculated by finding the derivative of the GRF curve and then finding the maximum derivative between the "time" at 20% and 80% of either the vertical impact peak or the active peak (Kluitenberg et al., 2012).
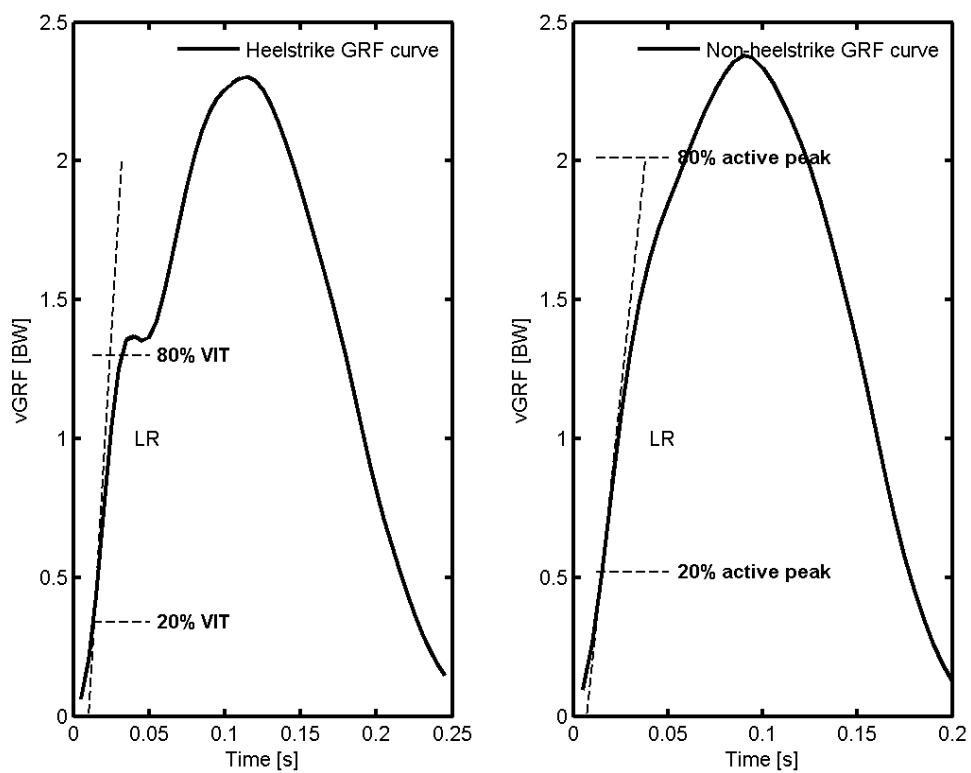
Figure 4.9 The GRF from a heelstrike and non-heelstrike situation. Maximum load rate and average load rate were calculated between 80% and 20% of either the vertical impact peak (VIT) or the active peak.
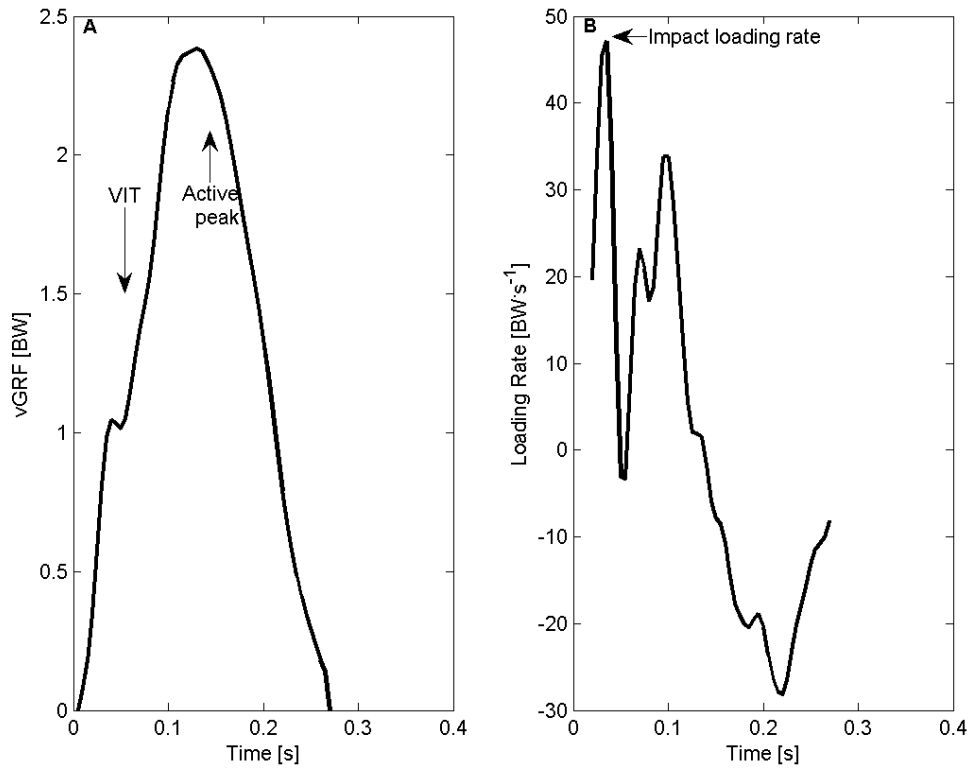
Figure 4.10 The vGRF during heel strike (A) with the impact loading rate calculated from the derivative of the GRF (B).

Vertical stiffness (kVert [kNm]) was calculated by dividing the maximum ground reaction force during the step (usually at mid-stance) by the maximum vertical displacement of the CoM (see Figure 4.6).

$$kVert = \frac{F_{max}}{\Delta_y} \tag{4}$$

Where; F_{max} = peak vGRF (N), Δ_y = max CoM – min CoM

Leg stiffness (kLeg (kNm)) was calculated by dividing the maximum ground reaction force during the step (mid-stance) by the delta change in leg length.

$$kLeg = \frac{Fmax}{\Delta L} \tag{5}$$

Where;

$$\Delta L = L - \sqrt{L^2 - \left(\frac{vTc}{2}\right)^2} + \Delta y \qquad\qquad (\mathbf{6})$$

Where; $\Delta y = 0 - \min$ CoM, L = initial leg length, The initial leg length was calculated as 0.53*height (Winter, 1979; Morin et al., 2005), v = velocity, Tc = contact time (Morin et al., 2005)

The peak anterior component of the hGRF data were used to calculate the mean hGRF (all peak hGRF values were collected and a mean of the values was obtained). A representation of the hGRF trace is shown below (Figure 4.11) to illustrate how the peaks of the anterior hGRF were detected. The hGRF force has been used by previous authors to calculate external work and mean hGRF (Belli et al., 2001; Cronin & Rumpf, 2014).
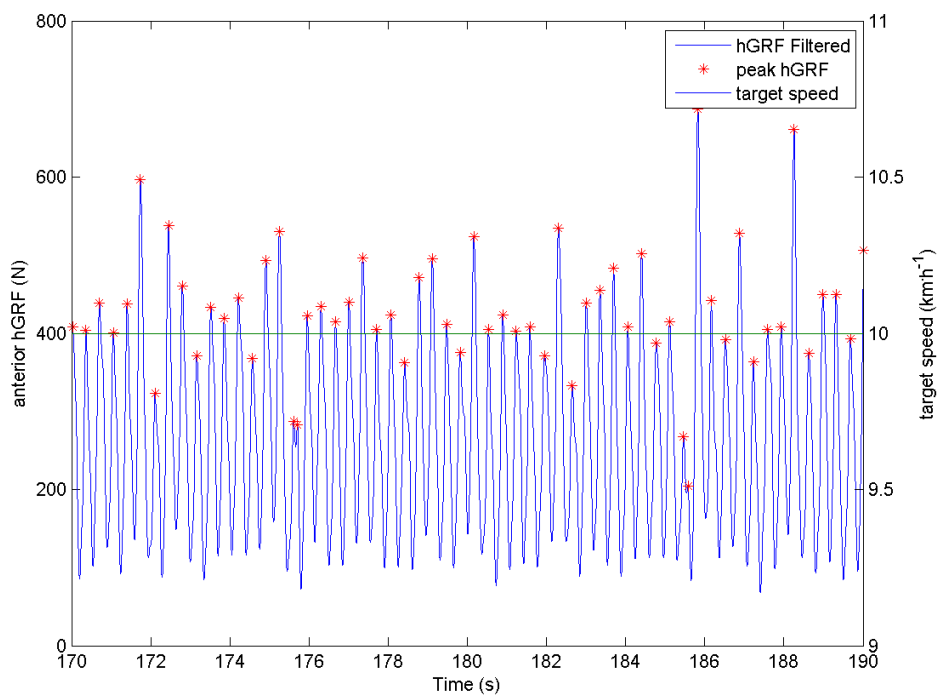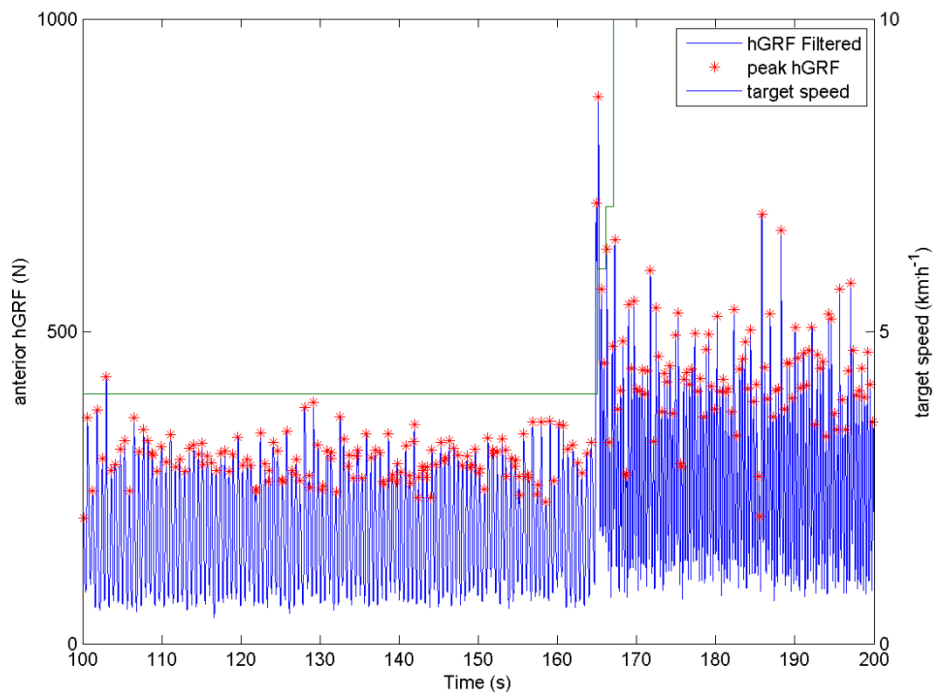
Figure 4.11 (top and bottom panel) displays an example of the horizontal anterior ground reaction force trace. The top panel displaces the hGRF trace as the target speed changes. The bottom panel displays the hGRF trace running at 10 km·hr^{-1}.

3.3.1 Statistical analysis

Descriptive data are presented as mean and standard deviation. The precision of the estimate of the outcome statistic is shown using 95% confidence intervals for the mean difference. All running mechanics data, including the % CV, steps above arbitrary thresholds and the percentage of impact peaks were analysed using a one-way (condition) repeated measures ANOVA and further delineated using pairwise comparisons. Sidak adjustments were performed to adjust for multiple comparisons. Data were checked for normal distribution using histograms, Q-Q plots and the Shapiro Wilk test. If sphericity was not assumed the P value for the Greenhouse Geiser test was chosen. Pearson's correlations (r) and 95% confidence intervals were used to represent the association between speed and running gait variables. As the Pearsons r value is not normally distributed and positively skewed (as it is either -1 to 1) the 95% confidence intervals were constructed using Fischers z transformation. All participant data across all trials were combined and displayed as a cross-sectional analysis. Due to unequal sample sizes, the Mann Whitney U test was used to compare participant characteristics between those included and those who failed to complete all exercise conditions (drop-outs). All analysis was performed using SPSS Version 22 (IBM, New York, USA).

4.4 Results

The results section is split into two sections; experiment **one** and experiment **two**. The mean, SD, mean difference, 95% confidence interval for the mean difference, and P value are presented.

Participant characteristics

Table 4.1 Mean (SD) participant characteristics (n=12).

Participant characteristics	Mean	SD
Age (years)	23	4
Height (cm)	179.6	4.4
Body mass (kg)	79.7	7.0
Body fat (%)	13.9	4.3
$\sum$ 7 skin folds (AU)	78.9	29.4
systolic BP (mmHg)	134	9
diastolic BP (mmHg)	73	6
Resting HR (beats·min^{-1})	58	13
predicted HR (beats·min^{-1})	191	3
Peak HR (beats·min^{-1})	186	9
$v\dot{V}O_{2max}$ (km·h^{-1})	15	1
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	53	7

There were no statistical differences for the main participant characteristics between those included ($n = 12$) and drop-outs ($n = 7$) for age ($P = 0.432$), height ($P = 0.592$), mass ($P = 0.697$) $\dot{V}O_2$ ($P = 0.902$), body fat % ($P = 0.384$).

Experiment One: The effect of varying the exercise-to- rest interval on the magnitude, rate and variability of the mechanical loading dose.

4.4.1 External load

Work, distance and mean velocity

Data for external work (kJ) and distance (m) were normally distributed. There were no significant differences in the means between conditions for both work [$F_{(2, 22)} = 3.099$, $P = 0.065$], or distance [$F_{(2, 22)} = 2.107$, $P = 0.145$] (Table 4.2).

Table 4.2 Mean, standard deviation, and raw mean difference [95% CI] for measures of external load (n = 12).

	5s-Int (1)		20s2s-Int (2)		80s-Int (3)		1 vs. 2	1 vs. 3	2 vs. 3
	Mean (SD)		Mean (SD)		Mean (SD)		MD [95% CI]	MD [95% CI]	MD [95% CI]
Distance (m)	4158.0	(376.6)	4200.3	(365.7)	4177.5	(388.1)	-42.3 [-108.6, 24.0]	-19.5 [-66.4, 27.3]	22.8 [-36.5, 82.1]
Work (kJ)	1041	(171)	1034	(143)	978	(133)	7 [-75, 89]	63 [-29, 154]	56 [1, 111]
Speed (m·s^{-1})	2.1	(0.2)	2.1	(0.2)	2.1	(0.2)	-0.001 [-0.25, 0.24]	0.013 [-0.007, 0.033]	-0.13 [-0.048 to 0.021]

Notes: m = meters, kJ = kilojoules, m·s^{-1} = meters per second, MD = raw mean difference, CI = 95% confidence interval

4.4.2 Running mechanics (kinematic and kinetic)

Kinetic data

The mean, standard deviation, mean difference (MD) and 95% confidence interval for running mechanics data are presented in Table 4.3 and 4.4. There was no significant mean differences between conditions for vertical impulse [$F_{(2, 22)} = 1.892$, $P = 0.175$], signal energy [$F_{(2, 22)} = 2.179$, $P = 0.137$], rate of peak force development [$F_{(2, 22)} = 0.096$, $P = 0.909$], maximum load rate (LR) [$F_{(2, 22)} = 2.513$, $P = 0.104$], average load rate (ALR) [$F_{(2, 22)} = 1.116$, $P = 0.345$], vertical stiffness [$F_{(2, 22)} = 2.929$, $P = 0.074$], leg stiffness [$F_{(2, 22)} = 0.522$, $P = 0.601$] or Δ CoM [$F_{(2, 22)} = 3.104$, $P = 0.064$].

Normalised (BW) mean vGRF and peak vGRF were significantly different between conditions ($F_{(2, 22)} = 7.204$, $P = 0.004$, and $F_{(2, 22)} = 4.488$, $P = 0.023$, respectively). For the pooled mean vGRF the 80s-Int condition was higher than the 5s-Int and 20s2s-Int. The peak vGRF intermittent protocols (5s-Int and 20s2s-Int) were higher than the 80s-Int. Mean anterior hGRF was also significantly different between means [$F_{(2, 22)} = 5.938$, $P = 0.009$], as was the peak anterior hGRF [$F_{(2, 22)} = 88.765$, $P = 0.0001$].

There was a significant effect of condition on %CV data (Table 4.5) for impulse [$F_{(2,22)} = 33.525$, $P = 0.0001$], load rate [$F_{(2,22)} = 30.153$, $P = 0.0001$], vGRF [$F_{(2, 22)} = 35.010$, $P = 0.0001$], kVert [$F_{(2,22)} = 61.032$, $P = 0.0001$] and kLeg [$F_{(2,22)} = 41.364$, $P = 0.0001$]. The variability was highest for the very intermittent 5s-Int condition compared to the 80s-Int condition.

Kinematic data

There were no significant mean differences for step length [$F_{(2, 22)} = 1.510$, $P = 0.243$], step frequency [$F_{(2, 22)} = 0.397$, $P = 0.677$], contact time [$F_{(2, 22)} = 1.578$, $P = 0.229$], or aerial time [F

$_{(2, 22)}$ = 1.523, P = 0.243]. There was a significant difference between means for total number of steps [$F_{(2, 22)}$ = 4.695, P = 0.020]. Post hoc analysis did not reveal significant differences between conditions. There was a significant main effect for MPO [$F_{(2, 22)}$ = 3.950, P = 0.034].

There was a significant main effect of condition on the %CV for step length [$F_{(2,22)}$ = 88.384, P = 0.0001] and aerial time [$F_{(2,22)}$ = 16.615, P = 0.0001].

Table 4.3 A Mean, standard deviation, mean difference [95% confidence interval] and P value for running mechanics variables ($n = 12$).

	5s-Int (1)		20s-Int		80s-Int		1 vs. 2		1 vs. 3		2 vs. 3	
	Mean (SD)		Mean (SD)		Mean (SD)		MD [95% CI]	P-value	MD [95% CI]	P-value	MD [95% CI]	P-value
kinetic												
I (BW·s)	0.35	0.02	0.35	0.02	0.35	0.02	0.004 [-0.007, 0.015]	0.636	-0.003 [-0.014, 0.009]	0.883	-0.007 [-0.016, 0.002]	0.120
E (BW·s)	0.59	0.06	0.58	0.03	0.61	0.06	0.011 [-0.027, 0.049]	0.831	-0.017 [-0.053, 0.019]	0.517	-0.028 [-0.066, 0.011]	0.188
RPFD (BW·s^{-1})	18.5	3.6	18.4	2.6	18.6	3.7	0.057 [-0.813, 0.928]	0.997	-0.108 [-1.203, 0.986]	0.990	-0.166 [-1.402, 1.070]	0.976
ALR (BW·s^{-1})	31.5	10	30.0	9.3	29.7	13.1	1.6 [-1.5, 4.7]	0.445	1.8 [-1.6, 5.3]	0.414	0.25 [-4.2, 4.8]	0.998
LR (BW·s^{-1})	42.5	10.9	41.1	9.4	39.8	12.7	1.4 [-1.7, 4.5]	0.560	2.7 [-0.69, 6.02]	0.136	1.3 [-2.3, 4.8]	0.697
vGRF (BW)	2.12	0.20	2.14	0.16	2.23	0.21	-0.03 [-0.10, 0.05]	0.76	-0.11 [-0.19, 0.03]	0.006*	-0.09 [-0.02, 0.20]	0.120
max vGRF (BW)	2.6	0.3	2.6	0.2	2.5	0.2	0.00 [-0.11, 0.12]	0.99	0.09 [0.01, 0.17]	0.022*	0.09 [-0.18, 0.00]	0.61
hGRF(BW)	0.40	0.052	0.41	0.06	0.42	0.055	-0.02 [-0.05, 0.00]	0.03*	-0.03 [-0.05, 0.00]	0.013*	0.00 [-0.02, 0.03]	0.99
max hGRF (BW)	2.0	0.3	1.7	0.4	1.2	0.2	0.27 [0.13, 0.40]	0.001*	0.77 [0.60, 0.93]	0.0001*	-0.50 [-0.68, -0.32]	0.0001*
MPO (W)	530.5	87.1	521.5	70.9	493.6	66.1	9.0 [-32.2, 50.1]	0.91	37.0 [-7.9, 81.8]	0.18	27.9 [0.7, 55.2]	0.04
kVert kNm	28.3	2.7	29.0	2.9	27.5	3.9	-0.54 [-2.1, 1.0]	0.734	0.82 [-0.87, 2.5]	0.490	1.4 [-0.13, 2.9]	0.077
kLeg kNm	18.5	3.0	18.8	3.4	18.9	3.7	-0.28 [-1.3, 0.78]	0.851	-0.40 [-1.7, 0.95]	0.805	-0.12 [-1.1, 0.84]	0.981
ΔCoM (m)	0.06	0.01	0.06	0.01	0.07	0.01	0.001 [-0.003, 0.006]	0.836	-0.003 [-0.008, 0.002]	0.401	-0.004 [-0.008, 0.000]	0.070

Notes: m = meters, BW = body weight, BW·s^{-1} = body weight per second, s = seconds, W = Watts, dim = dimensionless, I = vertical Impulse, E = Signal energy, RPFD = rate of peak force development, ALR= average load rate, LR = maximum load rate , MPO= mean peak power, kVert = vertical stiffness, kLeg = leg stiffness, vGRF = vertical ground reaction force, hGRF = anterior horizontal ground reaction force, ΔCoM = delta vertical centre of mass displacement

Table 4.4 Mean, standard deviation, mean difference [95% confidence interval] and P value for running mechanics variables ($n = 12$).

	5s-Int (1) Mean (SD)		20s-Int Mean (SD)		80s-Int Mean (SD)		1 vs. 2 MD [95% CI]	P-value	1 vs. 3 MD [95% CI]	P-value	2 vs. 3 MD [95% CI]	P-value
Kinematic												
SL (m)	0.99	0.06	0.99	0.06	0.97	0.07	-0.01 [-0.03, 0.02]	0.796	0.01 [-0.02, 0.05]	0.626	0.02 [-0.02, 0.06]	0.487
SF (#steps·s^{-1})	2.79	0.13	2.79	0.12	2.77	0.18	0.0 [-0.05, 0.05]	1.000	0.02 [-0.07, 0.11]	0.886	0.02 [-0.07, 0.11]	0.887
CT (s)	0.27	0.03	0.27	0.03	0.27	0.03	0.003 [-0.004, 0.010]	0.646	0.005 [-0.003, 0.014]	0.285	0.003 [-0.007, 0.012]	0.856
AT (s)	0.09	0.03	0.09	0.01	0.09	0.02	-0.001 [-0.008, 0.005]	0.893	-0.005 [-0.016, 0.005]	0.450	-0.004 [-0.013, 0.006]	0.610
No. Steps (#)	4498	675	4398	655	4198	582	100 [-60, 260]	0.286	299 [-21, 621]	0.069	200 [-127, 526]	0.304

Notes: **step length (SL), step frequency (SF), contact time (CT), aerial time (AT).**

Table 4.5 Mean, standard deviation, mean difference [95% confidence interval] and P value for CV data ($n = 12$).

	5s-Int (1) Mean (SD)		20s-Int Mean (SD)		80s-Int Mean (SD)		1 vs. 2 MD [95% CI]	P-value	1 vs. 3 MD [95% CI]	P-value	2 vs. 3 MD [95% CI]	P-value
kinetic												
CV_load rate	23	6	25	6	13	3	-1 [-5 to 2]	0.668	10 [5.6 to 14]	0.0001	11 [6 to 17]	0.001
CV vertical impulse	9	3	7	3	3	0.4	3 [0.4 to 5]	0.015	7 [4 to 9]	0.001	4 [2 to 7]	0.001
CV peak vGRF	13	4	13	4	8	5	0.2 [-1 to 2]	0.953	5 [3 to 8]	0.0001	5 [3 to 7]	0.0001
CV kVert	15	4	14	4	5	0.7	0.4 [-1 to 2]	0.896	10 [7 to 13]	0.0001	9 [6 to 12]	0.0001
CV kLeg	16	5	7	1	15	4	1 [-1 to 3]	0.454	9 [5 to 13]	0.0001	8 [5 to 11]	0.0001
Kinematic												
CV step length	22	6	24	7	4	0.6	-1 [-4 to 1]	0.467	18 [14 to 23]	0.0001	20 [14 to 26]	0.0001
CV aerial time	33	13	12	4	33	15	0.5 [-8 to 9)	0.998	21 [9 to 32)	0.001	30 (6 to 34)	0.005

Notes: CV = coefficient of variance, kVert = vertical stiffness, kLeg = leg stiffness, vGRF = vertical ground reaction force,

The mean and SD for the number of steps which peak vGRF exceeded 2.0, 2.2, 2.4 and 2.6 body weights (BWs) is shown in Table 4.6. There was a significant main effects for > 2 BW [$F (2,22) = 13.270$, $P = 0.0001$] with a greater number of peaks counted for the 80s-Int condition at >2 BW compared to 5s-Int ($MD = 715$ steps (358 to 1071), $P = 0.0001$) and a main effect for > 2.2 BW [$F (2,22) = 8.710$, $P = 0.002$] with 80s-Int being greater than the 5s-Int ($MD = 872$ steps (362 to 1382), $P = 0.002$. There were no significant main effects for the >2.4 BW category [$F (2,22) = 0.049$, $P = 0.952$] or > 2.6 [$F (2,22) = 0.660$, $P =0.527$].

Table 4.6 Mean (SD) number of steps that exceeded 2.0, 2.2, 2.4 and 2.6 BWs with the number (*n*) of participants who obtained the highest peaks.

	5s-Int		20s2s-Int		80s-Int	
	count	*n*	count	*n*	count	*n*
>2.0 BW	2827 (1022)	12	3017 (964)	12	3542 (1190)	12
>2.2 BW	1917 (1181)	12	2064 (868)	11	2789 (1512)	11
>2.4 BW	943 (1172)	11	994 (772)	11	952 (1225)	10
>2.6 BW	383 (628)	7	165 (286)	8	267 (883)	4

Experiment Two: The effect of varying the acceleration and deceleration rate on the magnitude, rate and variability of the loading dose.

4.4.3 External load

Work, distance and mean velocity

External work (kJ) and distance (m) were normally distributed. There were no significant differences in the means between conditions for both work [$F_{(2, 20)} = 0.224$, $P = 0.801$], or distance [$F_{(2, 20)} = 3.082$, $P = 0.064$] (Table 4.7).

Table 4.7 Mean, standard deviation, and raw mean difference [95% CI] for measures of external load ($n = 11$).

