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Bone health and risk of stress fracture in female endurance athletes

By

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Submitted in partial fulfilment of the requirements for the reward of

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Abstract

One in two women over the age of 50 will be diagnosed with an osteoporotic fracture during their life time (Van Staa et al. 2001). Osteoporosis is a condition in which the bone mineral density (BMD) is lost causing the bone to become weak and liable to fracture. It is well established that participation in weight bearing exercise (gymnastics and running) may be beneficial to BMD, due to the high mechanical loading (Drinkwater 1994; Kannus et al. 1994a; Marcus et al. 1992; Snow 1996).The high prevalence of amenorrhoea (1-44%) (Bennell et al. 1997b) in female athletes may result in poor bone health, leading to increased risk of premature osteoporosis or stress fracture injury, disabling an athlete's present and future career (Nattiv 2000; Nattiv et al. 1997). Oestrogen deficiency in amenorrhoeic athletes may compromise the beneficial effects of exercise, leading to lower BMD (Bass 2003; Saxon and Turner 2006), but it is unknown whether this is accompanied by structural differences such as changes to section modulus (Z). There is evidence that athletes display seasonal gains and losses in BMD with changes in training (McClanahan et al. 2002; Snow et al. 2001; Winters and Snow 2000), however, in amenorrhoeic athletes, it is possible that any seasonal losses may not be recovered thus contributing to lower BMD.

Studies have reported incidence rates of stress fracture to range between 8.7 -21.1 % in athletes with female endurance athletes at the greatest risk possibly due to the aforementioned high prevalence of menstrual dysfunction and a demand for thinness (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000). However prospective monitoring of stress fracture in female endurance athletes, the gold standard for measuring incidence, is limited with conflicting evidence of incidence and risk factors of stress fracture, possibly due to varying methodology and with no standard definition of stress fracture (Snyder et al. 2006). There is also limited robust evidence to determine whether psychological traits are associated with stress fracture history. There is solid evidence to suggest that the reoccurrence rate of stress fracture in athletes is high over the first 12-months following an initial stress fracture; this could be caused by retraining when the bone is at its weakest. Studies of other musculoskeletal injuries have shown bone loss following injury up to 12-months, however there is no research to suggest how much bone may be lost following a stress fracture injury. It is important therefore, to monitor, using robust methodology, the incidence and subsequent consequences for bone loss associated with stress fracture in athletes in order to provide support for potential intervention and treatment.

The main aims of this thesis are two-fold to: 1) determine prospectively the predictors of bone health and stress fracture in female endurance athletes, and 2) determine whether bone geometry and density change following a stress fracture. The specific objectives of the thesis were five-fold to: 1) determine the correlates of stress fracture history, 2) compare bone density and geometry according to menstrual function, 3) determine the incidence rates of stress fracture and identifiable risk factors, 4) quantify the seasonal variation in parameters of bone health and 5) determine the magnitude and timescale of bone loss and subsequent regain.

Seventy United Kingdom based female endurance athletes (runners and triathletes) aged 18-45 years were prospectively monitored for 12-months. At each assessment (baseline, 6-month and 12-months) questionnaires were used to assess menstrual, nutritional, eating psychopathology, exercise cognition, and injury histories. BMD, bone mineral content (BMC), hip geometric parameters, and body composition were assessed using dual x-ray absorptiometry (DXA), and anthropometric measures were taken. Training and stress fracture injury were prospectively monitored.

Retrospective data determined 19 (27%) of the athletes had a history of clinically diagnosed stress fracture. Athletes with a history of stress fracture had a significantly higher prevalence of current (47% vs 27%, p=0.008) and past (79% vs 53%, p=0.035) menstrual dysfunction and higher global scores on the eating disorders examination questionnaire (EDE-Q) (p=0.049) and the compulsive exercise test (CET) (p=0.016) compared to non-stress fracture athletes. Bone parameters by DXA, training duration, age, age at menarche and anthropometric measurements did not differ between groups. This study found a high prevalence of past stress fracture, and identified eating and exercise behaviour to be related to stress fracture risk independent of menstrual dysfunction.

To compare bone geometry and density according to menstrual function the athletes were classified as either a/oligomenorrhoeic athletes ($\leq$9periods/year) (AA) or eumenorrhoeic athletes ($\geq$10 periods/year) (EA) and compared to 88 eumenorrhoeic sedentary controls (EC). 30 athletes were AA and 40 EA. EC were significantly older, heavier and shorter than EA and AA who did not differ significantly. Femoral neck BMD was significantly higher in EA than AA and EC (mean (SE) EA: 1.117 (0.015), AA: 1.036 (0.020) and EC: 0.999 (0.014) g/cm^2 respectively; p<0.001). Section modulus (Z) was significantly higher in EA than EC (EA: 657 (20), AA: 639 (20), EC: 592 (10) cm^3 p=0.004), although AA did not differ significantly from EA and EC. Lumbar spine BMD was significantly lower in AA than EC (1.141 (0.019), AA: 1.105 (0.026) EC: 1.188 (0.014) g/cm^2, p=0.007). All differences persisted after adjustment for height, age, and body mass. Eumenorrhoeic athletes had significantly higher femoral neck BMD and Z than controls, consistent with previous research. Femoral neck Z and hence strength in bending was relatively maintained in athletes with menstrual dysfunction despite their lower BMD at this site, indicating possible structural adaptation.

Incidence of stress fracture was determined prospectively. Following withdrawal of 9 participants, 61 female athletes were monitored prospectively for the 12-month period. Among the 61 athletes, two sustained a stress fracture, both diagnosed by MRI, giving an annual incidence rate of 3.3%. The stress fracture cases were: both 800m runners aged 19 and 22 years, training on average 14.2 hours/week, eumenorrhoeic, and with no history of amenorrhoea. BMD, energy intake and EDE-Q and CET scores were similar to the mean values in the non-stress fracture group. Thus the incidence of stress fracture in this sample of female endurance athletes is lower than previously reported, possibly due to the increased awareness of stress fracture diagnosis, risk factors and athlete management.

Seasonal bone changes were determined in 61 female athletes. The greatest variation was observed in the endurance runners (n=52). The endurance runners were classified according to menstrual function (28 were EA and 24 AA). There were no significant differences at baseline or seasonal variation in height, weight, and body fat percentage. In EA, trochanter BMD increased over the summer (0.885 (0.019) to 0.947 (0.177) g/cm^2, p=0.002) with no significant change over the winter (0.880 (0.018) to 0.885 (0.018) g/cm^2 p=0.153). In AA femoral neck BMD decreased over the winter (1.065 (0.021) to