	20s2s-Int (1)		20s4s-Int (2)		20s6s-Int (3)		1 vs. 2	1 vs. 3	2 vs. 3
	Mean (SD)		Mean (SD)		Mean (SD)		MD [95% CI]	MD [95% CI]	MD [95% CI]
Distance (m)	4158.2	(351.6)	4106.6	(388.7)	4105.9	(391.1)	51.5 [-35.5, 138.7]	52.2 [-14.4, 118.9]	0.6 [-47.0, 48.3]
Work (kJ)	1016	(136)	1023	(192)	1002	(170)	-6 [-120, 107]	15 [-77, 106]	21 [-38, 79]
Speed (m·s^{-1})	2.1	(0.2)	2.1	(0.2)	2.1	(0.2)	0.028 [-0.018, 0.074]	0.029 [-0.009, 0.067]	0.001 [-0.023, 0.024]

m = meters, kJ = kilojoules, m·s^{-1} = meters per second, MD = raw mean difference, CI = 95% confidence interval

4.4.4 Running mechanics (kinematic and kinetic)

Kinetic

The mean, standard deviation, mean difference and 95% confidence interval for running mechanics data are presented in Table 4.8 & 4.9. Mean vertical impulse was significantly different between conditions [$F_{(2, 20)} = 8.334$, $P = 0.002$] with a lower mean vertical impulse for the 20s2s-Int condition compared to the 20s6s-Int. Signal energy [$F_{(2, 20)} = 2.911$, $P = 0.078$] and RPFD [$F_{(2, 20)} = 0.518$, $P = 0.603$] were not significantly different between conditions. There were no significant differences for mean LR [$F_{(2, 20)} = 0.286$, $P = 0.755$] or ALR [$F_{(2, 20)} = 0.501$, $P = 0.613$. Vertical [$F_{(2, 20)} = 12.570$, $P = 0.0001$] stiffness but not leg stiffness [$F_{(2, 20)} = 0.353$, $P = 0.707$] was significantly different between conditions. Sphericity for delta CoM was not assumed but was not significantly different between conditions [$F_{(1.277, 12.770)} = 4.085$, $P = 0.057$]. There was no significant difference for peak vGRF [$F_{(1.271, 12.709)} = 0.635$, $P = 0.477$], mean vGRF [$F_{(1.148, 11.479)} = 0.627$, $P = 0.466$], mean hGRF [$F_{(2, 20)} = 0.115$, $P = 0.892$]. Peak hGRF was significantly different between conditions [$F_{(1.271, 12.741)} = 46.699$, $P = 0.0001$] with the 20s2s-Int condition being greater than the 20s4s-Int and 20s6s-Int. There was no difference in peak hGRF between 20s4s-Int and 20s6s-Int (refer to Table 4.6 A).

There was no statistical difference between conditions for the %CV (See Table 4.10) for LR [$F_{(2, 20)} = 2.087$, $P = 0.153$], vGRF [$F_{(2, 20)} = 0.958$, $P = 0.401$], kVert [$F_{(2, 20)} = 1.336$, $P = 0.285$] and kLeg [$(2\ 20) = 2.980$, $P = 0.74$]. However, % CV was statistically different for vertical impulse [$F_{(2, 20)} = 4.486$, $P = 0.025$].

Kinematic

There were no significant differences for step length [$F_{(2, 20)} = 0.990$, $P = 0.389$] or aerial time [$F_{(2, 20)} = 1.695$, $P = 0.209$]. Step frequency [$F_{(1.326, 13.260)} = 4.623$, $P = 0.042$] and contact time [$F_{(2, 20)} = 6.804$, $P = 0.006$] were significantly different between conditions. There was no significant

difference in total number of steps [$F_{(2, 20)} = 0.519$, $P = 0.603$]. There was no significant difference for MPO [$F_{(2, 20)} = 0.257$, $P = 0.776$]. The % CV between conditions were not statistically different for step length [$F_{(2,20)} = 0.399$, $P = 0.677$] or aerial time [$F_{(2,20)} = 0.221$, $P = 0.803$] (See Table 4.10)

Table 4.8 Mean, standard deviation, mean difference [95% confidence interval] and P value for running mechanics variables ($n = 11$).

	20s2s-Int (1)		20s4s-Int (2)		20s6s-Int (3)		1 vs. 2		1 vs. 3		2 vs. 3	
	Mean (SD)		Mean (SD)		Mean (SD)		MD [95% CI]	P-value	MD [95% CI]	P-value	MD [95% CI]	P-value
kinetic												
I (BW·s)	0.35	0.02	0.36	0.02	0.36	0.02	-0.012 [-0.024, 0.000]	0.058	-0.015 [-0.026, -0.004]	0.010	-0.003 [-0.012, 0.007]	0.794
E (BW·s)	0.58	0.03	0.61	0.05	0.61	0.05	-0.029 [-0.074, 0.017]	0.270	-0.030 [-0.078, 0.018]	0.289	-0.001 [-0.022, 0.020]	0.999
RPFD (BW·s^{-1})	18.2	2.62	18.5	3.2	18.2	3.1	-0.341 [-1.68, 0.997]	0.862	0.014 [-1.16, 1.19]	1.000	0.355 [-0.453, 1.16]	0.557
ALR (BW·s^{-1})	30.2	9.7	31.3	10.9	30.8	12.4	-1.1 [-4.3, 2.1]	0.717	-0.62 [-3.8, 2.6]	0.934	0.49 [-0.36, 2.6]	0.961
LR (BW·s^{-1})	41.6	9.7	42.4	10.6	41.7	12.0	-0.75 [-4.0, 2.5]	0.888	-0.07 [-3.4, 3.2]	1.000	0.70 [-3.6, 2.2]	0.889
$\bar{x}$ vGRF (BW)	2.12	0.15	2.16	0.19	2.15	0.19	-0.039 [-0.170, 0.091]	0.793	-0.028 [-0.144, 0.088]	0.877	0.011 [-0.030, 0.053]	0.842
max vGRF (BW)	2.56	0.18	2.59	0.23	2.61	0.26	-0.033 [-0.171, 0.104]	0.878	-0.043 [-0.191, 0.100]	0.770	-0.013 [-0.073, 0.047]	0.915
$\bar{x}$ hGRF(BW)	0.42	0.07	0.42	0.06	0.42	0.06	0.005 [-0.029, 0.038]	0.973	0.005 [-0.038, 0.049]	0.980	0.001 [-0.024, 0.025]	1.000
max hGRF (BW)	1.66	0.37	1.19	0.23	1.13	0.20	0.467 [0.255, 0.680]	0.0001*	0.532 [0.343, 0.721]	0.0001*	0.065 [-0.024, 1.54]	0.180
MPO (W)	512.7	67.2	516.5	97.3	505.0	84.3	-3.7 [-62.6, 55.2]	0.997	7.7 [-38.6, 54.0]	0.955	11.4 [-18.1, 41.0]	0.649
$\bar{x}$ kVert kNm	28.5	2.3	27.2	2.1	26.8	2.0	1.4 [0.16, 2.6]	0.027*	1.8 [0.60, 3.0]	0.006*	0.42 [-1.1, 0.25]	0.284
$\bar{x}$ kLeg kNm	18.7	3.5	18.8	3.9	18.5	3.5	-0.16 [-1.3, 1.0]	0.974	0.11 [-0.66, 0.88]	0.971	0.26 [-0.49, 1.0]	0.711
ΔCoM (m)	0.06	0.01	0.07	0.01	0.07	0.01	-0.004 [-0.009, 0.002]	0.222	-0.004 [-0.009, 0.001]	0.139	0.00 [-0.003, 0.002]	0.914

Notes: m = meters, BW = body weight, BW·s^{-1} = body weight per second, s = seconds, W = Watts, dim = dimensionless, I = vertical Impulse, E = Signal energy, RPFD = rate of peak force development, ALR= average load rate, LR = maximum load rate , MPO= mean peak power, kVert = vertical stiffness, kLeg = leg stiffness, vGRF = vertical ground reaction force, hGRF = anterior horizontal ground reaction force, ΔCoM = delta vertical centre of mass displacement

Table 4.9 Mean, standard deviation, mean difference [95% CI] and P values for running mechanics variables (n = 11)

	20s2s-Int (1)		20s4s-Int (2)		20s6s-Int (3)		1 vs. 2		1 vs. 3		2 vs. 3	
	Mean (SD)		Mean (SD)		Mean (SD)		MD [95% CI]	P-value	MD [95% CI]	P-value	MD [95% CI]	P-value
Kinematic												
SL (m)	0.99	0.07	0.98	0.07	0.97	0.06	0.007 [-0.013, 0.044]	0.945	0.015 [-0.013, 0.044]	0.381	0.009 [-0.020, 0.037]	0.779
SF (#steps·s⁻¹)	2.78	0.13	2.75	0.13	2.74	0.13	0.036 [0.009, 0.063]	0.011	0.047 [-0.011, 0.104]	0.124	0.011 [-0.037, 0.058]	0.894
CT (s)	0.27	0.03	0.28	0.03	0.28	0.03	-0.006 [-0.013, 0.000]	0.040	-0.011 [-0.020, -0.003]	0.012	-0.005 [-0.015, 0.006]	0.553
AT (s)	0.09	0.02	0.09	0.02	0.09	0.02	0.001 [-0.006, 0.007]	0.989	0.004 [-0.003, 0.011]	0.388	0.003 [-0.002, 0.008]	0.261
No. Steps (#)	4423	681	4367	640	4347	611	56 [-106, 217]	0.723	76 [-208, 361]	0.844	21 [-179, 220]	0.988

Notes: step length (SL), step frequency (SF), contact time (CT), aerial time (AT).

Table 4.10 Mean, standard deviation, mean difference [95% confidence interval] and P value for CV data ($n = 11$).

	20s2s-Int (1) Mean (SD)		20s4s-Int (2) Mean (SD)		20s6s-Int (3) Mean (SD)		1 vs. 2 MD [95% CI]	P-value	1 vs. 3 MD [95% CI]	P-value	2 vs. 3 MD [95% CI]	P-value
kinetic												
CV_load rate	25	7	23	7	23	7	2 [-1 to 5]	0.400	2 [-2 to 5]	0.388	0 [-2 to 2]	0.994
CV vertical impulse	7	3	10	6	11	6	-3 [-7 to 1]	0.184	-4 [-7 to -0]	0.024	-1 [-5 to 3]	0.881
CV peak vGRF	13	5	13	4	13	4	1 [-1 to 2]	0.582	0 [-1 to 2]	0.805	0 [-1 to 1]	0.924
CV kVert	14	4	13	4	13	4	1 [-2 to 3]	0.881	1 [-1 to 3]	0.312	0 [-1 to 2]	0.662
CV kLeg	15	5	14	5	15	5	1 [-1 to 3]	0.205	1 [-1 to 2]	0.256	0 [-2 to 1]	0.801
Kinematic												
CV step length	24	8	23	5	23	5	1 [-3 to 5]	0.913	1 [-2 to 4]	0.801	0 [-3 to 3]	1.000
CV aerial time	33	16	33	9	35	11	0 [-9 to 9]	1.000	-2 [-10 to 6]	0.937	-2 [-8 to 5]	0.860

Notes: CV = coefficient of variance, kVert = vertical stiffness, kLeg = leg stiffness, vGRF = vertical ground reaction force, * denotes significance at P = < 0.05.

There was no statistical differences between conditions for the amount of steps above each arbitrary threshold for the > 2.0 BW [F (2,20) = 0.286, P = 0.764], >2.2 BW [F (2,20) = 0.505, P = 0.611], > 2.4 BW [F (2,20) = 0.656, P =0.530] and 2.6 BW [F (2,20) = 3.504, P = 0.05] (see Table 4.11).

Table 4.11 displays the mean (SD) number of steps that exceeded 2.0, 2.2, 2.4 and 2.6 BWs with the number (*n*) of participants who obtained the highest peaks. The percentage of steps with a discernible vertical impact peak is also presented.

	20s2-Int		20s4-Int		20s6-Int	
	count	*n*	count	*n*	count	*n*
>2.0 BW	2937 (968)	11	2955 (882)	11	2842 (746)	11
>2.2 BW	2004 (884)	10	2017 (1116)	11	1867 (955)	11
>2.4 BW	892 (720)	10	1067 (1239)	10	924 (1157)	11
>2.6 BW	94 (152)	7	451 (759)	5	454 (757)	5

The percentage number of steps that displayed a heel-strike pattern were not significantly different between conditions [F (2,22) = 2.8, P = 0.083] (See Table 4.12) .

Table 4.12 The percentage of steps within each condition that displayed a heel-strike pattern.

n	% of VITs detected											
	1	2	3	4	5	6	7	8	9	10	11	12
5s-Int	8 (2)	16 (6)	0 (0)	34 (3)	48 (11)	50 (17)	26 (16)	3 (1)	1 (1)	91 (2)	86 (11)	0 (0)
80s-Int	0 (0)	0 (0)	0 (0)	35 (16)	18 (8)	21 (8)	13 (4)	0 (0)	0 (0)	100 (1)	73 (12)	0 (0)
20s2-Int	8 (3)	7 (2)	0 (0)	36 (7)	55 (9)	9 (8)	31 (16)	4 (5)	0 (0)	87 (3)	84 (11)	0 (0)
20s4-Int	9 (3)	10 (5)	-	50 (10)	41 (7)	7 (6)	63 (20)	4 (3)	0 (0)	92 (4)	58 (16)	0 (0)
20s6-Int	6 (5)	13 (7)	-	57 (3)	34 (10)	23 (13)	40 (16)	3 (1)	0 (1)	94 (2)	54 (7)	0 (0)

Note: VIT = vertical impact transitent.

4.4.5 Cross sectional analysis: relationship between speed and the kinetics and kinematics of running gait

Figure 4.12 displays the relationship between speed and a selection of gait parameters across the five conditions. The steps for all individuals were pooled together into a pooled mean population value including more than 2500 steps. There was a strong positive correlation between speed and kVert and a strong negative association between speed and kLeg. Pearsons r for all other variables are presented in Table 4.13 below.
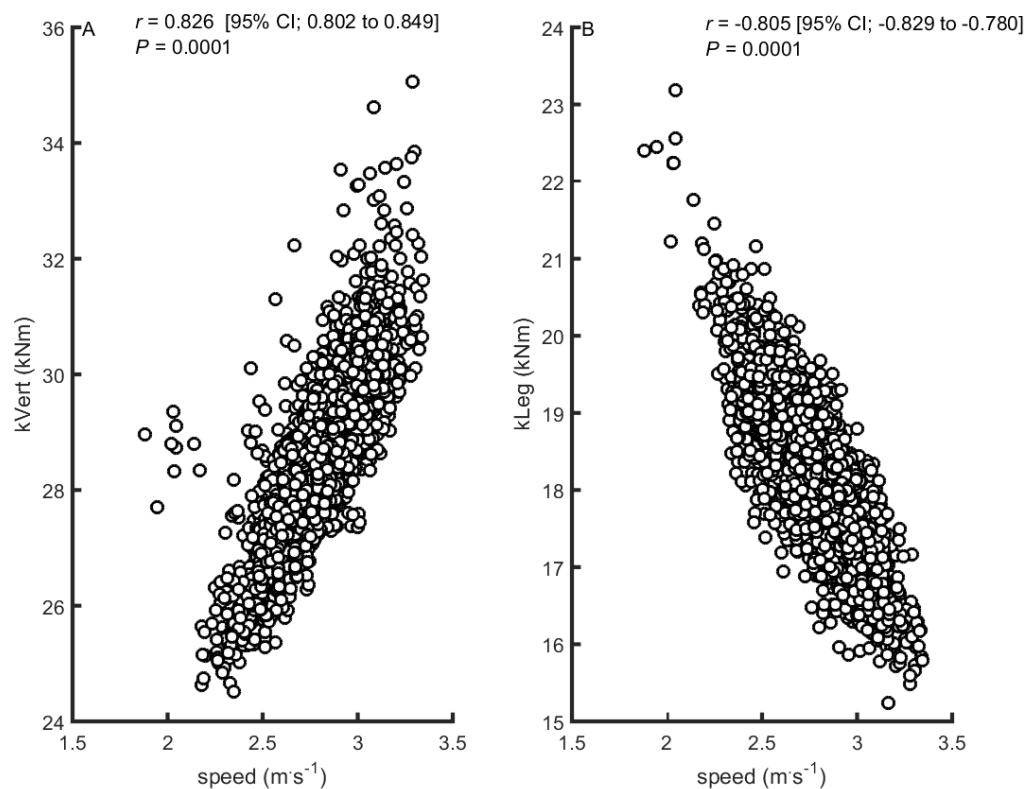
Figure 4.12 The relationship between kVert vs. Speed (A) and kLeg vs. Speed (B) for the 5s-Int protocol using a pooled mean for every step accounted for all 12 participants.

Table 4.13 Pearson's correlations (*r*) and bootstrapped 95% confidence intervals for speed vs. kinetic and kinematic variables.

Variable	5s-Int	80s-Int	20s2s-Int	20s4s-Int	20s6s-Int
LR (BW·s⁻¹)	0.608 [0.576 to 0.641]**	0.215 [0.181 to 0.249]**	0.793 [0.767 to 0.820]**	0.823 [0.799 to 0.846]**	0.806 [0.782 to 0.831]**
ALR (BW·s⁻¹)	0.548 [0.514 to 0.583]**	0.182 [0.148 to 0.216]**	0.740 [0.711 to 0.770]**	0.730 [0.702 to 0.758]**	0.748 [0.720 to 0.775]**
AT (s)	0.344 [0.305 to 0.383]**	0.250 [0.217 to 0.284]**	0.627 [0.593 to 0.661]**	0.616 [0.583 to 0.648]**	0.405 [0.366 to 0.443]**
CT (s)	-0.504 [-0.540 to -0.468]**	-0.452 [-0.483 to -0.421]**	-0.727 [-0.757 to -0.698]**	-0.528 [-0.563 to -0.493]**	-0.295 [-0.335 to -0.255]**
SL (m)	0.692 [0.663 to 0.722]**	0.635 [0.608 to 0.662]**	0.867 [0.845 to 0.889]**	0.833 [0.811 to 0.856]**	0.585 [0.551 to 0.619]**
SF (Hz)	0.416 [0.379 to 0.454]**	-0.015 [-0.049 to 0.020]	0.437 [0.398 to 0.475]**	0.439 [0.402 to 0.476]**	0.205 [0.164 to 0.246]**
COM (m)	-0.511 [-0.546 to -0.475]**	0.070 [0.035 to 0.104]**	-0.400 [-0.440 to -0.360]**	-0.420 [-0.457 to -0.382]**	-0.332 [-0.371 to -0.293]**
kVert (kNm)	0.826 [0.802 to 0.849] **	0.103 [0.068 to 0.137]	0.863 [0.841 to 0.885]**	0.941 [0.897 to 0.931]**	0.897 [0.879 to 0.916]**
kLeg (kNm)	-0.805 [-0.829 to -0.780]**	-0.228 [-0.262 to -0.194]**	-0.865 [-0.886 to -0.843]**	-0.732 [-0.760 to -0.703]**	-0.709 [-0.739 to -0.680]**

**. Correlation (*r*) is significant at the 0.01 level (2-tailed).

4.5 Discussion

The primary findings of the study was that; (1) the means (all steps detected for all participants) of the kinetic and (2) kinematic variables were similar between conditions (Table 4.3 and 4.4). The similarity in the means between the variables is not surprising given GRF is linearly proportional to the speed (Keller et al., 1996; Weyand et al., 2000). As hypothesised in the introduction, the higher forces imparted by the higher speeds were counteracted by the lower forces experienced during the lower speeds. Given the magnitude of the applied force is fundamental for bone remodelling (Frost, 1997a; Turner, 1998), the similarities in the mean vGRF characteristics might suggest that the osteogenic potential of the conditions are well matched and offer similar mechanical loading environments. Therefore, any changes in bone remodelling as a consequence of these training models might not be related to the mechanical load.

The peak vGRF achieved during running on the NMT are similar to those reported during running on a motorised treadmill (Kluitenberg et al., 2012) and over-ground running (Riley et al., 2008). The peak vGRF (highest vGRF of all steps) was greater for the more intermittent exercise conditions (5s-Int and 20s2s-Int) compared to the80s-Int. This can be explained by the higher speeds during the higher intensity running periods generating higher GRFs (Brughelli et al., 2011). A greater mean number of these peak vGRFs above 2.6 BWs are also evident in the intermittent protocols (Tables 4.6 and 4.11) compared to the more continuous protocol (80s-Int). However, this difference was not statistically different, and not all participants demonstrated greater peak vGRF with the greater intermittent running conditions.

Moreover, there was no statistical difference in percentages of vertical impact transients detected for the different running conditions. However, there was a large inter-individual variability in the amount of vertical impact transients detected between individuals and between conditions. This is due to the small sample size ($n = 12$) and the variability in running styles between participants. The lack of detected vertical impact transients in many of the participants is caused by using a forefoot strike pattern (Lieberman et al., 2010), which attenuates the impact transient. This finding

is common for NMT running because of the greater forward lean required to apply propulsive forces to overcome the inertia of the treadmill belt (Lakomy, 1987; Hagan et al., 2006). Despite this, some steps still represented a common heel-strike pattern (e.g. Figure 4.8) (Kluitenberg et al., 2012). The foot strike patterns of the participants were not measured before the study. Therefore, we cannot conclusively say that the NMT results in an attenuated/ absent VIT or whether this is simply a characteristic of the participants running styles. However, the attenuated VIT is confirmed by earlier research (Hagan et al., 2006) using an NMT. However, it is not clear on the type of model of NMT used or the process by which the VITs were analysed as data were included in a short report.

The intra-stride variability, as demonstrated by statistically significant differences in the %CV for kinetic variables was greater for the more intermittent protocols (5s-Int and 20s2s-Int) compared to the more continuous protocol (80s-Int condition). This is likely due to the variable speed ranges rather than the in-series number of exercise-to-rest intervals as the variability in vGRF was similar between the 20s2s-Int and 5s-Int and between the 20s2s-Int, 20s4s-Int and 20s6s-Int protocols. The greater variability and non-uniformity in loading pattern has been proposed as a potential mechanism to enhance bone adaptation as bone responds to unaccustomed loads (Moreno et al., 2008). Therefore, intermittent running could provide a more variable mechanical loading environment. Further work is warranted to investigate whether this variability is enough to induce positive bone adaptations over less variable mechanical loading environments

The threshold above which exercise becomes osteogenic has been suggested to be around 3.9-4 g (where g = the acceleration due to gravity) (Heikkinen et al., 2007). The impacts achieved during running on the NMT are below this threshold, and below impacts recorded during high-impact exercises such as jumping (Milgrom et al., 2000; Heikkinen et al., 2007). Therefore, it is likely that running is not the optimal mode of exercise to evoke the greatest gains in bone mass, especially on an NMT. However, using greater speed ranges during intermittent running might enable exercisers to induce the greatest achievable impacts even for a short period of time. Moreover, whilst the vGRF is a useful surrogate measure of strain, it does not represent local

strain at sites such as the tibia which have been shown to record higher strain during running compared to higher impact exercise such as jumping (Milgrom et al., 2000).

The activity of lower limb muscles (quadriceps and hamstrings) was also not assessed in this study. Potentially the muscle activity of the *rectus femoris* which aids in hip flexion and knee extension (Novacheck, 1998) could induce high levels of compressive axial strain on the femur (Cristofolini et al., 1995) during NMT running which is not accounted for from interrogation of the GRF components. Moreover, posterior muscles of the shank such as the *gastrocnemius* muscle and *Soleus* produce large flexion moments on the bone, and these bone loading patters vary throughout the stance phase of the gait cycle and at different speeds (Demes et al., 2001). In particular the two headed *gastrocnemius* muscle arises from the femoral condyles and insets onto the posterior surface of the calcaneus thus crossing two joints (knee and ankle). During the stance phase the gastrocnemius would contract eccentrically during dorsiflexion (Novacheck, 1998). Tension strain has been measured on the anterio-medial aspect of the tibia when the gastrocnemius muscle fatigued (Milgrom et al., 2007).

Moreover, It has been demonstrated that *vastus lateralis* (VL) activity during a vertical jump was much greater during running and that the activity of the VL contributed to greater compressive forces on the bone not accounted for by the GRF (Bassey et al., 1997). As yet no study has unequivocally demonstrated whether forces acting on the bone through gravitational loading are less important than forces acting through muscle activity (Judex & Carlson, 2009). However it is likely that because the body's centre of gravity is above the femur, absorption of energy by soft tissue and eccentric activity of antagonist muscles would contribute to a reduced GRF (Bassey et al., 1997). It is possible then that the NMT offers greater osteogenic benefits through higher forces generated by the knee extensor muscles required to propel the exerciser forward to overcome the inertia of the treadmill (Franks et al., 2012). This would be advantageous for rehabilitation purposes as the lower impacts through the reduced impact transients yet higher muscle forces might offer a positive loading environment for clinical populations.

The peak anterior (propulsive) hGRF data were also greater for the most intermittent protocol (5s-Int) being 40% greater than the 80s-Int and 15% greater than the 20s2s-Int. This is likely explained by the greater number of acceleration phases and greater acceleration required to reach the higher speeds. This greater propulsive horizontal force may induce different forms of mechanical strain (such as torsional) on the skeletal system. Furthermore, the changes in acceleration and deceleration would also likely contribute to greater eccentric activity of the knee extensor muscles in order to decelerate the body (Novacheck, 1998; Chang et al., 2001), and eccentric activity has been shown to be more osteogenic (Hawkins et al., 1999). The combination of both torsional and compressive strain created by bi-directional and variable mechanical loading environments is required for bone adaptation (Turner & Robling, 2003) and likely more osteogenic than those exercises which provide only one type of strain (Kohrt et al., 2009).

The number of loading cycles (# of steps) accounted for was 7% (300 steps), and 2% (100 steps) higher for the more intermittent protocol (5s-Int) compared to the most 80s-Int and 20s2s-Int protocol respectively. Whilst intuitively the number of loading cycles (cycles per second or per day) is important for bone adaptation an early study clearly showed that the effect of loading cycle diminished after ~100 cycles (Umemura et al., 1997; Turner & Robling, 2005b). It is unclear the true effect more cycles would have on bone for *in-vivo* loading protocols such as our protocol. A greater number of loading cycles would likely cause increased bone fatigue which has been argued to be deleterious for bone (Boudenot et al., 2015) due to the saturation of the mechanosensory system. However, the greater number of steps were performed in the intermittent protocols which might allow the bone to re-sensitise and therefore more cycles could be advantageous. It should be noted that post-hoc analysis did not reveal statistically significant differences between step counts but there was a large inter-individual variability as reflected by the wide confidence intervals (Table 4.3). The number of steps can and should be accounted for using the osteogenic index to establish the osteogenic potential of different exercises with different number of loading cycles (Turner & Robling, 2005b).

Frequency (steps per second) of loading is also an important stimulus for bone remodelling (Turner, 1998; Nagaraja & Jo, 2014) with lower loading magnitudes required if the loads are delivered at a sufficiently high frequency (Burr et al., 2002). The step frequency during running on the NMT was high for the speed compared to motorised treadmill and over-ground running (Kluitenberg et al., 2012). The step frequency reported on the NMT in the current study was also higher compared to previous studies (Brughelli et al., 2011). The greater step frequency is due to the very low aerial times recorded. Running at slow speeds on the NMT is difficult as individuals must overcome the inertia of the treadmill belt. To achieve this at lower speeds participants tended to reduce their step length and increase the step frequency through shortening the aerial time. The contact time reported in this study is similar to previous NMT studies (Brughelli et al., 2011; Cronin & Rumpf, 2014). Both studies used a similar NMT set-up, however, Cronin and Rumpf (2014) used younger adolescent population who were shorter than our population. It was also not clear how the data was processed and variables obtained in both studies.

The change in running technique on the NMT leading to an increased step frequency can reduce the loading on the foot during running (Wellenkotter et al., 2014). Given the loading rate is an indirect measure of the strain rate, and strain rate is important for bone adaptation (Cullen et al., 2001), it is likely that the NMT will reduce the osteogenic response on bone (Hagan et al., 2006). However, because continuous loading can be deleterious to bone, potentially the NMT could offer a reduced load bearing environment which might be advantages for rehabilitation (De Witt et al., 2009). Whilst step frequencies of > 2.0- 2.6 Hz have been shown to be related to accelerations and loading rates that are associated with bone health (Rowlands et al., 2014), this may not be apparent on the NMT where loading rates are attenuated.

The maximum load rate and average load rate obtained during all conditions on the NMT are much lower than those reported by previous studies (Kluitenberg et al., 2012). Loading rates of > 70-90 BW·s^{-1} reported by Kluitenberg et al. (2012) are more than two times those reported in the current study (Table 4.3). The contributing factors are likely the lower overall running speed

coupled with the dampening effects of running on a NMT (Belli et al., 2001; Brughelli et al., 2011). The reduced loading rate on the NMT compared to MT running supports previous comparative studies (Hagan et al., 2006).

The change in the vertical and leg stiffness which reflects the resistance of the body to deformation during running was used to further indirectly assess strain (Brughelli & Cronin, 2008). This is the first study to assess changes in stiffness during non-steady state locomotion. As we and others (Brughelli & Cronin, 2008) have demonstrated, vertical stiffness (kVert) increases with speed (Figure 4.11). This occurs as the centre of mass (CoM) moves over the base of support (BoS) and the displacement of the CoM reduces when doing this. Mean vertical and leg stiffness were not significantly different between conditions likely due to the relationship with speed. However, there was a large variability in the stiffness characteristics for the most intermittent condition. Interestingly, a lower degree of variability in kVert and kLeg is reported in the present study compared to previous literature (He et al., 1991) who reported > 25% CV for kVert and > 20% for kLeg. The lower CV reported in the present study could be due to a greater number of steps included in the current study (> 2500) compared to the latter. Furthermore, the greater range of speeds (2-6 m·s^{-1}) included by He et al. (1991) compared to the current study (1.5 -3.5 m·s^{-1}) could also explain the differences supporting our hypothesis that greater speed ranges could be more osteogenic due to the introduction of greater variability.

We demonstrated an inverse relationship with speed and kLeg (Figure 4.11). This is one of the few studies to demonstrate a relationship between kLeg and speed where others have demonstrated kLeg remains constant with increasing running speeds. The peak speeds achieved on the NMT were not greater than those previously used (> 5 m·s^{-1}). Moreover, we reported a mean kLeg of approximately 18 kNm which is in agreement with previous authors reporting kLeg values of ~ 20 kNm (McMahon & Cheng, 1990). Potentially the inverse relationship is due to a decrease in stride length that occurred as speed increased. Participants tended to take shorter more frequent steps on the NMT which might affect the change in leg length, as step frequency

increases have been shown to increase leg stiffness (Farley & Gonzalez, 1996) and shorten leg spring length. However, on a motorised treadmill it has been shown that as maximum vertical force increases the change in leg spring length increases which would reduce leg stiffness (Brughelli & Cronin, 2008)

As yet it is unclear how activities which increase stiffness may affect bone remodelling. However, an increase in the stiffness of the lower limbs may increase the compressive forces on the bone. It would be advantageous to understand how stiffness might affect bone remodelling. Participation in endurance running involves multiple repetitive loading cycles of a similar magnitude and strain rate (Milgrom et al., 2000) which have been shown to induce micro-damage leading to stress fractures (Milgrom et al., 2000). Successful endurance runners have been shown to have greater stiffness which increases running economy, as fatigue sets in the stiffness reduces. It is unclear how an increased variability in stiffness could affect bone remodelling. Potentially the variable change between high stiffness to low stiffness may increase hydrostatic pressure and increase intracellular fluid flow which is required for bone remodelling (Duncan & Turner, 1995). It also unclear whether there might be a threshold above which higher stiffness levels become deleterious to bone. Certainly as stiffness increases beyond a certain threshold there is an increase in injury risk for athletes (Brughelli & Cronin, 2008). Potentially, intermittent exercise may allow higher stiffness levels to be reached without detrimental effects, as the stiffness is only experienced for a short period of time interspersed with much lower stiffness levels.

Few differences between running mechanics were observed when the rate of acceleration and deceleration were manipulated. This suggests that changing the acceleration period has less an effect on running mechanics, assessed via GRFs, than the frequency of changes in exercise-to-rest conditions. This is demonstrated by the similar %CV for the 5s-Int and 20s2s-Int and also the comparisons of the 20s2s-Int, 20s4s-Int and 20s6s-Int. Therefore, greater differences between the lower and higher speeds will likely increase the variability and magnitude of the applied mechanical load.

4.5.1 Limitations and future recommendations

1. The reliability and reproducibility of the protocols have not been established. Whilst many studies have demonstrated good reliability of physiological variables on the NMT (Sirotic & Coutts, 2008; Aldous et al., 2014) few studies have looked at the reliability of the mechanical variables. It would be useful to determine the reliability and smallest worthwhile change (Hopkins et al., 2009) for the primary outcome measures.

2. Future studies should incorporate the use of motion analysis and measures of muscle activity. The effect of muscle activation on bone is not clear, nor how intermittent running might be affected by the protocol. Bone responds to physiological stress, and the nature of intermittent exercise has been shown to evoke different acute and chronic metabolic changes compared to more continuous exercise. The potency of this type of exercise on health and well-being has been investigated. It is not clear how these different mechanical stresses may impact on internal physiological stress.

4.6 Conclusion

Shorter and more frequent exercise-to-rest intervals during running does not impact on the mean loading magnitude and rate suggesting that the overall loading environment is not different when the mean speed and distance is well controlled. However, there is an increase in the step-step variability of the loading characteristics when the exercise-to-rest interval is manipulated. This is likely due to the changes in high and low speeds rather than the number of in-series changes in speed. Given variable and unpredictable loads are more osteogenic for bone, the shorter more frequent exercise-to-rest intervals may induce a more osteogenic response on bone.

CHAPTER 5:

Quantifying the osteogenic potential of high-intensity intermittent running with varying exercise-to-rest intervals: A re-examination of the osteogenic index.

5.1 Abstract

Aim: The osteogenic index (OI) is calculated based on one representative ground reaction force peak. However, given intermittent exercise is characterised by multiple brief high-intensity loading cycles interspersed with low-intensity loading cycles, the higher intensity loading is counteracted by the low intensity loading (as demonstrated in study 2). Therefore it is unclear how the OI as it is currently calculated could distinguish between the osteogenic potential of different intermittent protocols. A new method which quantifies the loading dose over multiple loading segments in the frequency domain may better estimate the osteogenic potential. However, this has only been quantified using accelerometers in continuous loading conditions. Therefore, the aim of the present study was to investigate the potential of a modified OI calculation on the loading characteristics of intermittent running with varying exercise-to-rest durations.

Method: Using the same participant population and study design from study 2 we calculated the OI using one representative GRF in both the time (OI_BW) and frequency (OI_fft) domain, and compared it to the new OI (OI_segFFT) approach. Experiment 1: used the vGRF data from the 5s-Int, 20s-Int and 80s-Int to calculate the traditional and new OI for the different exercise-to-rest intervals. For experiment 2 the OI was calculated for the protocols which differed in the acceleration and deceleration phases. Data are expressed as mean (SD) and were analysed using a one-way repeated measures ANOVA with mean difference and 95% confidence intervals (MD [95% CI]) for the traditional OI calculations. A three-way (condition*frequency*intensity) repeated measures ANOVA was used to examine the effect of condition on the new OI.

Results-Experiment one: There was a statistically significant difference between conditions for the OI_fft ($P = 0.0001$). The OI_fft was highest for the 80s-Int being 28% greater than the 5s-Int and 23% greater than the 20s-Int. There was a statistically significant 3-way interaction for condition*frequency band*intensity ($P = 0.012$). **Experiment two:** There was a significant main effect for condition for the mean differences of the OI_fft between conditions ($P = 0.033$). There was no significant effect of condition for the OI_BW ($P = 0.572$). There was no significant 3-way interact for condition*frequency band*loading intensity ($P = 0.870$).

Conclusion: The OI obtained from a single mean representative vGRF value is higher for the less intermittent protocol compared to the most intermittent protocol. However, when the magnitude, intensity and frequency of multiple loading segments are considered, intermittent locomotion allows the individual to obtain higher magnitudes of loading dose, shifting towards a higher frequency band. This is not apparent when the exercise-to-rest interval is controlled and again is likely explained by the differences in speed

5.2 Introduction

In study 2 we demonstrated that the mean loading dose is similar between exercise conditions with a fixed exercise-to-rest ratio and matched for speed. However, there is a greater variability in the mechanical loading dose. However, in this study we considered only the magnitude and rate of loading dose in isolation. Therefore, considering the combination of the magnitude, rate and frequency of the mechanical load might offer further insight into the nuances in the mechanical loading dose when intermittent mechanical loading is involved (Turner, 1998). Moreover, because of the diminishing returns associated with multiple loading cycles it would be pertinent to assess how the number of loading cycles may affect the osteogenic potential of the exercise.

The OI has been used to determine the osteogenic potential of a variety of exercise modes including jumping (Lester et al., 2009), running (Weeks & Beck, 2008; Lester et al., 2009), stepping exercise (Santos-Rocha et al., 2006), and more recently, the sport of cyclocross (Tolly et al., 2014). The majority of these studies have used predominantly continuous forms of exercise in which a period of steady-state loading is achieved. Yet, the OI may be useful to determine the osteogenic potential of intermittent forms of exercise (Tolly et al., 2014) which are known to be more osteogenic (Turner, 1998). However, as mechanical loading parameters during locomotion are inherently variable (Giakas & Baltzopoulos, 1997), the OI used in these aforementioned studies is potentially limited by the reliance on the use of a single representative vGRF curve. Therefore it is important to consider all loading cycles in the calculation to determine the nuances in the mechanical loading environment rather than one mean or peak data point.

To incorporate all loading cycles previous authors have decomposed the loading cycles into histograms (Ahola et al., 2010). However, as with the OI calculated in equation 7 (Section 2.5.2), only the magnitude of the loading dose is accounted for with this approach, which fails to incorporate the frequency (cycles per second [Hz]) and intensity (strain rate) of the loading dose. Different physical activities have been shown to change the interaction between the magnitude

and frequency of the mechanical load (Cappozzo, 1982), with greater speeds shifting the acceleration of the loads to the higher frequency spectra (Cappozzo, 1982). Both magnitude and frequency are important for bone adaptation (Burr et al., 2002), with higher frequencies enhancing the osteogenic potential of lower magnitude loads (Nagaraja & Jo, 2014). To account for the interaction of frequency and magnitude, the OI can be further developed to incorporate the frequency and rate of the mechanical load using the Fourier transformation (Turner, 1998).

Two groups (Rantalainen et al., 2009; Kelley et al., 2014) have opted to assess the OI with more complexity by combining the magnitude, frequency and intensity. However, when based on only one representative GRF curve (Rantalainen et al., 2009) the variability and discrete changes in the loading patterns during intermittent loading may not be observed. The development of a novel technique which determines loading dose magnitude at different intensities, within various frequency spectra (Kelley et al., 2014), may help to discriminate more appropriately between exercises which vary in intermittency. At present this technique has not been used to establish differences in loading dose between impact exercises of varying exercise-to-rest intervals. Moreover, the technique was developed to assess loading dose from accelerometers worn during daily activities. However, in principle this technique should be applicable to any force measuring device which measures force continuously.

Thus our aims were; (1) to establish whether a more intermittent bout of running may have a greater effect on the OI using the traditional OI calculation, (2) to establish whether the variability in the loading dose could be ascertained with the more recent time varying osteogenic index, and (3) to assess the osteogenic potential of manipulating the acceleration and deceleration rate during load bearing exercise. We hypothesised that the OI using one representative GRF curve would not be different between intermittent protocols. However, when all the loading cycles were considered including all components of the loading cycle (magnitude, intensity, frequency), we hypothesised that the OI would be higher for the very intermittent protocol compared to the less intermittent protocols.

5.3 Methods

Participants

These are the same group of participants used in Chapter 4, study 2.

Design

The randomisation procedures, exercise protocol and equipment set-up has been fully outlined in the methods section of Chapter 4 (4.2).

Calculation of the Osteogenic Index (OI)

The traditional OI was estimated both in the time and frequency domains. The OI calculated from the peak GRF in the time domain will be referred to as 'OI_BW'. To calculate this, every peak (excluding walking segments) for each step over the four minute exercise bout was detected using a bespoke step detection algorithm written using MATLAB (released 2012a, Mathworks, Inc) as outlined in Chapter 4. The vGRF curve was normalised to body weight (BW), with the peak of each step then averaged over the four minutes to obtain a mean vGRF (BW). The mean vGRF from each four minute bout was combined with all nine bouts to obtain a global mean vGRF.

To obtain the OI in the frequency domain, referred to as 'OI_fft', we used a previously published technique outlined by Rantalainen et al. (2009). Firstly, a 2nd order bi-directional Butterworth filter, optimised via residual analysis (20-30 Hz) (Winter, 2009), was applied to the data (described in Chapter 4). Then each step was time-normalised to the mean length of the ground contact time to produce a single representative vGRF curve. The time-normalisation was performed using the *interpft* function in MATLAB, which interpolates the data points using the fast Fourier transformation (FFT). The representative vGRF curve was then divided by body weight (Newtons) to normalise the data to each individual. Another FFT was performed on the acceleration curve in order to transform the data into the frequency domain. Prior to applying the FFT, the time domain data were padded with zeros at the end of the array, and a hanning window

was applied to the data. This reduces the amount of side lobes evident at the end of the FFT caused by spectral leakage, improving the resolution and efficiency of the FFT algorithm (Ramirez, 1985; Duhamel & Vetterli, 1990). The OI_fft was then calculated as follows:

$$OI = Ln\,(1 + N) * \sum_{i=1}^{f_i \leq 30\,Hz} \varepsilon_i f_i$$

Where:

$\varepsilon_i = A_i^2 + B_i^2$, $A_i = 1$: $th\ cosine\ of\ the\ Fourier\ series\ \&\ B_i = 1$: $th\ sine\ of\ Fourier\ series,\ f_i = 1$: $th\ frequency\ in\ Fourier\ series, and\ N = number\ of\ loading\ cycles$

This equation is adapted from Turner (1998). The integration of the Fourier series was performed up to a 30 Hz cut-off which was the average cut-off frequency established using residual analysis (Winter, 2009). An example of the frequency domain analysis of the vGRF curve in displayed in Figure 5.1.
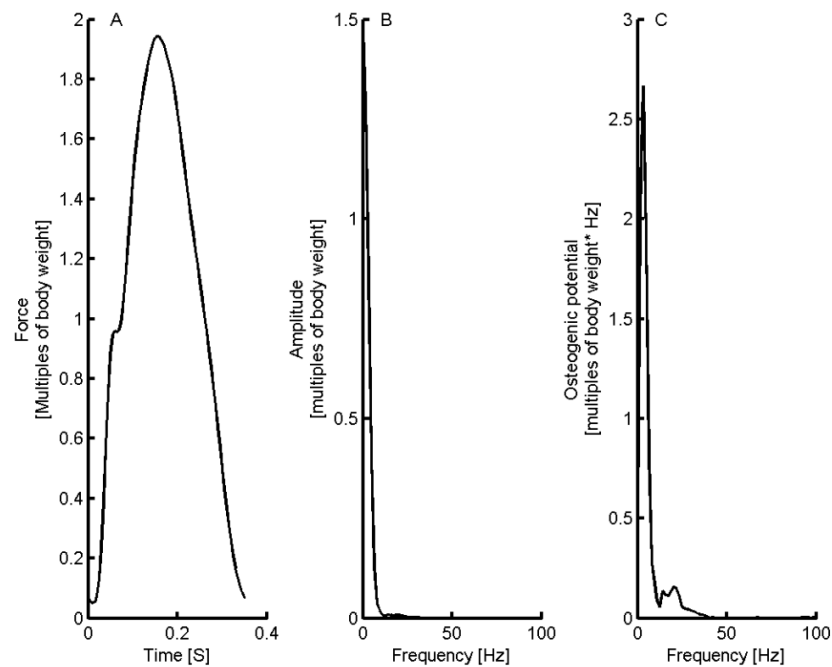
Figure 5.1 A representative vGRF in the time domain (A), transformed into the frequency spectra (B) and then with the amplitude of the FFT multiplied by the frequency (C).

The calculation of the new OI calculated over multiple loading segments which will be referred to as 'OI_segFFT' was calculated as described by previous authors (Chahal et al., 2014; Kelley et al., 2014). Firstly all the vGRF data were divided by the mass of the participant to obtain an acceleration curve according to Newtons second law of motion. Later on the data were divided by the acceleration due to gravity (9.81 m·s⁻²) to obtain body weights per second (BW·s⁻¹). Then each of the nine, 4 min exercise bouts was split into 48 separate segments of 5 s in length. These segments included loading at all speeds including walking. A FFT was applied to each 5 s segment. Prior to applying the FFT on each segment, the signal was zero padded and a hanning window was applied. A 30 Hz frequency band was used on each segment, unlike the use of a 6 Hz frequency band, which was used in the previous studies to account for the vibration of the skin impacting on the accelerometer (Kelley et al., 2014). The 30 Hz frequency bands were split into low and high frequency bands of equal lengths as follows: 0-6 Hz, 6-18 Hz, 18-24 Hz, 24-30 Hz. Each of the segments was assigned into four categories according to its loading intensity: (LI) - very light (≤ 5 BW·s⁻¹), light (5-10 BW·s⁻¹), moderate (10-15 BW·s⁻¹) and vigorous (≥ 15 BW·s⁻¹) (Chahal et al., 2014). The loading intensity can be calculated using the following equations:

$$LI_{B1} = \sum_{f_i = 0.1}^{6\,Hz} \frac{(A_i * f_i)}{g} \tag{1}$$

$$LI_{B2} = \sum_{f_i = 6}^{12\,Hz} \frac{(A_i * f_i)}{g} \tag{2}$$

$$LI_{B3} = \sum_{f_i = 12}^{18\,Hz} \frac{(A_i * f_i)}{g} \tag{3}$$

$$LI_{B4} = \sum_{f_i = 18}^{24\,Hz} \frac{(A_i * f_i)}{g} \tag{4}$$

$$LI_{B5} = \sum_{f_i = 24}^{30\,Hz} \frac{(A_i * f_i)}{g} \tag{5}$$

Where A_i = the magnitude (amplitude) of the FFT and f_i = frequency (Hz)

The loading dose was calculated as follows:

$$\text{LD}_{B1} = Ln\left(1 + \sum_k 5 * LI\_B1\right) \qquad (6)$$

$$\text{LD}_{B2} = Ln\left(1 + \sum_k 5 * LI\_B2\right) \qquad (7)$$

$$\text{LD}_{B3} = Ln\left(1 + \sum_k 5 * LI\_B3\right) \qquad (8)$$

$$\text{LD}_{B4} = Ln\left(1 + \sum_k 5 * LI\_B4\right) \qquad (9)$$

$$\text{LD}_{B5} = Ln\left(1 + \sum_k 5 * LI\_B5\right) \qquad (10)$$

The logarithmic function (Ln) is included in the calculation to account for the diminishing returns that coexist for prolonged loading on bone. An example of the multiple loading segments in the frequency domain is presented in Figure 5.2. To calculate the duration of time spent in each LI category, the number of segments in each category was multiplied by 5 s.
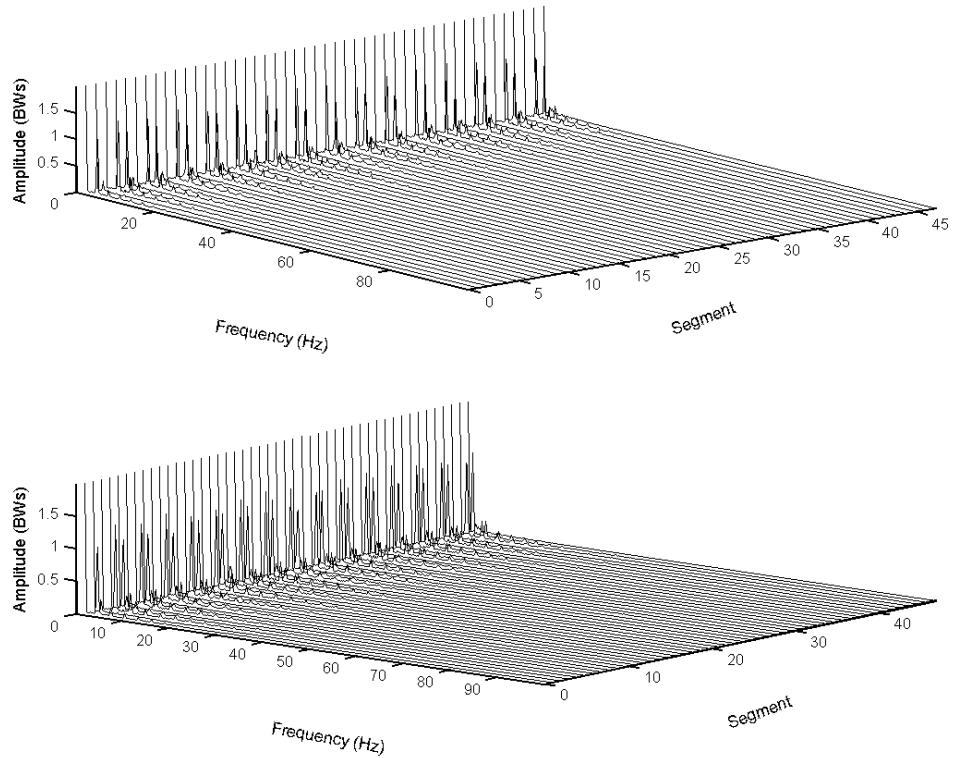
Figure 5.2 A representation of the frequency spectrum for each of the 48 segments, from two views obtained from one 4 min exercise bout (participant No. 2, 5s-Int).

5.3.1 Statistical analysis

Data are presented as mean and standard deviation (mean [SD]), unless stated otherwise. The precision of the estimate of the outcome statistics are shown using a 95% confidence interval. Both osteogenic index methods (eq. 1 and 2) were analysed using a one-way (condition) repeated measures ANOVA. The time varying loading dose data were analysed using a three way, ([3∗condition] x [5∗frequency band] x [4∗loading intensity]) repeated measures ANOVA. Where a three way interaction was found, further two way interactions were investigated. Sidak adjustments were performed to adjust for multiple comparisons.

5.4 Results

5.4.1. Time and frequency (FFT) domain OI

The mean (SD) and intra-individual variability (%CV) for the OI equations using one representative equation are displayed in Table 5.1.

Table 5.1 The mean (SD) for the OI_BW and OI_fft. The variance (%CV) of the OI_BW and OI_fft from over the nine segments is also presented.

	Condition	M	SD	%CV
	5s-Int	73.2	17.0	8.6
OI_fft	80s-Int	91.0	17.5	7.3
	20s2s-Int	69.8	15.0	6.6
	5s-Int	18.0	1.8	2.1
OI_BW	80s-Int	19.0	1.8	2.2
	20s2s-Int	18.0	1.4	2.0

There was a statistically significant difference between conditions for the OI_fft [$F_{(2, 22)}$ = 17.44, P = 0.0001]. The OI_fft was highest for the 80s-Int being 28% greater than the 5s-Int and 23% greater than the 20s2s-Int. However, the OI_fft for 5s-Int condition was 3% greater than the 20s2s-Int. There was a statistically significant difference between conditions for OI_BW [$F_{(2, 22)}$ = 5.638, P = 0.011]. The pairwise comparisons are displayed in Table 5.3. The 5s-Int protocol was statistically significantly lower than the 80s-Int but not the 20s2s-Int.

Table 5.2 The mean difference (MD), 95% confidence intervals (CI) and P-value between the three conditions for OI-fft and OI_BW ($n = 12$).

Condition		MD	95% CI		P-value
OI_fft			LB	UB	
5s-Int	80s-Int	-17.8	-31.9	-3.7	0.014
5s-Int	20s2s-Int	3.4	-4.0	10.8	0.536
80s-Int	20s2s-Int	21.2	11.2	31.1	0.0001
OI_BW					
5s-Int	80s-Int	-0.8	-1.4	-0.2	0.011
5s-Int	20s2s-Int	-0.2	-0.8	0.5	0.876
80s-Int	20s2s-Int	0.6	-0.21	1.5	0.165

5.4.2 Time varying loading dose [OI] and loading dose duration

The 3-way repeated measures ANOVA for loading dose is presented in Figure 5.3. There was a statistically significant main effect for condition [$F_{(2, 22)} = 29.437$, $P = 0.0001$], frequency band [$F_{(4, 44)} = 11.187$, $P = 0.0001$] and intensity [$F_{(3, 33)} = 122.203$, $P = 0.0001$]. There was a statistically significant two-way interaction for condition by intensity [$F_{(6, 66)} = 3.915$, $P = 0.002$]. Post hoc analysis showed that this difference occurred at the 5-10 BW and 10-15 BW intensities where the 5s-Int and 20s-Int had a higher loading dose compared to the 80s-Int (MD = 1.3; 95% CI = 0.5 to 2.1; $P = 0.003$, and MD = 1.4; 95% CI = 0.6 to 2.2; $P = 0.001$). There was no statistically significant difference between 5s-Int vs. 20s2s-Int (MD = -0.1; 95% CI = -1.0 to 0.7; $P = 0.96$). For the 10-15 BW intensity the 5s-Int was also statistically significantly higher than the 80s-Int (MD = 1.6; 95% CI = 0.1 to 3.2; $P = 0.042$) as was the 20s-Int (MD = 1.5; 95% CI = 0.3 to 2.7; $P = 0.013$).

There was also a statistically significant two-way interaction for frequency band by intensity [$F_{(12, 132)} = 9.272$, $P = 0.0001$], however, there was not a statistically significant interaction for condition by frequency band [$F_{(8, 88)} = 0.812$, $P = 0.594$]. There was also a statistically significant 3-way interaction for condition*frequency band*intensity [$F_{(24, 264)} = 1.837$, $P = 0.012$]. Therefore,

pairwise comparisons were performed on loading dose, in each frequency band to see the effect of frequency band and loading intensity on condition.

The loading dose at frequency band one (0-6 Hz), intensity one (<5 BW·s^{-1}), for 5s-Int vs. 20s2s-Int and 5s-Int vs. 80s-Int was significantly higher (MD = 0.153; 95% CI = 0.094 to 0.213; P = 0.0001, and MD = 0.212; 95% CI = 0.084 to 0.339; P = 0.002, respectively). There was no statistically significant difference in the mean loading dose for 20s2s-Int vs. 80s-Int (MD = 0.058; 95% CI = -0.44 to 0.161; P = 0.358). In frequency band two (6-12 Hz), loading intensity two, there was a significant difference in loading dose for 5s-Int vs. 80s-Int (MD = 2.042; 95% CI = 0.216 to 3.867; P = 0.028). In frequency band three, (12-18 Hz) there were no significant differences between loading doses at any of the loading intensities. For frequency band four (18-24 Hz) there was a significant difference between 5s-Int vs. 80s-Int and 20s2s-Int vs. 80s-Int (MD = 0.985; 95% CI = 0.262 to 1.708; P= 0.008, and MD = 1.503; 95% CI = 0.383 to 2.622; P = 0.009). In frequency band five (24-30 Hz), there was a statistically significant difference in loading dose at loading intensity two (5-10 BW·s^{-1}) for 5s-Int vs. 80s-Int and 20s2s-Int vs. 80s-Int (MD = 2.999; 95% CI = 0.106 to 5.892; P = 0.042, and MD = 3.191; 95% CI =1.285 to 5.096; P = 0.002, respectively).
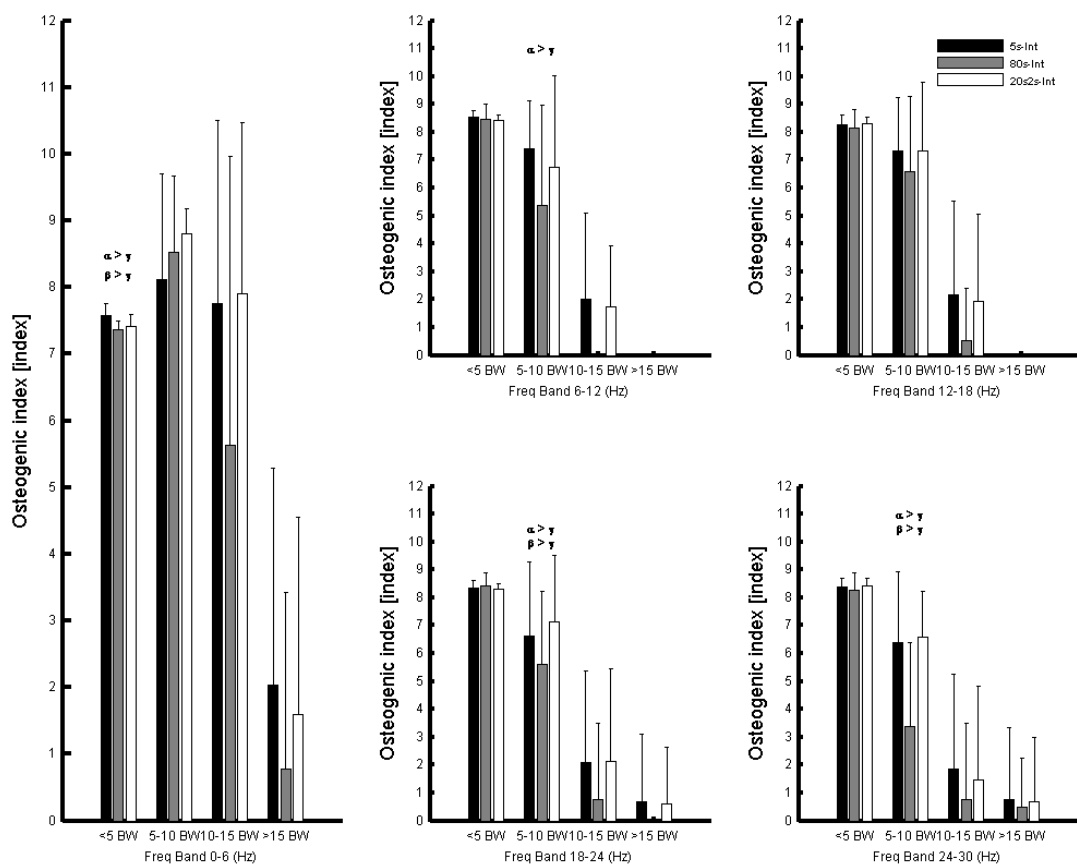
Figure 5.3 The mean (SD) for the magnitude of the loading dose within each loading intensity category, in each frequency band. α = 5s-Int, β = 20s2s-Int, γ = 80s-Int.

The mean (SD) for the 3-way (3 x condition, 4 x loading intensity, 5 x frequency band) repeated measures ANOVA for loading dose duration is presented in Figure 5.4. There was no significant main effect for condition [$F_{(2, 22)}$ = 2.233, P = 0.131] or frequency band [$F_{(4, 44)}$ = 1.672, P = 0.174]. However, there was a significant main effect for intensity [$F_{(3, 33)}$ = 126.314, P = 0.0001]. There was no significant 2-way interactions for condition by frequency [$F_{(8, 88)}$ = 0.710, P = 0.682], condition by intensity [$F_{(6, 66)}$ = 1.522, P = 0.185]. However there was a significant main effect for frequency by intensity [$F_{(12, 132)}$ = 15.322, P = 0.0001]. Finally, there was no significant three-way interaction for condition by frequency by intensity [$F_{(24, 264)}$ = 1.228, P = 0.217].

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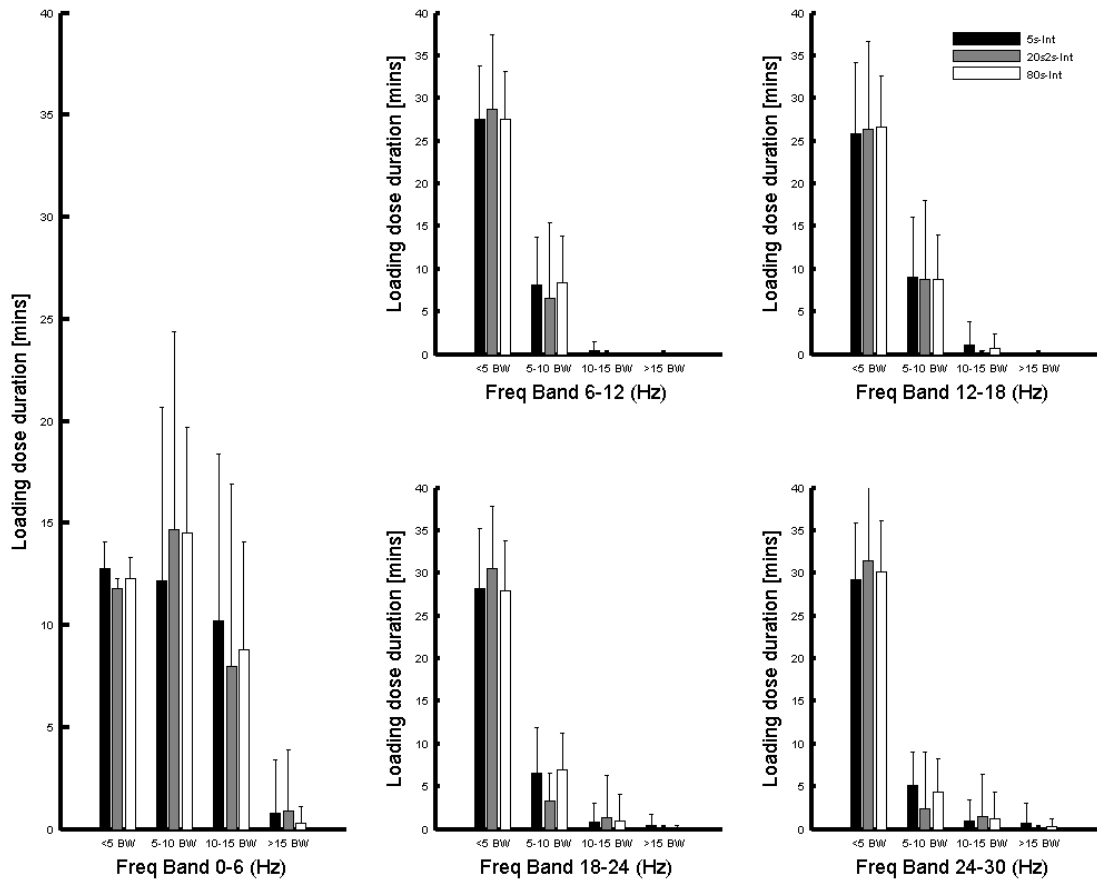
Figure 5.4 The mean (SD) for the duration of the loading dose within each loading intensity category, in each frequency band.

Table 5.3 A-C displays the number (*n* =12) of participants that obtained a loading dose in the five frequency bands (LDB1-LDB5) across the four intensity categories (< 5, 5-10, 10-15 and > 15 BW).

A 5s-Int	<5 BW	5-10 BW	10-15 BW	>15 BW
LDB1	12	12	11	4
LDB2	12	12	4	0
LDB3	12	12	4	0
LDB4	12	11	4	1
LDB5	12	11	3	1

B 20s2s-Int	<5 BW	5-10 BW	10-15 BW	>15 BW
LDB1	12	12	11	3
LDB2	12	10	5	0
LDB3	12	11	4	0
LDB4	12	11	4	1
LDB5	12	12	2	1

C 80s-Int	<5 BW	5-10 BW	10-15 BW	>15 BW
LDB1	12	12	8	1
LDB2	12	9	0	0
LDB3	12	11	1	0
LDB4	12	11	1	0
LDB5	12	8	1	1

Experiment Two: The effect of varying acceleration and deceleration rate of intermittent exercise on the osteogenic index.

5.4.3 Time and frequency (FFT) domain OI

Table 5.4 shows the mean (SD) for the OI_BW and the OI_fft. The variation (%CV) of the OI_BW and OI_fft over the nine segments is also presented.

Table 5.4 Mean, SD and coefficient of variation (%) for the OI_fft, and OI_BW (*n*=11).

	Condition	M	SD	%CV
OI_fft	20s2s-Int	70.4	15.5	6.6
	20s4s-Int	67.0	14.1	7.6
	20s6s-Int	64.2	12.9	6.6
OI_BW	20s2s-Int	89.3	22.2	2.0
	20s4s-Int	84.6	18.1	2.0
	20s6s-Int	82.4	18.6	2.0

There was a significant main effect for condition for the mean differences of the OI_fft between conditions [$F_{(2, 20)} = 4.071$, $P = 0.033$]. The 20s2s-Int condition was significantly higher than the 20s6s-Int condition. The MD (95% CI) is displayed in Table 5.5. There was no significant effect of condition for the OI_BW [$F_{(1.14, 11.4)} = 0.574$, $P = 0.572$].

Table 5.5 The mean difference, 95% CI, and P-values for the interaction between conditions for OI_fft, and OI_BW (*n* = 11).

Condition		MD	95% CI		P-value
OI_fft			LB	UB	
20s2s-Int	20s4s-Int	3.4	-3.9	10.8	0.508
20s2s-Int	20s6s-Int	6.2	1.1	11.4	0.019
20s4s-Int	20s6s-Int	2.8	-3.3	8.8	0.519
OI_BW					
20s2s-Int	20s4s-Int	-0.3	-1.4	0.8	0.814
20s2s-Int	20s6s-Int	-0.2	-1.1	0.7	0.920
20s4s-Int	20s6s-Int	0.1	-0.2	0.5	0.703

Notes: LB = 95% CI lower bound; UB: 95% CI upper bound

5.4.4 Time varying loading dose [OI] and loading dose duration

The mean (SD) for loading dose at each intensity category for each frequency band is presented in Figure 5.5. There was a significant main effect for frequency band [$F_{(1.63, 16.327)}$ = 7.601, P = 0.007] and a significant main effect for loading intensity [$F_{(2.43, 24.29)}$ = 95.324, P = 0.0001]. There was a significant frequency band by loading intensity interaction [$F_{(5.56, 55.61)}$ = 7.224, P = 0.0001]. There was no significant interaction for condition by frequency band [$F_{(7.54, 75.43)}$ = 0.727, P = 0.659] or condition by loading intensity [$F_{(4.59, 45.86)}$ = .886, P = 0.491]. There was also no significant 3-way interaction for condition by frequency band by loading intensity [$F_{(8.78, 87.85)}$ = .496, P = 0.870].
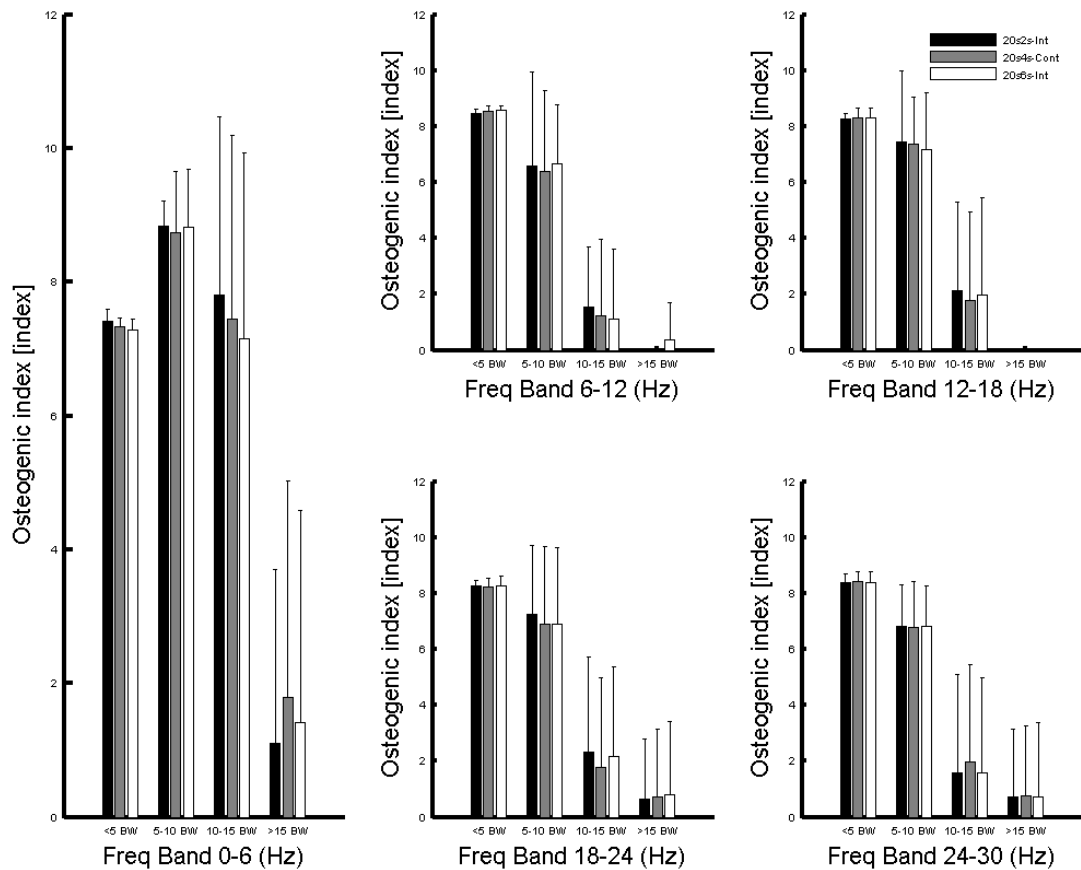
Figure 5.5 Mean (SD) for the magnitude of the loading dose within each loading intensity category, in each frequency band.

There was no significant main effect for condition [$F_{(1.00, 10.00)} = 0.994$, $P = 0.342$] and frequency band [$F_{(4, 40)} = 0.560$, $P = 0.693$]. There was a significant main effect for intensity [$F_{(1.75, 17.5)} = 119.699$, $P = 0.0001$]. There was a significant 2-way interaction for frequency band by loading Intensity [$F_{(2.53, 25.3)} = 17.971$, $P = 0.0001$] but no significant interaction for condition by loading intensity [$F_{(6.56, 65.69)} = 0.208$, $P = 0.897$] or condition by frequency band [$F_{(8, 80)} = 0.481$, $P = 0.835$]. However, there was a significant three-way interaction for condition by frequency band by loading intensity [$F_{(8.499, 84.99)} = 3.225$, $P = 0.002$].
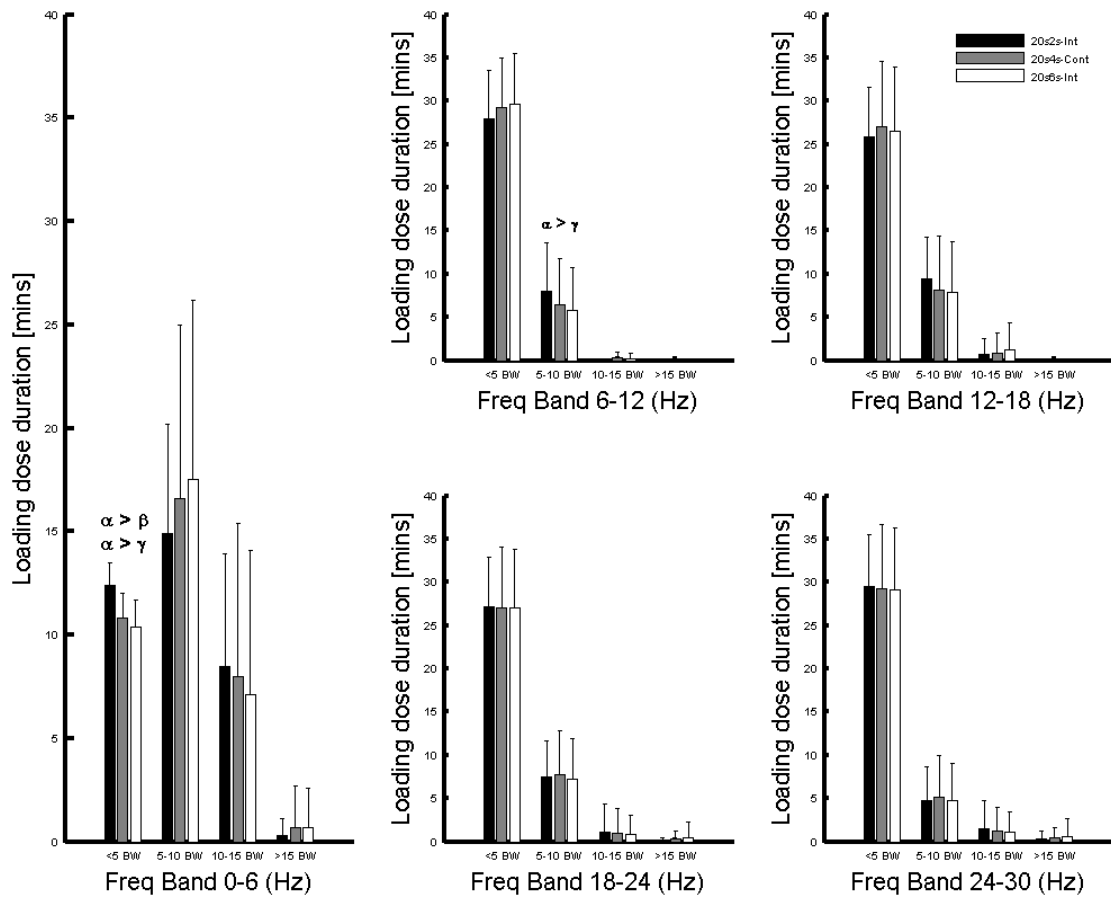
Figure 5.6 Mean (SD) for the duration of the loading dose within each loading intensity category, in each frequency band. α = 20s2s-Int, β = 20s4s-Int, γ = 20s6s-Cont.

Table 5.6 A-C The number (n =11) of participants that obtained a loading dose in the five frequency bands (LDB1-LDB5) across the four intensity categories (< 5, 5-10, 10-15 and > 15 BW).

A 20s2s-Int	<5 BW	5-10 BW	10-15 BW	>15 BW
LDB1	11	11	10	2
LDB2	11	9	4	0
LDB3	11	10	4	0
LDB4	11	10	4	0
LDB5	11	11	2	1

B 20s4s-Int	<5 BW	5-10 BW	10-15 BW	>15 BW
LDB1	11	11	10	3
LDB2	11	10	2	0
LDB3	11	11	3	0
LDB4	11	10	3	1
LDB5	11	11	3	1

C 20s6s-Int	<5 BW	5-10 BW	10-15 BW	>15 BW
LDB1	11	11	10	2
LDB2	11	11	2	1
LDB3	11	11	3	0
LDB4	11	10	4	1
LDB5	11	11	3	1

5.5 Discussion

The primary findings of the study was that both the OI_BW and OI_fft, estimated via one representative vGRF curve were statistically greater for the 80s-Int compared to the 5s-Int and 20s2s-int protocols. This is not surprising given the more continuous protocol had a greater overall mean vGRF when all loading cycles were averaged together (results in Chapter Four, Table 4.3). Interestingly, when the OI_BW was compared between the intermittent protocols there was a 5% and 1% difference for the 5s-Int and 20s2s-Int respectively compared to the more continuous protocol. The magnitude of percentage difference for OI_fft between 80s-Int vs. 5s-Int, and 80s-Int vs. 20s2s-int was 28% and 21% respectively. This suggests that the frequency and rate of the load combined in the equation does provide more important information about the nature of the loading dose when using only one representative curve.

The OI_BW obtained from different impact exercises are compared in Figure 5.7. As most of the current studies have normalised the GRF to body weights using equation 1, we have also compared our data using equation 1.
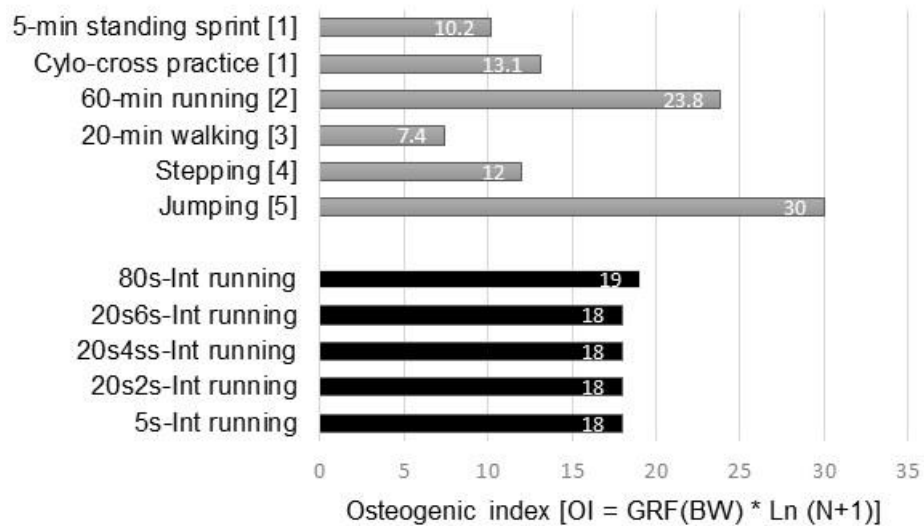
Figure 5.7 A comparison of the OI_BW calculated from the intermittent running protocols together with the calculated OI_BW from previous research using impact exercise. N.B. [1] (Tolly et al., 2014), [2] (Weeks & Beck, 2008), [3] (Turner & Robling, 2003), [4] (Santos-Rocha et al., 2006), [5] (Rantalainen et al., 2009).

The OI_BW obtained from the 45 min running protocols are much lower than the OI_BW obtained from jumping activities. Intuitively, this makes sense as jumping produces greater impact forces than running (Janz et al., 2003). The OI_BW obtained from 45 min of intermittent running is lower than the OI_BW obtained from the estimation from 60 min of running (Weeks & Beck, 2008). This might be due to the lower peak vGRF obtained whilst running on the NMT caused by the lower running speeds, generating lower GRFs (Weyand et al., 2000). Moreover, due to the nature of the NMT, the individual must apply greater propulsive horizontal GRFs to the treadmill belt in order to overcome its inertia (Brughelli et al., 2011). This moves the centre of mass (CoM) over the base of support and reduces the displacement of the (CoM), attenuating the peak vGRF (Brughelli et al., 2011) as also demonstrated in Chapter 4.

The mean OI_fft from all five protocols were lower than the mean OI_fft [110 (26)] reported by Rantalainen et al. (2009) during exhaustive bilateral jumping. This again provides evidence that the OI_fft and OI_BW are capable of distinguishing between discrete modes of exercise which

vary in their peak impact, even when less loading cycles are performed. Given the magnitude of the mechanical strain is related to greater gains in BMD (Vainionpää et al., 2007), this would suggest that the more continuous protocol in the current study provides a greater osteogenic stimulus. This might seem counterintuitive, as the more intermittent protocols produced greater peak speeds and produced a greater number (but not statistically) of higher peak vGRFs as a result, compared to the more continuous protocol (refer to Chapter 4, Table 4.3). Moreover, whilst the OI was higher for the 80s-Int, and this difference reached statistical significance it is not clear whether this would translate into a clinically meaningful change in bone.

Nevertheless, given higher impact loads are most important for bone adaptation (Martyn-St James & Carroll, 2010), and activities which produce greater impacts are associated with changes in bone geometry, even if these high impacts are rare (Deere et al., 2012), one might conclude that the OI calculated from a single GRF curve is misleading and incapable of distinguishing between intermittent loading protocols, contradicting the hypothesis of previous authors (Tolly et al., 2014). Therefore, longitudinal studies are warranted to determine the effect of various impact-exercises with standardised OIs on BMD. Furthermore, the OI may not be able to isolate the nuances of intermittent locomotion in its current form.

The current study challenges the use of the single OI measure and provides useful information on a new method for calculating loading intensity that was originally designed for accelerometers (Kelley et al., 2014). This study extends the previous data investigating the effect of speed of locomotion (Kelley et al., 2014) by comparing different running conditions which alternate between high speeds, generating high forces, and low speeds, generating lower forces. The observations demonstrate that whilst the magnitude of the loading dose between conditions is similar at lower intensities (< 5 BW·s^{-1}), the magnitude of the loading dose is greater at higher intensities, in higher frequency bands for the more intermittent protocols. Given bone responds to dynamic (Lanyon & Rubin, 1984), high frequency loading (Turner & Robling, 2003), the more intermittent protocols may be considered more osteogenic compared to the less intermittent

protocol. In previous studies (Chahal et al., 2014) greater movement speeds produced greater magnitudes of loading and the frequency of the loading dose contributed to the greater magnitude and intensity of the load. Consistent with these findings, the present study demonstrates that the magnitude of the loading dose was greater when the frequency band shifted towards the higher frequencies (24-30 Hz). Moreover, as no significant differences were observed between loading doses when the same exercise-to-rest interval was used, but the rate of the acceleration and deceleration was changed, it suggests that the peak running speed may be of more importance. Therefore, assessing multiple segments of the loading dose over different frequency spectra may be more appropriate at distinguishing between non steady-state exercise conditions. It is pertinent here to stress the inter-individual variability for the loading dose measures. Not all participants attained loading doses in high frequency bands (Table 5.4 A-D and Table 5.7) likely due to the differences in running styles and inter-individual differences in speed thresholds. Moreover, animal studies have demonstrated that whilst higher frequency loads are osteogenic (Hsieh & Turner, 2001), loads beyond 10-30 Hz did not result in any further change (Warden & Turner, 2004).

As the duration of the mechanical load is important for bone adaptation (Turner, 1998), the time spent at each loading intensity was calculated. The greatest time was spent at the lower intensities ($<5 \text{ BW·s}^{-1}$) which is consistent with previous studies (Chahal et al., 2014). Greater time spent at this intensity was shown not to be associated with improvements in BMD (Chahal et al., 2014). As the frequency increased, the duration of time spent in each intensity category also reduced. Interestingly, there was no significant difference between conditions for duration. As shorter loading bouts are more osteogenic than longer loading bouts, owing to osteo-desensitisation (Turner, 1998), only short periods of time in the high frequencies may be required to be osteogenic.

It is not clear how the calculation of the OI will reflect actual changes in bone remodelling. Currently, the reported correlations between the OI and markers of bone turnover are either small (Rantalainen et al., 2009) or non-existent (Lester et al., 2009; Erickson & Vukovich, 2010). The

effect of lifetime participation in various impact sports on bone properties has been quantified using the OI (Daly & Bass, 2006). High OI values correlated (standardised regression coefficients & 95% CI) with a greater mid-femur cortical area (0.16, 95% CI = 0.01 to 0.31), cortical bone mineral content (0.18, 95% CI = 0.04 to 0.33) and velocity of sound (m·s^{-1}) (0.22, 95% CI = 0.07 to 0.37) (from quantitative ultrasound of the calcaneous). Interestingly the duration of participation did not correlate with these parameters. The OI calculated via the segmented approach (OI_segFFT) correlated with strength and also calcaneal BMD in post- menopausal females (Chahal et al., 2014). In view of these aforementioned studies, the OI shows promise at dosing the osteogenic potential of various modes of exercise. However, the validity of the measure to establish a true response on bone is unknown and requires further examination in longitudinal randomised controlled trials.

At present the OI is based on forces imposed by gravitational loading rather than muscle forces. Given joint reaction forces deliver torsional shear stresses as well as axial compressive forces on the bone (Kohrt et al., 2009) compared to the compressive strain via gravitational forces, a combination of the two forms of loading would be beneficial to optimise bone adaptation (Kohrt et al., 2004) and should be included in the OI equation. A further limitation is that the OI does not include a measure of the individual's BMD which will likely cause intra-individual variation on the bone's response to mechanical stimuli. Finally the OI only includes a measure of vertical GRF, The combination of medial-lateral (ML) and anterio-posterior (AP) assessment of the force may also help to improve the sensitivity of the OI measure. Therefore, as an addition to the current equation the authors offer a possible alternative:

$$OI = \sum OI_{xGRF} + OI_{yGRF} + OI_{zGRF} \qquad (13)$$

Where OI$_{xGRF}$, OI$_{yGRF}$ and OI$_{zGRF}$ refer to the OI calculated from the peak ML, AP and vertical GRF using equation 3. This progression of the equation requires appropriate testing and as the ML data are often minute and inherently noisy, it may only offer useful information when cutting manoeuvres are included in the analysis.

The current study is also limited by the use of the NMT which uses strain gauges to measure forces which are limited in their ability to measure impacts. Whilst piezoelectric force plates are better adapted at measuring impacts, these devices are limited by continuous drift in the force data and therefore strain gauges provide far better long term stability. The body posture adopted on an NMT also requires a much greater forward lean and an affinity for the participant to forefoot strike. Thus, the NMT will likely attenuate the impact transients (Hagan et al., 2006) leading to lower loading doses across frequency bands. Moreover, the NMT does not allow multi-directional movements like those characterised in soccer. Multi-directional movements are likely to be important in promoting bone adaptation (Krustrup et al., 2010b). Finally, there is likely a large intra- and inter-individual variation in the loading dose which will be dependent on the running characteristics of each participant (Masani et al., 2002; Riva et al., 2014). For example, we demonstrated that only a certain number of participants were able to obtain loading doses in the higher frequency bands which was likely a combination of variability in running style, and speed. Nevertheless, the potential for the new method to distinguish between exercises which induce different loading magnitudes and frequencies is encouraging, providing a clear application in a real-word setting.

5.3.1 Practical applications and future recommendations

1. This OI_segFFT could be used in sporting situations in which accelerometers are used to dose training load.

2. Investigating the application of the use of the OI_segFFT over a longitudinal study to assess whether training enables individuals to spend longer at higher intensities and frequencies.

3. Investigation of the application of the OI_segFFT calculation on accelerometers placed in different positions to assess the loading dose at different loading sites.

4. The implementation of the OI_segFFT to assess its internal validity at assessing the osteogenic potential of intermittent loading patterns.

5.6 Conclusion

The OI obtained from a single mean representative vGRF value (OI_BW and OI_fft) is statistically higher for the less intermittent protocol compared to the most intermittent protocol. However, when the magnitude, intensity and frequency of multiple loading segments (OI_segFFT) are considered, intermittent exercise allows the individual to obtain higher magnitudes of loading dose, shifting towards a higher frequency band. The new method (OI_segFFT) may therefore be more appropriate to distinguish between loading characteristics of different activities. Moreover, more intermittent mechanical loading may induce different loading characteristics in both frequency and intensity despite similar mean speeds and work done.

CHAPTER 6:

The effect of intermittent running on biochemical bone turnover markers

6.1 Abstract

Aim: Intermittent exercise (e.g. soccer) has been reported to be more osteogenic than continuous exercise. However, the mechanisms responsible have not been established. The alternating high and low mechanical loads, allowing the mechanostat to resensitise, might enhance the osteogenic potential of intermittent exercise. Whilst brief continuous load-bearing exercise increases bone remodelling in favour of resorption, it is unclear how intermittent exercise effects acute bone remodelling. Our aim was to investigate the effect of varying degrees of intermittent exercise on acute bone remodelling, as measured by bone turnover biomarkers.

Method: Twelve healthy males (mean age 23 (4) years, height 179.6 (4.4) cm, body mass 79.7 (7.0) kg) completed one control protocol (no exercise), and three 45 min intermittent running protocols (5 s intervals [5 s], 20 s intervals [20 s] and 80 s intervals [80 s]) over total of 4 weeks. Protocols were matched for total distance and mean speed, on a Force 3 non-motorised treadmill. Venous blood samples were collected at the same time of day following a 12 h fast at baseline (BASE), 1 h, 2 h and 24 h post-exercise. Carboxyterminal crosslinked telopeptide (CTX-I) and procollagen type 1 amino terminal propeptide (P1NP) were used as markers of bone resorption and formation, respectively. Data are presented as mean difference (MD) and 95% confidence intervals. Raw mean data were analysed using a two-way univariate repeated measures ANOVA.

Results: There was a significant main effect for time ($P = 0.0001$), condition ($P = 0.032$) and a significant condition by time interaction ($P = 0.001$) for CTX-I. At 1 h the 20 s-Int and 5s-Int conditions were higher than the control condition (20s-Int: MD = 0.351, 95% CI = 167 to 534, $P = 0.0001$; 5s-Int MD = 0.221, 95% CI = 0.038 to 0.405, $P = 0.010$ respectively). The 80s-Int was not statistically higher than the control at 1 h (MD = 0.106, 95% CI = -0.018 to 0.349, $P = 0.099$). The 20s-Int was statistically higher than the 80s-Int (MD = 0.185, 95%CI = 0.008 to 0.362, $P = 0.035$). At 2 h post all exercise conditions remained higher but not statistically. There was no statistically significant interaction for P1NP.

Conclusion: The results confirm that bone remodelling is stimulated acutely by load-bearing exercise, in favour of resorption, attenuating the normal decline in diurnal response in CTX-I, which lasts up to 1 h post exercise This decline is more apparent for shorter (5 s and 20 s) intermittent exercise-to-rest durations. Therefore, shorter intermittent exercise intervals may stimulate more bone turnover acutely despite similar mean speeds, exercise durations and similar external work.

6.2 Introduction

The findings from study two suggest that the mean loading dose when all cycles are considered is similar across the 3 exercise conditions. However, the intra-step variability in the loading dose is greater for the severe and moderate intermittent running conditions. It is not clear from these studies how the varied mechanical loading conditions might affect acute bone turnover and physiological responses.

To assess the osteogenic response of different exercise conditions we must obtain an objective measure of the turnover of bone tissue properties. Changes in bone remodelling may take between $6 - 12$ months for discernible changes in bone mineral density (BMD) to be detected using current scanning technologies (Kemmler & Engelke, 2004). The development of biochemical bone turnover markers (BTMs), which reflect the activity of osteoblast (formation) and osteoclast (resorption) cells, offers a unique opportunity to assess the mechanisms of the bone's response to exercise (Seibel, 2005; Banfi et al., 2010).

To date, BTMs have been used to assess the acute responses to various modes of exercise including endurance running (Scott et al., 2011; 2013), jumping (Rantalainen et al., 2009; Rogers et al., 2011), cycling (Guillemant et al., 2004; Barry & Kohrt, 2007), resistance exercise (Ashizawa et al., 1997; Whipple et al., 2004) and whole body vibration (WBV) (Bemben et al., 2015). Bone turnover markers appear to be intensity (Scott et al., 2011), duration (Kristoffersson et al., 1995; Woitge et al., 1998), and exercise type specific - hence responses in the literature are non- uniform. However, continuous impact (Scott et al., 2011) and non-impact (Barry & Kohrt, 2007) exercise modes have been shown to induce acute changes in bone remodelling, in favour of resorption, up to 1-2 h post exercise.

Recently, the effect of intermittent exercise on BTMs has been investigated (Mezil et al., 2015) demonstrating an increase in bone turnover response from baseline values, in favour of formation. However, the study design did not include a comparison between different exercise-to-rest

durations of intermittent exercise. Given intermittent exercise can impose different physiological and metabolic stresses depending on the duration and frequency of the exercise-to-rest intervals (Laursen & Jenkins, 2002; Akubat & Abt, 2011; Tschakert & Hofmann, 2013), determining the effect of different exercise-to-rest durations using a fixed ratio (1:):1) might facilitate the development of intermittent training programmes providing relevant information on the effect of different exercise interval durations on biochemical responses of the musculoskeltal system which are lacking in the "programming puzzle" (Buchheit & Laursen, 2013b). Furthermore, the study by Mezil et al. (2015) failed to compare data to a non-exercising control condition. Therefore, it is unclear whether the post-exercise changes were due to the exercise stimulus, or circadian variation in BTMs. Finally, whilst not a limitation, the study used a low-impact mode of exercise (cycle ergometry). Cycling produces mainly concentric muscle activation whereby the muscle shortens during its action (Bijker et al., 2002). However, running intermittently involves more eccentric muscle activation whereby the muscle lengthens during its action (Greig & Siegler, 2009), potentially increasing the forces acting on the bone (Hawkins et al., 1999; Bodor & Jarosz, 2015). As such, the non-load-bearing exercise mode would not be comparable to intermittent load-bearing activities. The effect of intermittent exercise with varying durations of exercise-to-rest intervals on markers of acute bone metabolism therefore requires further investigation.

Therefore, the primary aim of the study was to establish the acute effect (and magnitude of response) of intermittent exercise incorporating moderate-high impacts, varying in the duration of the exercise-to-rest interval with a fixed ratio and matched for mean speed, distance and duration, on traditional bone turnover markers compared to a non-exercising control condition. It was hypothesised that protocols with greater intermittency (e.g. greater frequency of exercise-to-rest intervals) would increase the osteogenic effect of exercise, and increase acute bone turnover compared to the more continuous exercise protocols.

6.3 Methods

Participants

This is the same group of participants as outlined in Chapter 4 (Section 4.2). Briefly, 22 healthy males were initially recruited to the study (Figure 6.1). However, seven participants failed to complete the full 45 min exercise session, terminating the exercise due to exhaustion on at least one occasion. Therefore, these participants were excluded from the final analysis. One participant was excluded in week one as they were a casual smoker, and one participant failed to complete the study due to an injury incurred outside of the confines of the study, one participant was deemed inappropriate for the study as they did not perform enough exercise per week as specified in the inclusion criteria. Therefore, 12 healthy male participants (mean age 23 (4) years, height 179.6 (4.4) cm, body mass 79.7 (7.0) kg) successfully completed all testing sessions. The inclusion criteria for participants was as follows: participants were non- smokers, between the age of 18-30 years, had not recently (last 12 months) suffered from a broken bone or fracture, were not regularly ingesting non-steroidal anti-inflammatory medication, or other medications that may affect bone metabolism, and participated in at least three sessions of weight-bearing exercise per week including both continuous and intermittent forms of exercise. The study was approved by the Departmental Ethics Committee and conformed to the Declaration of Helsinki. All participants provided written informed consent after having all experimental procedures explained to them both verbally and in writing.
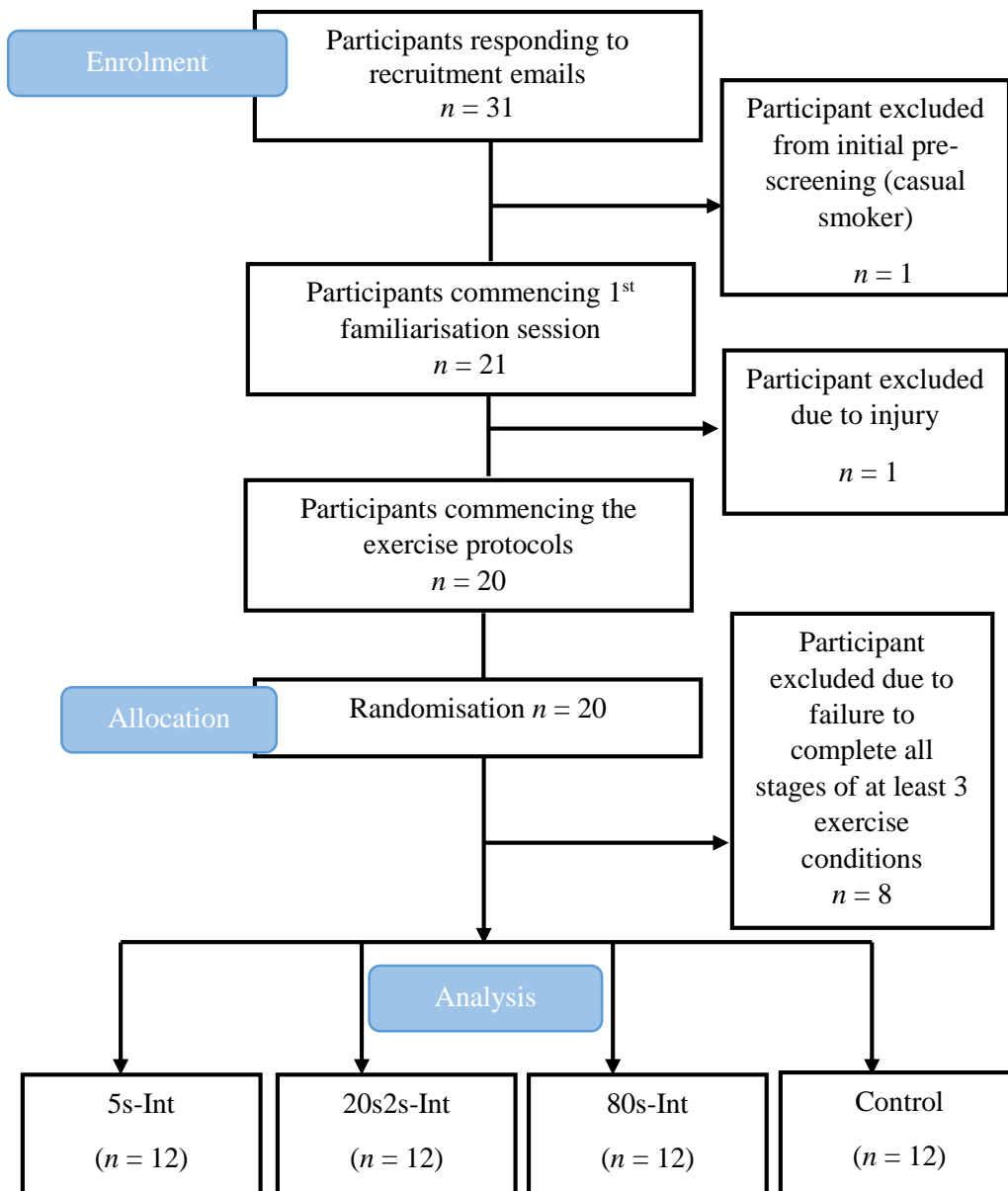
Figure 6.1 Participant recruitment and total *n* included in all testing conditions.

Design

Participants attended the exercise physiology laboratory over a seven-week period (Figure 6.2).

However, the inclusion of data from the final two exercise visits was not required for the purpose

of this study which used only the first three exercise trials and one non-exercise control. The final two exercise conditions were not included because bone turnover markers could not be analysed for these two trials. Moreover, study 2 and 3 demonstrated a very similar loading environment between conditions and therefore the magnitude of changes in bone turnover are likely to be similar. In week one, participants completed three preliminary testing sessions, consisting of medical screening, habituation to the non-motorised treadmill (Woodway Force 3, Woodway Ltd), assessment of the participant's maximal aerobic fitness ($\dot{V}O_{2max}$), velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$), and completion of a three-day food diary using the MyFitnessPal application. All participants reported the food diaries in the 3 days before the first experimental week and in-between week 3 and 4. In the following weeks, participants completed three 45 min exercise trials, of varying exercise-to-rest intervals, and one non-exercise trial (control). Protocols were performed over a four-week period to allow for appropriate wash-out between exercise trials. The design followed a randomised within-subject crossover design.

To ensure adequate control of confounding factors on bone markers, participants were required to adhere to the following pre-exercise guidelines:

- 12 h fasted state prior to each visit (Vasikaran et al., 2011a).

- Avoid any exercise at least 48 h prior to the testing session and limit physical activity the morning of the testing session (Vasikaran et al., 2011a).

- Light-moderate walking may be performed on the previous day prior to testing.

- Remain in a euhydrated state by drinking to thirst.

- Avoid alcohol and any psychoactive substances at least 24 h prior to the exercise testing session.

Participants attended the laboratory at the same time of day for all four protocols to control for the effects of diurnal variation; two participants were tested in a day and were staggered by approximately 1 h, thus, participants completed their pre-exercise (BASE) blood between the hours of 06:30-08:00. Consequently, 2 h post exercise blood samples were obtained between

10:00-11:30. Therefore, the intra-individual differences were controlled for because the participant had the blood taken at the same time of data for all conditions and all time points. However, because of the staggered design the inter-individual differences were less well controlled with a 1 h delay for certain participants. On attending the laboratory for the exercise visits, the participants signed the pre-exercise questionnaire and provided verbal consent to commence the testing procedure. The researcher also obtained verbal confirmation that the participants were 12 h fasted.
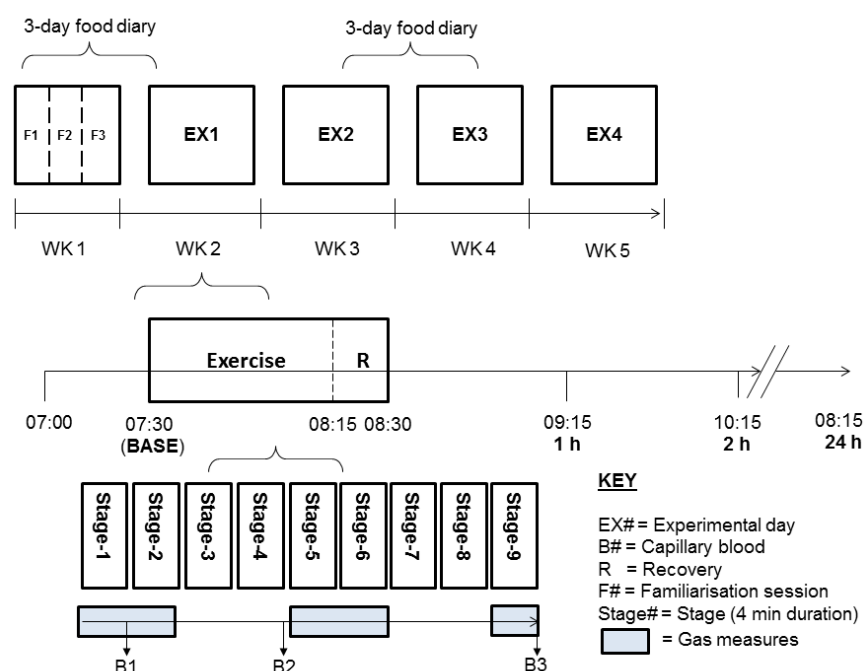
Figure 6.2 The timing of each blood sample in relation to exercise, and the timing of blood and physiological measures during each exercise trial.

Exercise testing

The exercise protocols have been fully described in the Methods section of Chapter 4 (Section 4.3).

Dietary analysis

A three-day food diary was completed on three separate occasions over a seven week period. Participants were requested to record all food and drink consumed over three consecutive days.

The use of a smartphone (iPhone or Android) or iPod that had access to the MyFitnessPal application (MyFitnessPal Inc) was used, and all products were recorded directly by the participant into the app. Participants were then required to send the list of micro and macro-nutrients consumed each day, total calories, and the percentages of fat, carbohydrate and protein. An example of the data can be seen in Appendix 1.

Oxygen consumption and heart rate measurement

Respiratory data were recorded using an online gas analyser (Oxycon Pro, Jaegger, Hoechberg, Germany). The set-up and calibration of the equipment has been outlined in the Methods section of Chapter 3. Respiratory data were collected over three phases during the 45 min exercise session: 0-9 min, 25-34 min and 40-45 min. Pilot testing revealed that ratings of perceived exertion were higher when wearing the face mask, and so to increase the completion rate of the full 45 min protocol it was deemed appropriate to not continuously measure respiratory data throughout the entire protocol. Heart rate was monitored continuously throughout exercise by a Polar chest strap, with heart rate sent wirelessly to the Oxycon Pro during the same collection periods. Oxygen consumption and heart rate data were obtained directly from the Oxycon Pro with a 60 s mean calculated for each minute. The 60 s means were pooled together to obtain a global mean for each 4 min exercise phase.

Biochemical analysis

Venepuncture was performed using a butterfly needle to obtain blood samples from the vein, situated near the antecubital fossa of either the right or left arm. Six, 5 microliter (μL) samples of blood were collected in glass vacutainers, 30 min prior to exercise (BASE), 1 h, 2 h and 24 h post exercise. Three samples were collected in red serum separating tubes, were gently inverted five times, and then left to clot at room temperature for 30 min. Samples were placed in a centrifuge and spun at 1800 g for 10 min at 4°C. The remaining three samples were collected in purple top, Ethylenediaminetetraacetic acid (EDTA) tubes, were gently inverted 8-10 times and were

immediately placed into a centrifuge and spun at 2500 g for 15 min at 4°C to obtain the blood plasma. Plasma and serum samples were aspirated from the vacutainers, with 500 µL of the sample alloquoted into two separate 1000 µL cryotubes and stored immediately at -80°C for analysis (range, 6-12 months storage time).

Plasma was used for the measurement of C-terminal telopeptides of Type I collagen (CTX-I) and procollogen type 1 amino terminal propeptide (P1NP) on the IDS-iSYS multi-discipline automated analyser. The IDS-iSYS intact P1NP and CTX-I assays are based on chemiluminescence technology. Given type I collagen makes up nearly 90% of the organic matrix of bone (Seibel, 2005; Wheater et al., 2013) an important step in bone formation and resorption is the synthesis and degradation of type 1 collagen. During synthesis, propetides are released from the amino- and carboxyterminal of the procollagen molecule. Conversely, the degradation is seen where small peptide fragments are released. These fragments are then secreted into blood circulation. Amino terminal propeptide of type 1 procollagen (P1NP) and CTX-I represent these processes and are therefore specific and sensitive markers for bone formation and resoption. Both CTX-I and P1NP are currently recommended for the measurement of bone resorption and formation respectively (Vasikaran et al., 2011a)

Samples of CTX-I were measured in singleton, but duplicate for P1NP. The reportable range of the IDS-iSYS CTX-I assay kit is 0.033-6.000 ng·mL^{-1}. The expected mean (95% CI) range for healthy males (fasted) is reported as 0.294 ng·mL^{-1} (0.115-0.748). Intra-assay precision for CTX-I ranged from 3.2 to 3.5%, with the inter-assay precision of the assay range from 4.4 to 5.3%. For P1NP the detectable reference range is 27.7-127.6 ng·mL^{-1}, the intra-assay precision was 3.4 -5.3% and the inter-assay precision range 3.9-5.5% (full information in Appendix C).

All blood markers (excluding pH and acid-base balance) were adjusted for plasma volume (PV), the corrected data are presented in the main text and the outcome from the ANOVA for the uncorrected data are presented in appendices D. Shifts in PV have been reported to affect the

response of biomarkers, including protein markers of bone turnover (Brahm et al., 1997a; Kargotich et al., 1998). In order to account for the shift in PV, as a direct response of the exercise, haematocrit (Hct) and haemoglobin (Hb) were obtained from both fingertip and venous blood samples. The venous samples were taken at the same time as the venous blood draw. A sample of blood was obtained from one EDTA tube and measured in duplicate for both Hct and Hb, with a mean taken from the two measures. Haemoglobin was measured on an automated haemoglobin analyser (Hemocue Ltd, Sheffield, UK). The method of microcentrifugation was used for the measurement of Hct. Blood. markers were adjusted for percentage changes in PV (%ΔPV) shifts using the following equations (Dill & Costill, 1974; Bemben et al., 2015):

$$PV\ change = \left[\left(100 - \frac{Hb_b}{Hb_a}\right) \times \frac{1 - (Hct_a - 100)}{1 - (Hct_b - 100)}\right] - 100 \qquad \textbf{Equation 1}$$

$$Corrected\ concentration = \frac{Uncorrected\ x\ 100}{100\ \pm PV\ change} \qquad \textbf{\textit{Equation 2}}$$

Capillary blood samples (finger-tip) were obtained at baseline, 4 min, 24 min and immediately post exercise for measurements of blood lactate, pH, glucose, bicarbonate and base excess. Blood was collected from the finger-tip in a heperanised capillary tube and run in singleton using a multi-array blood gas and electrolyte analyser (Radiometer, West Sussex, UK).

6.3.3 Statistical analysis

Data are reported as means and standard deviations. The precision of the population estimate of the outcome statistics is shown using 95% confidence intervals. A one-way repeated measures ANOVA was used to detect differences between data across three time points for the food diaries. Due to one missing venous sample (participant number 2, control, 1 h), and two missing capillary samples, all blood data were analysed using a univariate two-way (condition by time) repeated

measures ANOVA. Subject ID was used as a random effect factor, with condition and time as the fixed effects factors. The pairwise comparisons were adjusted using a Sidak adjustment for multiple comparisons. The studentised residuals from the repeated measures ANOVA were checked for normality using Q-Q plots and histograms. Where data failed to meet assumptions of normality and uniformity, data were corrected using log transformations. An analysis was performed on the raw data for both plasma volume corrected and uncorrected data. Bone turnover marker data are presented as a raw mean values and percentage change from baseline as performed by previous authors (Brahm et al., 1997a). All analysis was carried out using the statistical software package SPSS (Version 22.O for Windows; SPSS Inc, Chicago, IL, USA).

6.4 Results

Participant characteristics have been reported in Chapter 4, section 4.4.

6.4.1 Food diaries

Dietary data for the three-day food diaries is presented in Table 6.1. There were no significant differences between pre, mid and post intervention for the macro nutrients (fat, carbohydrate and protein), calcium or total calories (kCal) consumed. However, the variance (% CV) in the estimates of the dietary intake over the three days was very large.

Table 6.1 The micro and macro nutrient data as mean (SD), coefficient of variation (% CV), raw mean difference (MD), 95% confidence intervals and P values across the three time points. (n = 12).

	PRE			MID			POST			Pre Vs. Mid		Pre Vs. Post		Mid Vs. Post	
	Mean (SD)		CV	Mean (SD)		CV	Mean (SD)		CV	MD [95% CI]	P value	MD [95% CI]	P value	MD [95% CI]	P value
Fat (g)	81	(19)	34%	83	28	27%	79	(18)	35%	-2 [-25, 21]	0.865	3 [-15, 20]	0.745	4 [-15, 24]	0.632
CHO (g)	296	(81)	21%	292	85	19%	269	(47)	22%	5 [-39, 48]	0.821	27 [-18, 73]	0.214	23 [-39, 85]	0.439
Protein (g)	115	(46)	28%	117	48	27%	113	(36)	29%	-2 [-21, 18]	0.853	1 [-18, 19]	0.954	2 [-20, 24]	0.827
Total cal (kCal)	2388	(413)	15%	2409	524	9%	2264	(328)	15%	-21 [-282, 240]	0.861	124 [-209, 456]	0.431	145 [-300, 589]	0.489
Calcium (% RDA) [range]	74 [6, 221]	(72)	55%	78.9 [9, 245]	(76)	65%	71 [8, 168]	(50)	58%	-5 [-50, 41]	0.830	4[-27, 34]	0.787	8 [-38, 55]	0.698

Notes: grams (g), carbohydrate (CHO), recommended daily allowance (RDA).

6.4.2 Cardiorespiratory data

The mean and SD for HR and % $\dot{V}O_{2max}$ are displayed in Figure 6.3 A and B, respectively. There was no significant main effect for condition for HR [F $_{(2, 22075)}$ = 2.002, P = 0.159] or % $\dot{V}O_{2max}$ [F $_{(2, 22.038)}$ = 2.303, P = 0.124]. However, there was a significant main effect for time for HR [F $_{(4, 46.088)}$ = 23.910, P = 0.0001] and % $\dot{V}O_{2max}$ [F $_{(4, 45.955)}$ = 4.087, P = 0.006]. There was no significant condition by time interaction for HR [F $_{(8, 83)}$ = 1.405, P = 0.207] or % $\dot{V}O_{2max}$ [F $_{(8, 85)}$ = 1.681, P = 0.115]. The mean difference for % $\dot{V}O_{2max}$ for the time points (1-5) between the 80s-Int vs. 20s2s-Int were not significantly different (-3.3, -3.0, -4.9, -4.4, 1-5 % $\dot{V}O_{2max}$, respectively). Similarly, the mean difference (% $\dot{V}O_{2max}$) across the five time points between 80s-Int and 5s-Int were also not significantly different (-5.6, -3.9, -4.6, -4.6, 0.5 respectively).
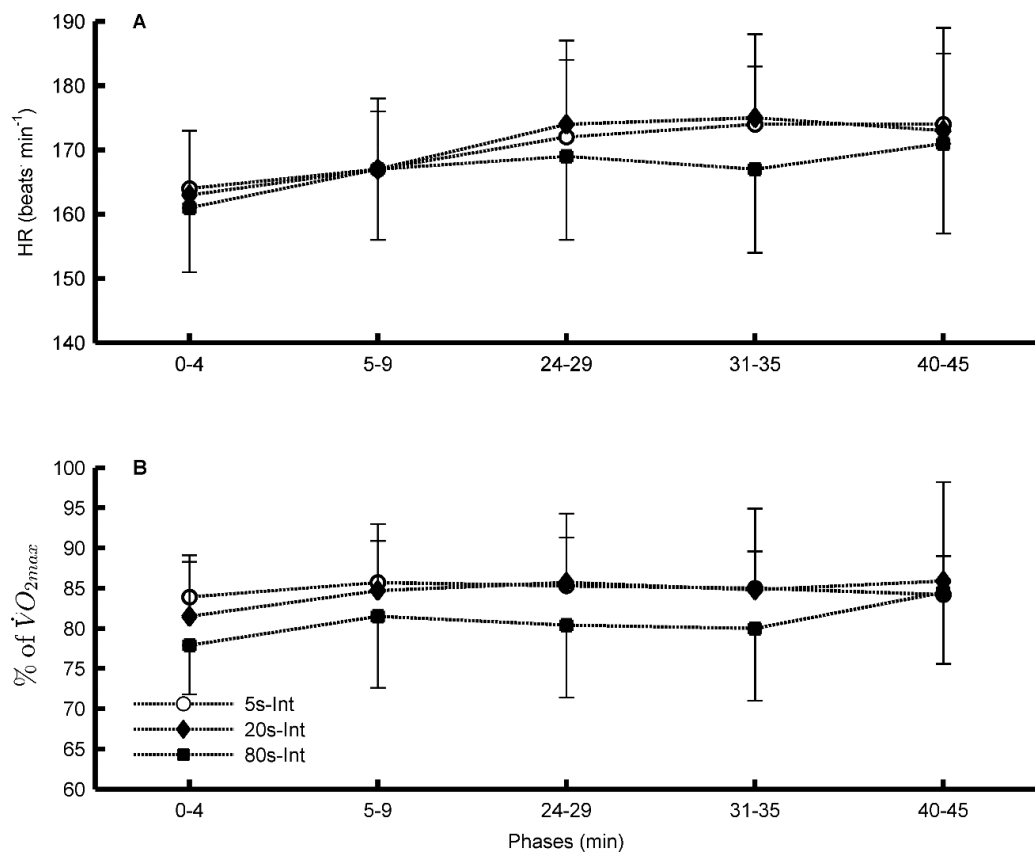
Figure 6.3 The mean (SD) for HR (Panel A) and % $\dot{V}O_{2max}$ for the five 4 min phases.

6.4.3 External training load

Data for mean speed ($km \cdot h^{-1}$), distance (m) and work (kJ) have been presented previously in Chapter 4 (Section 4.3).

6.4.4 Metabolic responses (capillary blood samples)

Plasma Volume

Plasma volume change from venous blood [mean (SD)] is presented in Figure 6.4. There was a significant main effect for time [$F_{(2, 22)} = 18.763$, $P = 0.0001$]. However, there was no significant main effect for condition [$F_{(3, 33)} = 1.779$, $P = 0.170$], nor a significant condition by time interaction [$F_{(6, 66)} = .528$, $P = 0.785$].
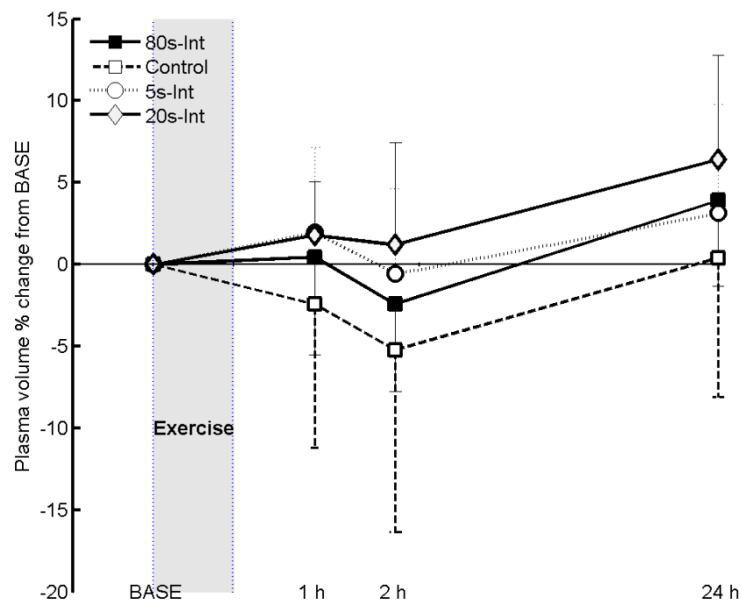
Figure 6.4 Mean (SD) for plasma volume (% change) responses at BASE, 1 h, 2 h and 24 h post exercise.

Blood lactate [Bla-]

The means (SD) for corrected blood lactate are presented in Figure 6.5. There was a significant main effect for time [$F_{(3, 33.107)}$ = 28.819, P = 0.0001], condition [$F_{(2,22.073)}$ = 4.478, P = 0.023], and a significant condition by time interaction [$F_{(6,64)}$ = 2.812, P = 0.017].. At 4 min the 5s-Int (4.5 [1.4]) was lower than the 20-Int (5.7 [1.5]), (MD = -1.1 mM·L^{-1}; 95% CI = -2.2 to -0.18; P = 0.045). At 24 min the 5s-Int (4.3 [2.0]) was lower than the 20s-Int (6.0 [2.7]), (MD = -1.8 mM·L^{-1}; 95% CI= -2.9 to -0.7; P = 0.001) and the 80s-Int (6.8 [4.1]), (MD = -2.5 mM·L^{-1}; 95% CI = -3.6 to -1.4; P = 0.0001). The blood lactate concentration at 45 min (immediately post exercise) remained lower for the 5s-Int (4.3 [2.5]) vs. the 20s-Int (6.0 [3.0]), (MD = -1.8 mM·L^{-1}; 95% CI = -2.9 to -0.7; P = 0.001) and vs. the 80s-Int (6.5 [4.3]), (MD = -1.6 mM·L^{-1}; 95% CI =-2.7 to -0.5; P = 0.002).
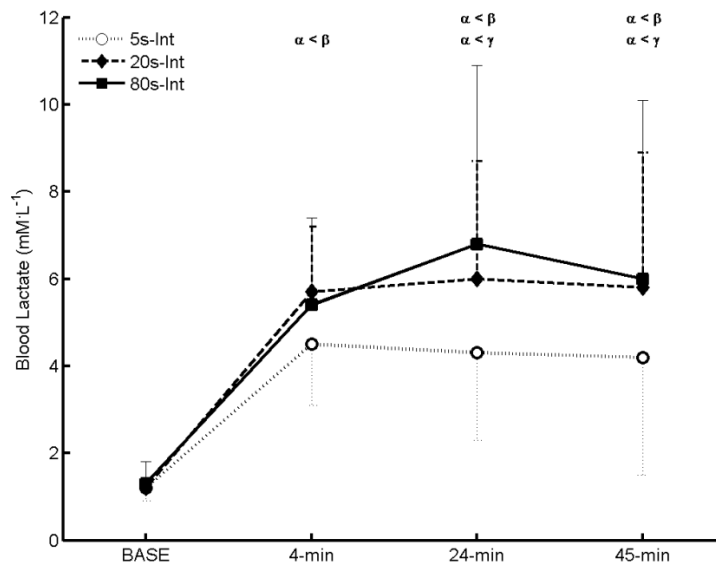
Figure 6.5 Mean (SD) blood lactate (mM·L^{-1}) responses during the 3 exercise conditions; 5s-Int (α), 20s2s-Int (β) and 80s-Int (γ). Symbols denote a statistically significant interaction between the conditions; alpha level was set at P < 0.05.

Blood glucose

The mean (SD) for blood glucose is presented in Figure 6.6. There was a significant main effect for time [$F_{(3, 33.186)}$ = 12.515, P = 0.0001] across all time conditions. There was no significant main effect for condition [$F_{(2, 22.088)}$ = 2.310, P = 0.123] nor a significant condition by time interaction [$F_{(6, 65)}$ =0.945, P = 0.469].
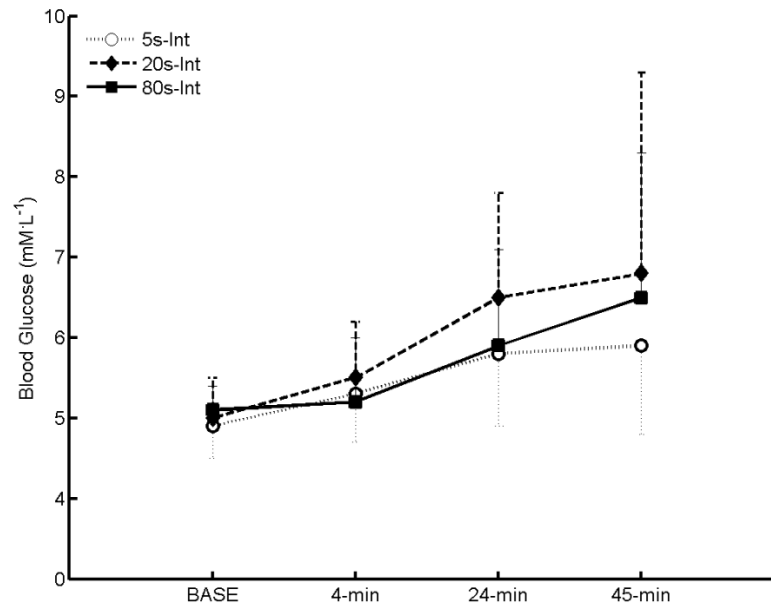
Figure 6.6 Mean (SD) for blood glucose (mM·L^{-1}) responses during the 3 exercise conditions; 5s-Int (α), 20s2s-Int (β) and 80s-Int (γ). Symbols denote a significant interaction between the conditions; alpha level was set at P < 0.05.

There was a significant main effect for time [$F_{(3, 33.073)}$ = 17.818, P = 0.001] but not for condition [$F_{(2, 22.021)}$ = .696, P = 0.509]. However, there was a significant condition by time interaction [$F_{(6, 65)}$ = 3.299, P = 0.007]. At 24 min and 45 min pH was significantly lower for 80s-Int compared to 5s-Int (MD = 0.024; 95% CI = 0.006 to 0.044; P = 0.006, and MD = 0.024; 95% CI = 0.006 to 0.031; P = 0.007, respectively).
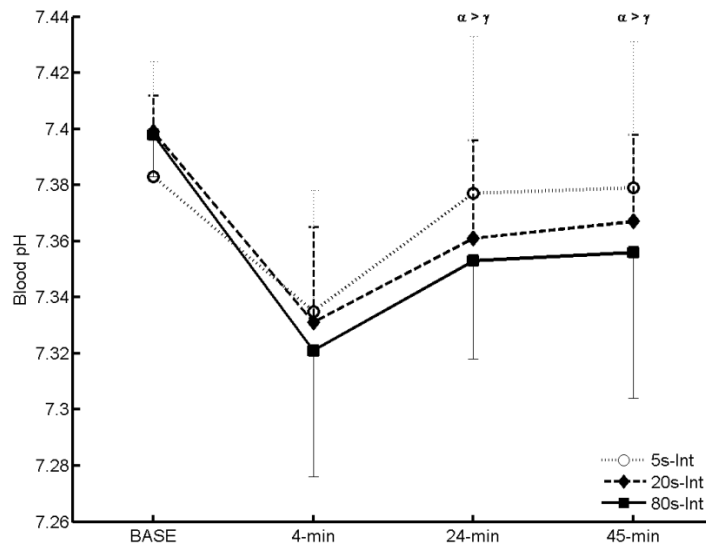
Figure 6.7 Mean (SD) for blood pH responses during the 3 exercise conditions; 5s-Int (α), 20s2s-Int (β) and 80s-Int (γ). Symbols denote a significant interaction between the conditions; the alpha level was set at $P < 0.05$.

Bicarbonate [HCO3-] and Base excess

Mean (SD) for Base excess & HCO3- are presented in Figure 6.8 A and B respectively. There was a significant main effect of time for both Base excess and HCO3- [$F_{(3, 33.279)} = 34.556$, $P = 0.0001$, and $F_{(3, 33.279)} = 34.159$, $P = 0.001$ respectively] but not condition [$F_{(2, 22.464)} = .818$, $P = 0.454$, and $F_{(2, 22.464)} = .871$, $P = 0.432$ respectively]. However, there was a significant condition by time interaction for both Base excess and HCO3- [$F_{(6, 62)} = 4.431$, $P = 0.001$, and $F_{(6, 62)} = 5.051$, $P = 0.0001$ respectively].
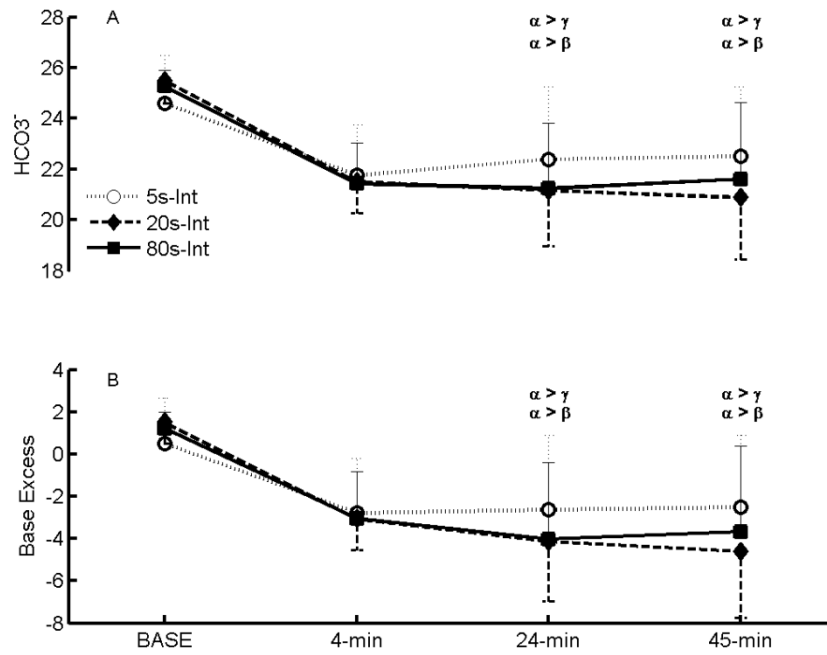
Figure 6.8 Mean (SD) for b HCO3- (top panel) and Base Excess (lower panel) responses during the 3 exercise conditions; 5s-Int (α), 20s2s-Int (β) and 80s-Int (γ). Symbols denote a significant interaction between the conditions. Alpha level was set at $P < 0.05$.

6.4.3 Bone Turnover Markers

All bone turnover markers were adjusted for PV changes. The unadjusted data were processed in an identical way to the adjusted blood markers. Only the adjusted data are presented as there were no differences in the outcomes of the ANOVA between adjusted and unadjusted data.

C- terminal teleopeptide of type 1 collagen (CTX-I)

There were no significant differences between baseline concentration values for CTX-I between the four conditions [5s-Int, 1.07 (0.47), 20s2s-Int, 1.2 (0.66), 80s-Cont, 1.04 (0.50) and Control, 1.12 (0.50) ng·mL^{-1}; $P = 0.227$].

There was a significant main effect for time [F (3, 33.059) = 17.978, P = 0.0001], condition [F (3, 33.039) = 3.321, P = 0.032] and a significant condition by time interaction [F (9, 98) = 3.460, P = 0.001]. Pairwise comparisons showed that at 1 h the 20 s-Int and 5s-Int conditions were higher than the control condition (20s-Int: MD = 0.351, 95% CI = 167 to 534, P = 0.0001; 5s-Int MD = 0.221, 95% CI = 0.038 to 0.405, P = 0.010 respectively). The 80s-Int was not statistically higher than the control at 1 h (MD = 0.106, 95% CI = -0.018 to 0.349, P = 0.099). The 20s-Int was statistically higher than the 80s-Int (MD = 0.185, 95%CI = 0.008 to 0.362, P =0.035). At 2 h post all exercise conditions remained higher but not statistically higher than the control condition. At 24 h post the control condition and the 20s-Int condition were statistically higher than the 80s-Int.
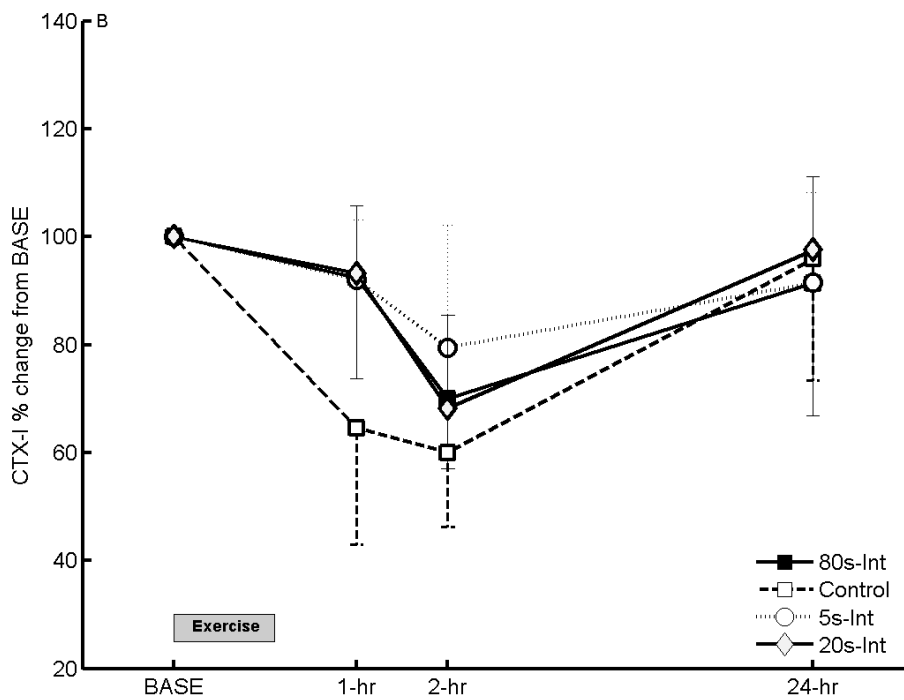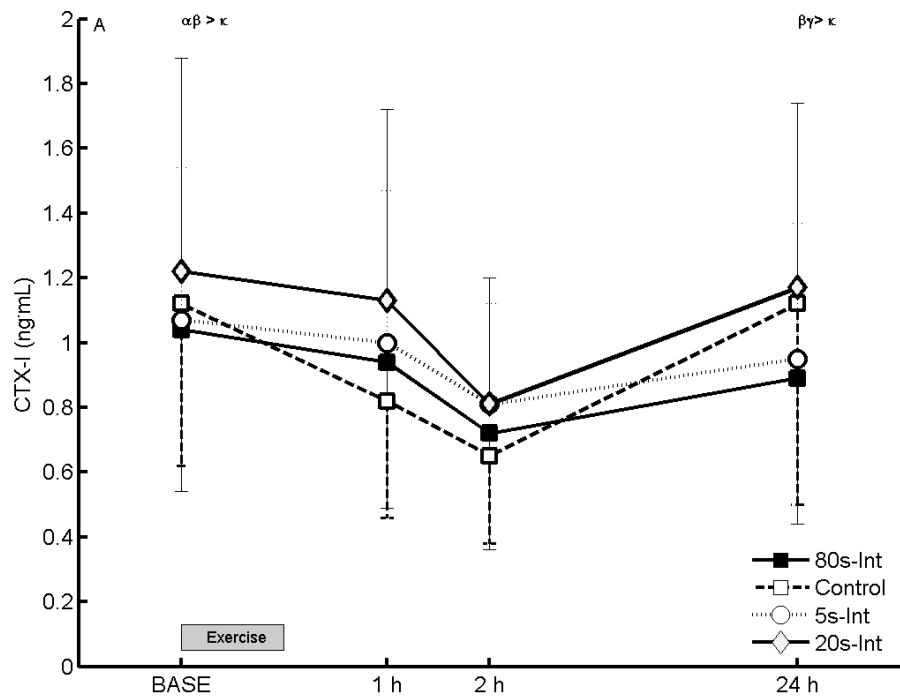
Figure 6.9 (A) displays the raw mean values for CTX-I. 5s-Int (α), 20s-Int (β) and 80s-Int (γ),

control (κ). Symbols denote a significant interaction between the conditions. Alpha level was set

at P < 0.05. Panel B displays data expressed as a percentage change from baseline.

Procollagen type 1 amino-terminal propeptide (P1NP)

Baseline concentration values for P1NP between the four conditions were as follows; 5s-Int = 102.5 (50.3), 20s2s-Int = 104.0 (57.6), 80s-Int = 103.7 (50.3) and control = 108.4 (53.3) ng·mL^{-1}]. P1NP Data were log transformed, the main effect for condition was not statistically significant [$F_{(3, 33.022)}$ = 0.253, P =0.859]. There was a statistically significant main effect of time [$F_{(3\ 33.097)}$ = 7.323, P = 0.001]. The condition by time interaction was not statistically significant [$F_{(9, 98)}$ = 1.365, P = 0.214].
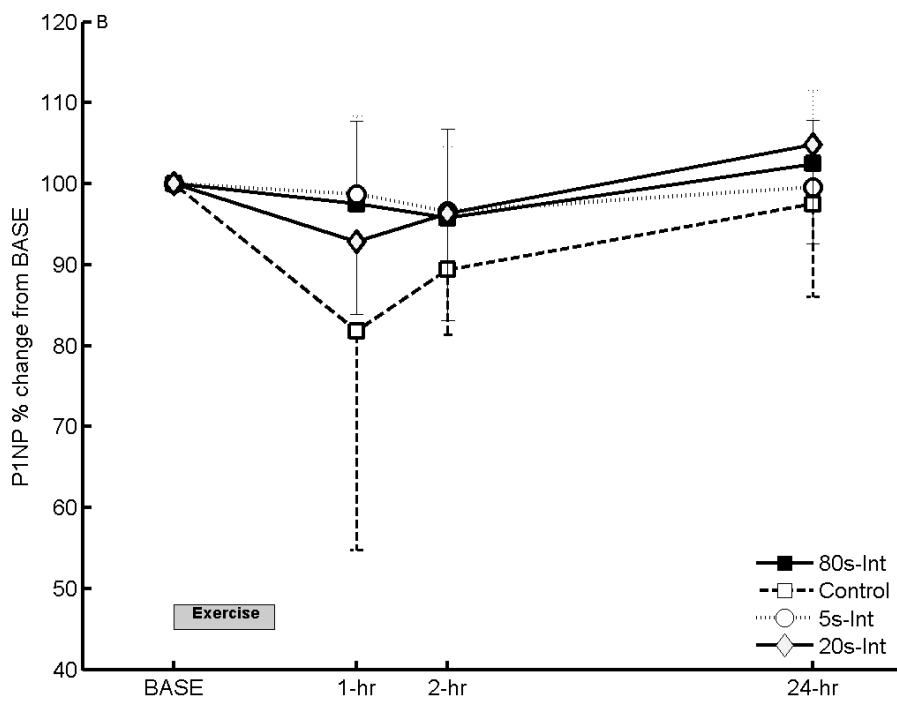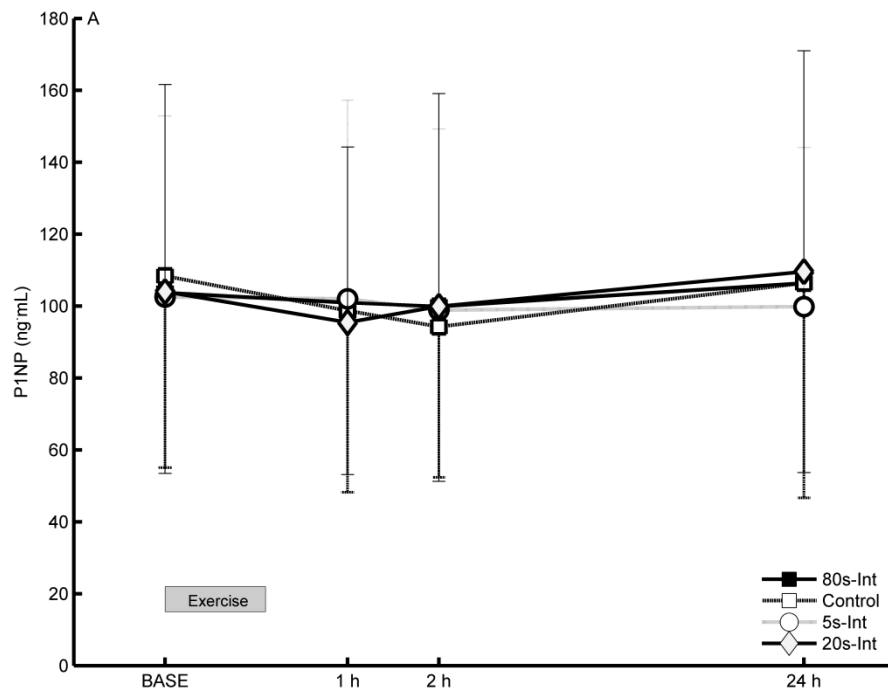
Figure 6.10 (A) displays the raw mean values for P1NP. Panel **B** displays the mean (SD) for % change from BASE for P1NP.

6.5 Discussion

The primary finding(s) of the study was that exercise had an effect on circulating concentrations of CTX-I, a biomarker of bone resorption, compared to the control condition. CTX-I was statistically higher at 1 h post exercise for the 5 s and 20 s conditions, compared to the non-exercising (control) condition. However, the 80 s conditions was not statistically higher than the control condition suggesting a greater effect of short interval intermittent exercise on circulating CTX-I concentration.

Exercise-induced elevations in CTX-I have been observed in previous studies investigating either endurance running (Scott et al., 2011) and plyometric jumping (Rantalainen et al., 2009) at 1 h post exercise. Whilst in our study the circulating concentrations of CTX-I for exercise were higher than the control, we did not observe an overall increase in CTX-I concentration above baseline. In contrast, Scott et al. (2011) observed a 3% elevation in CTX-I above baseline immediately post exercise which remained up to 1 h post exercise for the 75% $\dot{V}O_{2max}$ condition compared to the lower intensity (55% and 65% $\dot{V}O_{2max}$) conditions. As there was no control condition included in this study, it is not clear whether the 55 and 65% conditions still elicited an augmented response in CTX-I above normal diurnal variation. Previous authors, Guillemant et al. (2004) and Rogers et al. (2001) also observed a 45-50% and ~10% increase above BASE, respectively. The difference observed by the two latter studies can be explained by the provision of a standardised meal provided to all participants. It has been demonstrated that fasting attenuates the circadian rhythm of CTX-I (Clowes et al., 2002) and pre-feeding reduces CTX-I to its lowest diurnal point (Henriksen et al., 2003). Therefore, the provision of a meal 3 h prior would allow a full stimulatory effect of exercise on CTX-I concentrations.

It is unclear why Scott et al. (2011) observed an increase in CTX-I above baseline, and the present study did not. Our participant characteristics were very similar, and the exercise intensity used was similar (75% $\dot{V}O_{2max}$), if not higher for the present study. Potentially, the discontinuous nature of all the exercise protocols in the present study coupled with overall shorter exercise

durations (36 min excl. rest in our study compared to 60 min) may explain the finding. This suggests an interaction effect of exercise duration and intensity, and thus overall volume of exercise likely dictates acute responses of BTMs to exercise. Moreover, NMT treadmill running attenuates the vertical impact transients experienced while running, compared to motorised treadmill running (Hagan et al., 2006). Therefore, the ergometer may have attenuated the osteogenic potential of the exercise due to the attenuated impacts transients (Rantalainen et al., 2011a). As we demonstrated in Study 1, the speeds used to elicit the same relative oxygen consumption on an NMT would be much lower than the speeds used on an MT at the same relative intensity. Therefore, lower impacts may have been experienced in the current study compared to the previous study (Scott et al., 2011). Potentially the combination of higher impacts and longer durations at a high physiological intensity induces increases in CTX-I

Scott et al. (2011) reported a reduction from baseline of 39-42% from 2-3 h post-exercise. The ~40% reduction is similar to the reduction we observed in our control group at 2 h, and is consistent with circadian variation in CTX-I (Bjarnason et al., 2002) and other exercise studies (Zittermann et al., 2002) demonstrating a similar magnitude in reduction over a 4 h period. At 2 h post exercise circulating CTX-I was not statically different between conditions which is similar to previous studies showing a similar decline between conditions differing in intensity (Scott et al., 2011). When data are expressed as a change from baseline the 5 s intermittent condition demonstrates a smaller decline in circulating CTX-I compared to the control condition, and the other exercise conditions. Whilst previous studies have expressed data as a delta change from baseline (Brahm et al., 1997a) this might be a limitation as the authors would not account for the phenomenon of regression to the mean (Vickers, 2001).

The lower blood lactate concentrations, and metabolic acidosis associated with the very short exercise-to-rest intervals compared to longer exercise-to-rest intervals is supported by previous research (Christmass et al., 1999a; Belfry et al., 2012b) and likely reflects less anaerobic glycolytic load (Buchheit & Laursen, 2013a) compared with longer exercise-to-rest intervals. Moreover, the reduction in blood lactate could also be explained by the active recovery phases

since blood lactate is oxidised by the working muscles at lower intensities and there is a redistribution of lactate via blood flow changes (Gladden, 2004). The higher blood lactate accumulation with longer exercise intervals is also likely exacerbated by the enhanced anaerobic contribution exerted by running on the NMT (De Witt et al., 2009). The reduced metabolic acidosis, coupled with elevations in CTX-I for the more intermittent protocol, supports previous work demonstrating no association between blood lactate concentration and BTMs (Herrmann et al., 2007). This appears to confirm that metabolic acidosis does not stimulate osteoclast activity *in vivo*, disproving previous hypotheses (Ashizawa et al., 1997).

At 24 h post exercise, CTX-I concentrations were statistically higher for the control conditions and 20 s condition compared to the 80 s condition. Several previous authors have demonstrated a prolonged elevation in CTX-I (Kerschan-Schindl et al., 2009; Scott et al., 2010; Rogers et al., 2011). The longer duration, higher exercise intensities, and greater mechanical stresses imparted by ultra- endurance exercise (264 km run) could be the explanation for the prolonged elevations in CTX-I demonstrated by Kerschan-Schindl et al. (2009). However, it is not clear how well controlled the post exercise follow-up blood sampling was between participants. In the current study, the sampling time was well controlled. However, diet during this period was not controlled between conditions which could account for some variation in the data. Moreover, when expressed as a change from baseline the 24 h time points are not statistically different from each other. Therefore, the response could simply be due to diurnal variation and may be an artificial response due to the difference in baseline values between conditions.

The observations by Rogers et al. (2011) are likely the combined effects of greater mechanical strain imparted by plyometric exercise, coupled with the effects of prior feeding on CTX-I. Interestingly, Rantalainen et al. (2009) observed a delayed rise in CTX-I occurring 48 h post exercise. This again may have been as a result of the increased mechanical strain imparted by the continuous fatiguing plyometric exercise mode but is also coupled with poor control of diet during the time frame, and also poor control of sampling time points. Therefore, the data should be treated with caution. However, the nature of the exercise in which participants were required to

perform the exercise to volitional exhaustion is similar to the exhaustive intermittent running protocol by Scott et al. (2010) who also demonstrated prolonged elevated CTX-I levels beyond 24 h. Therefore, total energy expenditure may also be a contributing factor to increase bone turnover (Fong et al., 2013).

The increase in CTX-I in the hours following exercise suggests an increase in osteoclast activity. However, the clinical significance of prolonged osteoclast activity in response to acute exercise remains unclear. The responses on BTMs are driven by both endogenous (hormonal) and exogenous (mechanical loading) factors. For example, the increase in CTX-I may be as a result of a decrease in serum calcium in response to exercise stimulating an increase in parathyroid hormone (PTH) to maintain calcium homeostasis to which bone is an important calcium reservoir. Although this cannot be confirmed in the present study, previous research has demonstrated transient increases in PTH in response to exercise, peaking in the first few minutes of recovery following exercise (Scott et al., 2011; Townsend et al., 2016). In a well- controlled exercise study utilising a high temporal frequency of sampling time points Townsend et al. (2016) conclude that the combination of changes in ionized calcium and phosphorus during exercise and recovery might control changes in PTH. However, CTX-I was not measured in this study. However, the provision of a calcium rich meal prior to exercise has been shown to attenuate the exercise induced rise in PTH and CTX-I (Haakonssen et al., 2015)

With respect to the current study, comparisons between studies relating to BTMs should be made with caution as the lack of harmonisation of results from different laboratories is a limitation. For example, systematic bias exists for the IDS iSYS autoanalyser used in the current study when compared to Roche assays used in the aforementioned studies (Rantalainen et al., 2009; Rogers et al., 2011; Scott et al., 2011) and an ELISA calibrator (Chubb et al., 2015). The iSYS system has difficulty in detecting low bone turnover rates, conversely when values are above 0.3-0.5 ng·mL the iSYS gave higher values than Roche and ELISA methods (Chubb et al., 2015).

As P1NP reflects the synthesis of type I collagen, it was used as a measure of bone formation (Seibel, 2005). There was no statistically significant condition by time interactions for P1NP at any time point. This is supported by previous research which has shown no change in P1NP immediately after 30 min of walking (Tosun et al., 2006), cycling at 95% of the ventilatory gas exchange (Pomerants et al., 2008), and no difference from BASE following 60 min of continuous running at 55, 65 and 75% $v\dot{V}O_{2max}$ (Scott et al., 2011) or 60 min of continuous running followed by intermittent exhaustive running (Scott et al., 2010). However, Hermann et al. (2007) showed a decrease at 3 h post exercise, cycling for 60 min at 75% of the anaerobic threshold. The difference could be due to the difference in loading between exercise modes. Cycling is non-weight-bearing and consists mainly of concentric muscle activity whereas running involves weight-bearing eccentric activity (Bijker et al., 2002). Whilst we did not measure P1NP responses during exercise it is possible that P1NP concentration increased above BASE during exercise as demonstrated by Scott et al. (2011).

It is unclear whether an increase in P1NP reflects an increase in type 1 collagen bone formation. Potentially, prolonged blood sampling time points in the days following may demonstrate changes in P1NP, likely beyond 4 days (Scott et al., 2010). The time course of formation after mechanical loading has been well documented with new osteoblasts appearing on the bone surface 24-48 h after initiating mechanical loading (Turner et al., 1998). Finally, whilst the measure is sensitive as a marker of bone formation, it may also reflect the response of other tissues such as tendon (Langberg et al., 2000) and skeletal muscle (Crameri et al., 2004). However, it has been shown that these fragments are released quicker from bone and therefore other tissues contribute less to the circulating pool (Seibel, 2005).

6.6 Conclusions

The study confirms previous findings that moderate impact exercise has an acute effect on bone turnover, in favour of resorption, lasting up to 1 h post exercise. At 1 h post exercise both the 5 s and 20 s intermittent exercise conditions were higher; however, the 80 s intermittent condition

was not. Therefore, intermittent exercise of shorter exercise-to-rest intervals and greater frequency of changes in speed stimulated bone turnover more than longer exercise-to-rest intervals. If the prolonged concentration of CTX-I reflects increased osteoclastogenesis, the stimulatory effect may result in greater osteoblast activity in the following days. When prescribing intermittent exercise programmes for health and fitness benefits, the duration of the exercise and rest bouts must be considered based on the aim of the programmes.

CHAPTER 7:

Synthesis of findings, limitations, and future recommendations for further investigation

7.1 Synthesis of findings

This final chapter is intended to summarise the primary aims and findings from the individual studies, synthesising the contributions of the thesis and providing recommendations for future work. The thesis is the result of two separate randomised counterbalanced crossover studies using a group of healthy regularly active males. The objectives of the thesis were:

1. To develop intermittent protocols matched for external load (mean speed, distance and duration) on a non-motorised treadmill but which vary proportionally in the frequency of exercise-to-rest intervals and durations but use a fixed 1:1 exercise to rest ratio.

2. To investigate the effect of intermittent exercise of different exercise-to-rest durations on the magnitude and variability of kinetic and kinematic variables of running gait to determine how well the external load is controlled.

3. To assess the utility of the osteogenic index (OI) as a measure of the osteogenic potential of intermittent exercise and establish the use of a novel OI model which incorporates magnitude, frequency and intensity of mechanical loading across multiple loading segments using Fourier analysis.

4. To investigate the effect of intermittent exercise of different exercise-to-rest durations, but fixed exercise-to-rest ratios, on traditional biochemical markers of bone metabolism.

The central theme of the thesis was to investigate potential mechanisms to explain the positive effects of intermittent exercise (e.g. small-sided soccer games) on bone health as noted by previous authors (Helge et al., 2010). Due to the limitations of controlling external load (mean speed, work done, distance), and the unpredictable exercise-to-rest ratios characterised by small-sided soccer games, we attempted to develop intermittent protocols within a laboratory environment to investigate the possible mechanisms of action. Moreover, in a recent opinion

article (Boudenot et al., 2015) the use of interval or intermittent running was proposed as an alternative and potentially more favourable mode of running to overcome the bone fatigue associated with continuous running. As yet literature supporting the use of intermittent running for bone health is confined only to animal studies (Gross et al., 2004), which demonstrates a novel aspect of our work.

Study 1

The non-motorised treadmill (NMT) used throughout this thesis was chosen for a number of beneficial attributes detailed in Chapter 2 and Chapter 3 (see Table 2.3). Whilst the treadmill has been used extensively to assess physiological responses to intermittent protocols (Sirotic & Coutts, 2008; Aldous et al., 2014), at the time of completing this thesis there were no studies using high-intensity intermittent exercise on the NMT using percentages of the velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$) to dose the exercise intensity. Given this is a common method to dose the intensity of exercise (Buchheit & Laursen, 2013b), and enables the mean speed to be standardised across conditions, this approach was adopted in this thesis. The $v\dot{V}O_{2max}$ is obtained from a graded exercise test (GXT) (Midgley & Mc Naughton, 2006) performed on the target ergometer, in this case, the NMT.

However, there are few studies (Davies et al., 1984; Mauger et al., 2013) which have assessed peak cardiorespiratory responses on the NMT via a GXT. Both of the aforementioned studies demonstrated that whilst the NMT enabled exercisers to reach a similar (Davies et al., 1984) or greater (Mauger et al., 2013) $\dot{V}O_{2max}$ compared to a motorised treadmill (MT), there was a reduction in peak speed by ~ 30% on the NMT. Therefore, the NMT may not provide a valid environment to establish a maximal cardiorespiratory performance, especially if performing a continuous GXT. Moreover, the validity of a continuous GXT to establish reference speeds for intermittent exercise programmes has been questioned (Bangsbo, 1994; Castagna et al., 2005). This resulted in a number of important research questions to answer related to the NMT:

1. Could a similar or higher $\dot{V}O_{2max}$ be achieved on the NMT compared to the MT using a continuous GXT.

2. Could an intermittent GXT produce a similar or higher $\dot{V}O_{2max}$ together with smaller reductions in peak speed and peak heart rate?

Therefore, the primary aim of study one was to investigate the effect of both continuous and intermittent GXTs on a NMT compared to a continuous GXT on a motorised treadmill (MT) which is traditionally used to establish reference speeds and dose exercise intensity for exercisers who engage in running based exercise.. A similar $\dot{V}O_{2max}$ was obtained on the NMT using a continuous GXT when compared to the MT. However, $v\dot{V}O_{2max}$ was reduced by approximately 30%, with a very large reduction in time to exhaustion and a moderate reduction in peak heart rate (HR). When an incremental protocol was performed intermittently (15 x 15 s) on the NMT, a similar $\dot{V}O_{2max}$ can be achieved, together with smaller reductions in $v\dot{V}O_{2max}$, and peak HR. We postulated that the higher peak speeds achieved might be due to shorter peak exercise time (Bangsbo, 1994). Moreover, the active rest periods may enhance blood buffering capacity of hydrogen ions (H^+) and encourage replenishment of phosphocreatine stores (PCr) through enhanced oxidative phosphorylation (Billat et al., 2001). However, we did not have direct evidence for this to confirm our assertions and this warrant further investigation.

The longer exercise-to-rest intervals (30 x 30 s) resulted in greater reductions in $\dot{V}O_{2max}$ and peak HR. Longer exercise phases during intermittent exercise have been shown to result in greater reliance on anaerobic metabolism, resulting in greater metabolic strain and increased perceived exertion despite similar times to exhaustion (Price & Moss, 2007b). Our data supports this, as such, we decided to use the shorter 15 by 15 s NMT GXT for the assessment of $v\dot{V}O_{2max}$ which was used in the subsequent studies.

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In summary:

- The study is the first to utilise intermittent GXTs on the NMT.

- The study adds further support to the existing literature demonstrating the greater submaximal energetic requirements of NMT running when compared to an MT. This is important because higher physiological intensities can be reached at lower speeds. The lower speeds would suggest less impact on the musculoskeletal system in comparison to running at a similar exercise intensity on an MT or indeed over –ground which would lead to higher speeds and higher impacts.

- The study contributes to the existing literature related to the different physiological responses that occur as a result of manipulating the exercise-to-rest duration of intermittent exercise.

- An intermittent GXT (15 x 15 s exercise-to-rest intervals) may establish more appropriate reference speeds to dose future intermittent protocols.

Study 2

In both Chapter 1 and 2 we highlighted the importance of quantifying the osteogenic potential of exercise to facilitate the prescription of an appropriate exercise dose for the target population, which is yet to be defined (Tobias et al., 2014). The 'optimal' dose of exercise is unlikely to be universal given bone responds differently depending on sex, age, loading site, and genetics. Moreover, quantifying the mechanical loading environment of different exercise modes will help to establish the external load delivered to the musculoskeletal system. If the external load is well controlled, any differences in osteogenic response between conditions might be due to another variable.

The studies using small-sided soccer games failed to quantify the mechanical loading environment (Helge et al., 2010). Therefore, it is not possible to establish how the mechanical loading environment in small-sided soccer games might offer a greater osteogenic stimulus. To quantify the mechanical load, the strain or deformation of bone can be indirectly quantified using the surrogate measure of the vertical and horizontal components of the ground reaction force (vGRF, hGRF) (Ebben et al., 2010). Specifically, the peak of the vGRF, area under the v|GRF curve (vertical impulse) and loading rate of the vertical impact transient can be used to assess the magnitude and rate of strain (Ebben et al., 2010) imparted by the exercise on the musculoskeletal system. Both strain rate and magnitude are fundamental for bone remodelling (Turner & Robling, 2005b). Given intermittent running is characterised by brief periods of high-intensity loading interspersed with periods of low-intensity loading (Bangsbo, 1994), the mechanical loading environment for intermittent exercise may provide a more osteogenic response compared to continuous exercise (Turner & Robling, 2005b). However, this has not been fully investigated. Furthermore, if the mean speed and duration of the exercise conditions are well-controlled, the high-intensity loading phases may be counteracted by the low-intensity phases, and thus the loading environment would be similar.

Therefore, the aims of study 2 was to assess the mechanical loading environment of intermittent exercise that varied in the exercise-to-rest duration but was controlled for mean speed and exercise duration and thus similarly matched for distance and external work. The study was segmented into two experiments: the first investigated the mechanical loading dose elicited by intermittent exercise protocols of differing exercise-to-rest durations, while the second manipulated the acceleration and deceleration rate whilst controlling the duration of the exercise to rest interval.

We demonstrated that the higher-intensity loading phase was indeed counteracted by the lower intensity loading phase across conditions when the loading dose was expressed as a mean. However, there was greater intra-step variability for the more intermittent exercise conditions, likely due to the multiple changes in high and low speeds. Animal studies (Robling et al., 2000; Burr et al., 2002) have demonstrated the bone's unique phenotypic response to mechanical loads

which are variable and of a certain strain magnitude, and loading rate (Moreno et al., 2008). This variable loading environment induced by intermittent running could stimulate a greater bone remodelling response as bone responds to loads which are unaccustomed and variable.

In summary:

- This is the first study to quantify the mechanical loading environment of running conditions which vary in their intermittency through manipulation of the exercise-to-rest duration.

- Whilst the difference in mean forces were largely unremarkable, the greater variability in the mechanical loading dose may be a key factor in enhancing the osteogenic potential of intermittent exercise.

- This study adds to the current literature on running mechanics and the limited body of work on the NMT.

- This study also provides useful information on vertical and leg stiffness changes when running on an NMT

Study 3

Study 2 investigated the magnitude and loading rate of the mechanical loading environment. However, animal models have also demonstrated that mechanical strains of greater frequency (Hz) (Hsieh & Turner, 2001) enhance the osteogenic potential of the loading dose. There also appears to be an optimal threshold for the number of loading cycles which stimulate the osteogenic response (Umemura et al., 1997), which when surpassed the mechanical loads are no longer effective. The diminishing returns of multiple loading cycles should therefore be accounted for in the quantification of loading dose. Therefore, obtaining one single measure of mechanical loading

dose which combines frequency, magnitude, intensity (rate) and also accounts for the diminishing returns of multiple cycles would be advantageous for prescribing the optimal exercise mode.

To achieve this, the osteogenic index (OI) was developed which combines all the characteristics of mechanical load into one measure (Turner & Robling, 2003). The ability of the OI to distinguish between different intermittent mechanical loading environments has yet to be established. A novel calculation which utilises multiple loading segments assessing the contribution of frequency and intensity of load to the overall magnitude of load might be more effective at distinguishing between conditions (Kelley et al., 2014). Therefore, the aim of study 3 was to investigate the utility of the OI at distinguishing between intermittent exercise of different exercise-to-rest durations.

We demonstrated that the OI obtained from a single mean representative vGRF value was similar between conditions. This is likely due to the previously mentioned issues of using a mean representative value where the higher intensity loading is counteracted by the lower intensity loading. When the magnitude and intensity of multiple loading segments were calculated across a number of frequency bands (Chahal et al., 2014), intermittent exercise allows the individual to obtain higher magnitudes of loading dose in the higher frequency bands which corroborated previous findings (Chahal et al., 2014). However, this response is highly variable between individuals, with some individuals not obtaining loads in the higher frequency bands. This could be explained by differences in an individual's running gait and the differences between forefoot strikers compared to heel strikers (Lieberman et al., 2010). The greater loading dose in the higher frequency bands at higher loading intensities is likely caused by the greater running speeds (Kelley et al., 2014) creating greater peak vGRF. Moreover, it is not clear if higher frequencies beyond 10 Hz are more osteogenic for humans (Warden & Turner, 2004). These authors demonstrated no changes beyond 10 Hz in cells using a controlled *in vitro* experiment

In summary:

- This was the first study to utilise the segmented OI approach to different intermittent exercise conditions.

- This is also the first study to examine the utility of the OI at distinguishing between different intermittent mechanical loading environments.

- The study highlights the limitations of the OI at distinguishing between intermittent mechanical loading patterns and offers new insights for further research in developing the algorithm.

Study 4

In studies 2 and 3 the mechanical loading environment, assessed by the magnitude, rate and frequency of the loading cycles were quantified. Whilst the difference in the mean mechanical loading dose was largely unremarkable between conditions, the variability in speed, resulting in greater intra-step variability of the loading dose for the more intermittent protocols is interesting. Given bone responds to mechanical loading environments which are variable, and include higher peak impacts of a greater strain rate, even if those peaks are rare (Tobias et al., 2014), the intermittent protocols could induce a greater osteogenic response. Moreover, bone tissue also responds to the intensity of the exercise (Scott et al., 2011). Given manipulation of the exercise-to-rest duration can induce a different physiological 'stress' (Buchheit & Laursen, 2013b), it is pertinent to establish how exercise of various exercise-to-rest durations might affect bone metabolism acutely.

At the time of completing this thesis only one published study (Mezil et al., 2015) had assessed the acute effects of an intermittent protocol on markers of bone turnover. However, Mezil et al

(2015) did not compare the intermittent protocol to other modes of exercise or variations of intermittent exercise. Moreover, the study failed to compare to a control condition to establish the effect of exercise on circadian variation which is so important in these types of studies. Therefore, the aim of the final experiment was to investigate the effect of different exercise-to-rest durations using a 1:1 exercise-to-rest ratio on bone turnover markers (BTMs).

The results confirmed that bone remodelling is stimulated acutely by load-bearing exercise, in favour of bone resorption. At 1 h post exercise there was a greater bone turnover for the 5s-Int and 20s-int compared to the 80s-Int condition. Therefore, shorter more frequent exercise-to-rest intervals might result in a greater bone turnover. However, the mechanisms responsible for the elevation in CTX-I concentration are not clear.

The greater frequency of the acceleration and deceleration phases in the 5 s condition may have contributed to greater eccentric muscular actions and ground reaction forces generating higher torsional and bending loads on the bone (Milgrom et al., 2000; Bodor & Jarosz, 2015). Moreover, greater variability of the mechanical loading environment has been shown to offer osteogenic benefits, at least in animal models (Moreno et al., 2008; Wallace et al., 2013) and supported to a lesser extent in cross sectional human studies (Nilsson et al., 2013).

The inter-individual variability as demonstrated by the wide confidence intervals across conditions requires further investigation to assess the repeatability and reproducibility of the response. The use of the intensive protocols required to induce shifts in bone tissue would mean that the protocols would not be transferable to clinical, sub-clinical or elderly. However, the intensity of the protocols could be reduced and made relative to the specific population. Moreover, the protocols are designed to maximise bone adaption and accrual. Therefore, younger populations that would require and be able to tolerate higher intensities might benefit from high intensity intermittent protocols over more continuous load bearing protocols. It is likely that in

order to prevent poor bone health in later life, the performance of high impact exercise should be performed from a young age to maximise peak bone mass.

We believe that the use of the different intermittent exercise conditions combined with the non-exercising control group, which is lacking in the literature, adds novelty to our findings. Therefore, further work is required to firstly attempt to reproduce our findings and secondly to optimise the exercise-to-rest ratio to maximise the osteogenic response. Moreover, the clinical significance of the elevated CTX-I across exercise conditions is unclear. The enhanced bone turnover, in favour of resorption, suggests a catabolic effect on bone acutely after exercise (Falk et al., 2015). Whether this will lead to greater bone formation in the following days is unclear (Scott et al., 2013). However, as bone remodelling follows an activation → resorption → formation cycle (Robling & Turner, 2009) it is likely that the catabolic response should be followed by an anabolic phase. More studies are required to confirm this response.

7.2 General conclusion

In summary, when performing intermittent impact exercise of varying exercise-to-rest ratios the mean mechanical load, assessed via the vertical and horizontal components of the GRF, is similar across conditions. This is due to the higher intensity loads imposed by higher speeds being counteracted by the lower intensity speeds imposed by lower speeds. However, the variability in changes between the high to low speeds increases the intra-step variability of the mechanical loading components (magnitude, frequency and rate). Moreover, the inclusion of higher speeds and therefore higher intensity loading can result in a higher proportion of loading cycles entering into the higher frequency spectra. This variable loading environment with greater magnitudes of loading across a wider frequency band could provide a more favourable osteogenic environment for bone. However, the acute responses of bone remodelling, assessed via bone turnover marker changes, did not reveal detectable changes between exercise conditions in the recovery hours following exercise. However, this suggests that exercise has an effect on bone remodelling, in favour of resorption, which remains elevated for 2 h post exercise.

7.3 General limitations

There are a number of general limitations of note which should be addressed:

1. Reliability and reproducibility

Reliability, which relates to the magnitude of the measurement error (Hopkins, 2000), and repeatability, which refers to the variation in the repeat measurements made on the same participant under identical conditions (Hopkins, 2000), were not established for any of the primary outcome measures throughout the thesis. The lack of reliability and reproducibility was due in-part to time constraints due to the number of conditions included in each experiment. For example, in order to appropriately perform reliability testing for the first experiment we would have needed to assess the reliability of all 4 GXTs on ~20 participants, as recommended by Hopkins (2009). Similarly, the exercise protocols used in study 2 would require the same treatment. As such, reliability testing over a minimum of two repeats would need to be completed on five intermittent protocols on ~ $n = 20$. Whilst we had proposed to assess the reliability of the biomarkers to a number of different intermittent protocols, it was decided that the cost of completing the study did outweigh the benefits of completing such a study.

2. Sample size

The sample size for studies 2, 3 and 4 was smaller than initially anticipated. This was largely due to the number of participants who failed to complete the full 45 min exercise trial, terminating exercise early due to fatigue. Whilst we attempted to recruit more participants, the study design dictated a large commitment from participants requiring the completion of 15 separate visits to the laboratory over a 7-week period. The inclusion criteria of 18-30 years, Caucasian males, who participated in both intermittent and continuous exercise, meant that those individuals suitable for inclusion trained regularly. Participants were unwilling to commit to a study that disrupted their normal training programme.

3. The NMT

The use of the NMT likely introduced a number of limiting factors. Firstly, a truly continuous model of exercise was not included in this thesis due to the physiological and biomechanical constraints of running on the NMT. Secondly, the requirement to overcome the inertia of the treadmill belt puts a greater emphasis on smaller muscle groups (Brown et al., 2007) increasing the energetic requirement at submaximal exercise intensities and reducing running economy (De Witt et al., 2009). Moreover, as the belt begins to slow between stance phases, due to the inertia of the belt, the intra-stride variability is increased affecting the economy of movement. It is likely the unfamiliar environment of NMT running for some exercisers resulted in a large inter-individual variation for both physiological and biomechanical responses as demonstrated in Chapter 6.

The NMT is limited in its ability to assess impact as the maximum sampling frequency is only 200 Hz. Usually sampling frequencies of 1000-2000 Hz would be more appropriate to measure impacts (Winter, 2009). Moreover, the strain gauges are less sensitive to measure impact peaks. Piezoelectric force plates would be more appropriate (Winter, 2009) and maybe an avenue for future work. Moreover, the NMT was only capable of measuring the anterior horizontal force. The potential utility of assessing both the anterior and posterior ground reaction force would be interesting.

4. The use of velocity at $\dot{V}O_{2max}$ for dosing intermittent exercise

Whilst the use of percentages of $v\dot{V}O_{2max}$ to dose intermittent exercise is a common approach in the literature (Price & Halabi, 2005; Scott et al., 2011; Buchheit & Laursen, 2013b), and at the time of the development of the experiments it was recommended for intermittent exercise (Buchheit & Laursen, 2013b), this technique to dose exercise intensity may have contributed greatly to the variability between individuals and conditions (Mezzani et al., 2012). This is largely

due to the loss of the linear relationship between $\dot{V}O_2$ and workload that occurs for constant-work-rate above the first ventilatory threshold (Mezzani et al., 2012). Therefore, $\dot{V}O_2$ steady-state is not reached for exercise which is performed in the high to severe-intensity domain, such as that performed during our studies.

It has been observed that individuals can possess a similar $\dot{V}O_{2max}$ yet markedly different ventilatory thresholds (Ghosh, 2004). In such a case, using percentages of $\dot{V}O_{2max}$ or percentages of $v\dot{V}O_{2max}$ would likely result in one participant exercising at a greater percentage above their anaerobic threshold than another participant. Thus, due to the changes in the loss of linear relationship of $\dot{V}O_2$ and workrate (Mezzani et al., 2012) the $\dot{V}O_2$ would continue to rise exponentially as a consequence of the slow component of $\dot{V}O_2$ kinetics (Scott, 1999), creating a disparity in $\dot{V}O_2$ between individuals. To address this issue future studies would benefit from utilising speed thresholds related to the first and second ventilatory threshold (Wolpern et al., 2015). In doing so, some of the inter-individual variability may be accounted for.

5. Bone turnover markers

Bone turnover markers provide only a surrogate measure of bone turnover, providing an indirect assessment of osteoblast and osteoclast activity (Banfi et al., 2010). Moreover, the two BTMs used in this study are not synonymous with bone tissue and may also reflect the turnover of other tissues such as tendon, skin and dentin (Seibel, 2005). As with all biochemical measures there also exists a large inter-individual variability, and whilst attempts were made to reduce the variability, it is not possible to account for all the variability. Clearly a larger sample size is therefore warranted with biochemical measures, however, this is not always feasible due to the cost of the biomarkers.

A further limitation of the study was that calcium, phosphorus and parathyroid hormone were not measured. Potentially, the increase in CTX-I may be as a result of a decrease in serum calcium in

response to exercise stimulating an increase in parathyroid hormone (PTH) (Bouassida et al., 2006) to maintain calcium homeostasis. Therefore, measurement of these markers would likely help explain some of the potential findings, adding strength to the design. The lack of measurement of these markers were dictated by the financial cost of measuring these extra samples. Whilst the author made attempts to obtain external funding from a number of grant applications, no extra money could be obtained.

6. The P-value

Given the recent debates and emerging published evidence critiquing the 'usefulness' (or lack thereof) of the P-value (Cumming, 2013; Colquhoun, 2014; Batterham & Hopkins, 2015), it seems pertinent to include a brief narrative on its use in this thesis. Whilst the author agrees that the reliance on the P-value in isolation is inappropriate, especially when using $P = 0.05$ can lead to a false-discovery rate of ~30% (Colquhoun, 2014), the use of a P-value combined with a raw mean difference and 95% confidence interval still has merit in scientific research. Firstly the mean difference can be seen as an absolute effect size providing the reader with a value that describes the magnitude and direction of the effect. The use of the confidence interval then provides important information on the precision of the population estimate and the variability within the data. Whilst the author attempted to include a standardised effect size (e.g Cohens d) with confidence intervals throughout the thesis, the complexity of the within-subject crossover design, issues of non-normally distributed data, and missing data points for the final experiment, proved challenging to find a more appropriate statistical approach. Therefore, to preserve continuity in the statistical approach across experiments, the traditional null hypothesis significance testing was utilised.

7.4 Recommendations for future work

As a result of the novel findings from the research and limitations of the current thesis, there are a number of avenues which require further investigation.

1. Markers of bone turnover

The inclusion of parathyroid hormone (PTH) and calcium are important for further work to establish potential mechanisms of action. The primary effect of PTH on bone is to induce bone resorption liberating calcium from the mineralized matrix (Bouassida et al., 2006). However, the response of PTH on bone is biphasic, exerting both catabolic and anabolic effects (Gardinier et al., 2015) depending on the temporal sequence of its release. That is, if PTH release is sustained continuously it exerts a catabolic effect whereas intermittent PTH exerts an anabolic response (Bellido et al., 2014). The intermittent PTH response is achieved through daily injections of teriparatide [recumbent human PTH; rhPTH(1-34)] (DiMeglio & Imel, 2014). An excessive elevation in PTH has been shown to result in excessive production of osteoclasts and an enhanced activity of osteoclasts outweighing osteoblast activity. Conversely intermittent PTH promotes osteoblast precursor proliferation and reduces osteoblast apoptosis (Bellido & Hill Gallant, 2014).

A potential mechanism for exercise exerting anabolic responses on bone is due to mimicking the temporal sequence of PTH on bone (Gardinier et al., 2015). Exercise has been shown to increase PTH acutely. With continuous exercise above a certain intensity ($> 65 - 75\% \, \dot{V}O_{2max}$) PTH has been shown to increase and remain elevated following exercise for 1-2 h post exercise (Bouassida et al., 2006). This elevation is in-part explained by the requirement to maintain Ca^{2+} levels which have been shown to decrease with exercise (Barry & Kohrt, 2007). However, the rise in PTH cannot be explained completely by its contribution to maintain calcium levels (Scott et al., 2014). Conversely anaerobic exercise has been shown to result in an initial reduction in PTH followed by a rise (Kristoffersson et al., 1995).

At present there is limited information on PTH responses to a truly intermittent protocol. However, when exercise is separated into two discrete bouts of exercise PTH responses mimic that of pharmacological treatment albeit lower peak levels, and therefore intermittent exercise might be anabolic for bone via this mechanism (Bouassida et al., 2003). PTH is intensity and duration dependent and may be affected by blood lactate and catecholamines (Bouassida et al., 2006).

Given the information presented on the different responses of intermittent exercise of different exercise-to-rest durations which effect catecholamine release, blood lactate, $\dot{V}O_2$ etc differences in acute bone remodelling may also exist through mechanisms explained by PTH. Moreover, the response may serve to suppress sclerostin which inhibits wnt signalling pathway allowing load-induced bone formation (Falk et al., 2015).

2. The osteogenic index

Currently the OI includes only a measure of the vertical component of the ground reaction force. Given bone responds to bi-directional loading (Moreno et al., 2008), the development of an OI which integrates the loading dose from the medio-lateral, anterior-posterior and vertical components of the ground reaction force may improve the internal validity of the equation. As such, we propose a possible equation for future studies to apply where:

$$OI = \sum OI_{xGRF} + OI_{yGRF} + OI_{zGRF}$$

Where, OI_{xGRF}: medio-later OI; OI_{yGRF}: anterior-posterior OI, and OI_{zGRF} is the vertical OI.

The OI would also benefit from being assessed at different load bearing sites and potentially the use of accelerometers or wearable monitors could be implemented to achieve this.

3. Different exercise-to-rest ratios

As discussed in detail in Chapter 2, intermittent exercise is a complex model which can be manipulated in a variety of ways all of which result in different physiological, neuromuscular and biomechanical stresses. To inform future training programmes for intermittent exercise there is a need to establish how bone may respond to manipulating the exercise mode (impact vs. non-impact) and also the manipulation of different exercise to rest ratios. In this thesis we controlled the ratio at 1:1, however soccer has been reported to be closer to a 3:1 ratio (Bangsbo, 1994). The

phenomenon of the rest interval in re-sensitising bone has only really been addressed in rodents and animal studies. Therefore more work is required manipulating the exercise-rest interval further Furthermore, the NMT allows only movements in the sagittal plane, and therefore movements such as rapid changes of direction, and jumps cannot be included in the intermittent protocol which would affect the physiological and biomechanical response (Buchheit & Laursen, 2013b; 2013a). Undoubtedly these will have an effect on the magnitude of the effect of different intermittent exercise programmes.

4. **Transference of intermittent exercise to clinical/sub-clinical populations**

The current study was a 'proof-of-concept' study with the purpose of challenging and investigating the premise that intermittent running might be more osteogenic than more continuous exercise. The high intensity protocols used in this study are designed for healthy individuals to maximise bone health. In a clinical or sub clinical population (post-menopausal females) this exercise would likely be too high intensity. However, the study demonstrates, to an extent, that there may be different osteogenic responses to intermittent exercise (of varying exercise-to rest intervals) in humans, somewhat supporting animal models. Therefore, intermittent exercise could be performed as an additive therapy for clinical and subclinical population. What the appropriate mode, intensity, exercise-to-rest duration should be requires further work.

Future work might also look to establish how intermittent exercise might be advantageous to younger adults and/or children to maximise peak BMD. Current exercise guidelines for children utilise continuous forms of exercise therefore evidence is needed to promote the potential potency of intermittent exercise on bone health.

5. Exercise mode

In the thesis we focused on running to match the mode of exercise used in soccer, and to offer evidence to either support and/or reject the opinions on interval running stated by previous authors (Boudenot et al., 2015). However, running is likely not the most osteogenic mode of exercise (Weeks & Beck, 2008). The concept of intermittent exercise should be applied to other modes of bone building exercise such as jumping. As resistance exercise is often characterised by different exercise to rest intervals, this might also be another avenue to establish the effect of intermittency.

CHAPTER 8:
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8.1 References

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CHAPTER 9:
Appendices

Appendix A: Non-motorised treadmill characteristics

The Woodway Force 3, Non-motorised treadmill (NMT) (Woodway Ltd, Weil an rhein, Germany) characteristics are detailed below in Table 9.1 and 9.2.

Table 9.1 Physical and performance specifications of the Woodway Force 3 Non-Motorised Treadmill

Belt type	60 individual slats
Drive system	114 precision ball bearings with 12 guide rollers (4 mm lateral tolerance)
Running surface	Vulcanized rubber (38-43 shore hardness)
Load/resistance system	Electromagnetic breaking system provides 15-150 lbs resistance
Unit weight	560 lbs
Power supply	110 V power supply (dedicated circuit and NEMA 5-20R outlet receptacle required)
User weight capacity	800 lbs
Running surface area	22" x 68"
Performance indicators	Speed/load/distance
Load cell	4 vertical load cells under belt
	1 horizontal load cell attached to vertical strut
Tachometer	XPV7 PCB 20 pulse treadmill tachometer function

Table 9.2 details the technical specifications of the 4 load cells (strain gauges) used to obtain the vertical force.

Non linearity	± 0.0170 %
Hysteresis error	± 0.0170 %

Pacer performance software and creating bespoke protocols

Programmes can be developed in stages using the Pacer performance software. The stage was specified with a series of numbers from zero-ith stage, the start time and speed was manipulated dependent on the required programme. The load was also specified as zero which is the equivalent to 15 lbs of resistance on the belt according to manufacturers' specifications. An instruction and sound file was not included in the programme. The participants obtained visual and audio information on when to change their velocity. This information was presented to them via a PC screen

Appendix B: Maximal oxygen update criteria

Table 9.3 A-D Presents $\dot{V}O_{2max}$ criteria data for all participants (n=16) across all trials for Cont-MT, Cont-NMT, 15s-NMT and 30s-NMT.

A Participant (*n*)	Plateau (< 0.2 L·min⁻¹)	RER (> 1.15)	HR (± 10 beats·min⁻¹)	$\dot{V}O_{2verif}$ (2 % $\dot{V}O_{2max}$)	RPE (>8)
1	Yes	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	No	Yes
5	Yes	Yes	No	No	Yes
6	Yes	Yes	Yes	No	Yes
7	No	Yes	Yes	No	Yes
8	Yes	Yes	Yes	No	Yes
9	Yes	Yes	Yes	No	Yes
10	Yes	Yes	Yes	No	Yes
11	No	Yes	No	Yes	Yes
12	No	Yes	Yes	Yes	Yes
13	Yes	Yes	No	Yes	Yes
14	No	Yes	Yes	No	Yes
15	No	Yes	Yes	No	Yes
16	Yes	Yes	Yes	No	Yes

B Participant (*n*)	Plateau (< 0.2 L·min⁻¹)	RER (> 1.15)	HR (± 10 beats·min⁻¹)	$\dot{V}O_{2verif}$ (2 % $\dot{V}O_{2max}$)	RPE (>8)
1	No	Yes	Yes	Yes	Yes
2	No	Yes	Yes	Yes	No
3	Yes	Yes	No	Yes	Yes
4	Yes	Yes	No	No	Yes
5	Yes	No	Yes	No	Yes
6	Yes	Yes	Yes	Yes	Yes
7	Yes	Yes	Yes	No	Yes
8	Yes	Yes	No	No	Yes
9	No	Yes	No	No	Yes
10	Yes	Yes	No	Yes	Yes
11	Yes	Yes	No	No	No
12	No	Yes	Yes	Yes	Yes
13	Yes	Yes	No	Yes	Yes
14	No	Yes	Yes	No	Yes
15	No	Yes	Yes	No	Yes
16	Yes	Yes	Yes	Yes	No

C Participant (n)	Plateau (< 0.2 L·min^{-1})	RER (> 1.15)	HR (± 10 beats·min^{-1})	$\dot{V}O_{2verif}$ (2 % $\dot{V}O_{2max}$)	RPE (>8)
1	No	Yes	Yes	Yes	Yes
2	No	Yes	Yes	No	Yes
3	No	Yes	Yes	No	Yes
4	Yes	Yes	Yes	Yes	Yes
5	No	Yes	No	No	Yes
6	No	Yes	Yes	No	Yes
7	Yes	Yes	Yes	No	Yes
8	Yes	Yes	Yes	No	Yes
9	Yes	Yes	No	Yes	Yes
10	Yes	Yes	Yes	No	Yes
11	Yes	Yes	No	Yes	Yes
12	Yes	Yes	Yes	Yes	Yes
13	Yes	Yes	No	No	Yes
14	No	Yes	Yes	No	Yes
15	No	Yes	Yes	Yes	Yes
16	Yes	Yes	No	No	Yes

D Participant (n)	Plateau (< 0.2 L·min^{-1})	RER (> 1.15)	HR (± 10 beats·min^{-1})	$\dot{V}O_{2verif}$ (2 % $\dot{V}O_{2max}$)	RPE (>8)
1	Yes	Yes	Yes	Yes	Yes
2	No	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes	Yes
4	No	Yes	Yes	No	Yes
5	Yes	Yes	No	No	Yes
6	No	Yes	Yes	Yes	Yes
7	No	Yes	Yes	No	Yes
8	No	Yes	No	No	Yes
9	No	Yes	Yes	No	Yes
10	No	Yes	Yes	No	Yes
11	No	Yes	Yes	Yes	Yes
12	No	Yes	Yes	Yes	Yes
13	No	Yes	No	No	Yes
14	No	Yes	Yes	No	Yes
15	Yes	Yes	Yes	Yes	Yes
16	Yes	Yes	Yes	No	Yes

Note: $\dot{V}O_{2max}$ criteria for continuous motorised treadmill (Cont-MT), Continuous non-motorised treadmill (Cont-NMT), 15 x 15s non-motorised treadmill (15s-NMT) and 30 x 30s non-motorised treadmill (30s-NMT) protocols. Abbreviations: Respiratory exchange ratio (RER), Heart rate

(HR), Ratings of perceived exertion (RPE), verification ($\dot{V}O_{2verif}$. A 'Yes,' signifies that the criteria were successfully met, a 'No,' signified that the criteria were not successfully met.

Appendix C: Intra- and inter-assay precision information

This Information was provided by a biomedical scientist at Manchester Hospital

Method

The precision of the CTX-I assay was assessed using the IDS-iSYS CTX-I control set which contains 3 levels of serum based controls. In order to assess intra-assay precision, all 3 levels of control material were run 15 times as part of the same batch. For inter-assay precision, all 3 levels of control material were analysed on 10 separate days, as part of different batches. The results were subsequently analysed and compared to the assay performance quoted in the kit insert.

Table 9.4 Intra-Assay Precision CTX-I

Sample No.	CTX-I (ng/mL)		
	IQC1	IQC2	IQC3
1	0.236	0.922	2.244
2	0.239	0.924	2.246
3	0.248	0.957	2.286
4	0.239	0.936	2.261
5	0.232	0.949	2.252
6	0.232	0.889	2.136
7	0.228	0.889	2.104
8	0.236	0.879	2.145
9	0.222	0.874	2.134
10	0.232	0.870	2.134
11	0.223	0.879	2.086
12	0.227	0.894	2.131
13	0.238	0.908	2.247
14	0.232	0.910	2.177
15	0.220	0.864	2.034
Mean	0.232	0.903	2.174
SD	0.007	0.029	0.076
%CV	**3.2**	**3.3**	**3.5**

Intra-assay precision ranged from 3.2 to 3.5%. The within-batch precision quoted by the manufacturer ranges from 2.1% (at a concentration of 0.877 ng/mL) to 4.9% (at a concentration of 0.216 ng/mL). Although the precision of the assay in our laboratory was slightly worse at the

upper end (3.5% compared to 2.4%), it also appeared better than that quoted by the manufacturer at the lower end (3.2% compared to 4.9%). Overall the intra-assay precision of the assay is acceptable for an automated immunoassay.

Table 9.5 Inter-Assay Precision

Sample No.	CTX-I (ng/mL)		
	IQC1	**IQC2**	**IQC3**
1	0.230	0.860	2.083
2	0.238	0.908	2.247
3	0.239	0.874	2.047
4	0.233	0.869	2.074
5	0.250	0.904	2.08
6	0.242	0.852	2.071
7	0.256	0.896	2.141
8	0.215	0.802	1.981
9	0.234	0.949	2.242
10	0.220	0.931	2.247
Mean	0.240	0.880	2.120
SD	0.012	0.042	0.094
%CV	5.27	4.80	4.44

The inter-assay precision of the assay ranged from 4.4 to 5.3%. This performance was actually better than that quoted by the manufacturer whose between-batch precision varies from 4.7 to 8.8%

The intra and inter-assay precision for the intact P1NP assay is shown below Table 9.6

Table 9.6 The intra and inter-assay precision for the intact P1NP assay

	Within-batch precision (n=20)			Between-batch precision (n=10)		
Mean	26	44.7	109.5	27.6	46.8	117.2
SD	1.4	1.5	5	1.5	2.5	4.6
CV	5.30%	3.40%	4.60%	5.5	5.3	3.9

Appendix D: Uncorrected (for plasma volume change) blood data

P1NP uncorrected

The main effect for condition was not statistically significant F $_{(3, 33.032)}$ = 1.161, P =0.339, time [F$_{(3\ 33.031)}$ = 6.593, p = 0.001. The interaction was not statistically significant F $_{(9, 98)}$ =0.989, P = 0.457].

CTX_I uncorrected

Condition (F$_{(3, 33.033)}$ = 3.073, P = 0.041, time F $_{(3, 33.061)}$ = 16.890, P = 0.0001, condition by time F $_{(9,98)}$ = 3.144, p = 0.002.

Blood lactate uncorrected

The means (SD) for blood lactate are presented in Figure 6.5. There was a significant main effect for time [F $_{(3, 33.103)}$ = 29.594, P = 0.0001], and condition [F $_{(2, 22.066)}$ = 4.635, P = 0.021]. There was also a significant condition by time interaction [F $_{(6, 64)}$ =3.187, P = 0.008]

Uncorrected blood glucose

There was a significant main effect for time [F $_{(3, 33.157)}$ = 17.989, P = 0.0001] across all time conditions. However, there was no significant main effect for condition [F $_{(2, 22.081)}$ = 2.882, P = 0.077] nor a significant condition by time interaction [F $_{(6, 65)}$ =1.306, P = 0.267].

RATING	DESCRIPTOR
0	REST
1	VERY,VERY EASY
2	EASY
3	MODERATE
4	SOMEWHAT HARD
5	HARD
6	
7	VERY HARD
8	
9	
10	MAXIMAL

Figure 9.1 Fosters category ratio rating of perceived exertion scale (Foster et al., 2001).
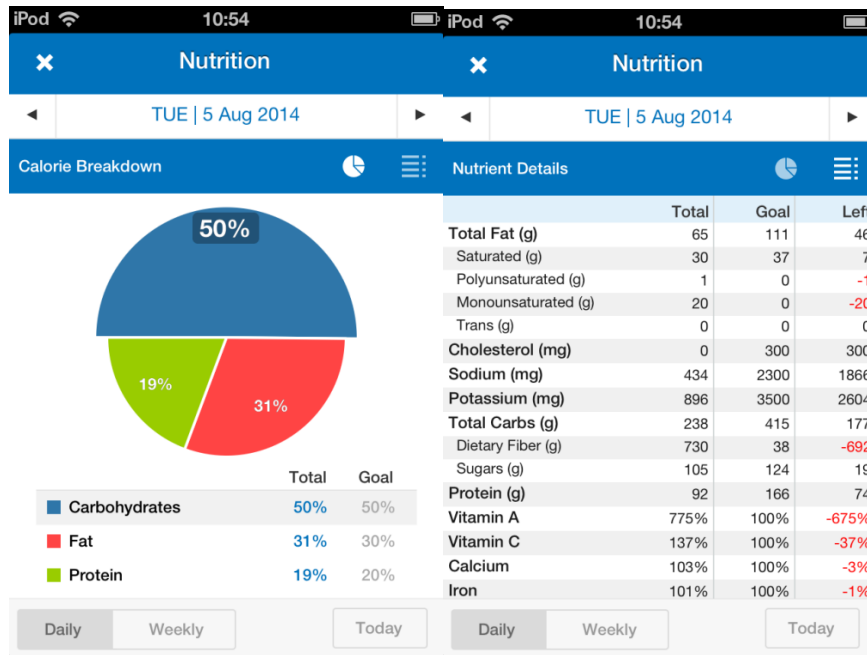
Appendix F: Food diary outputs

Figure 9.2 Example of food diary outputs from MyFitnessPal application.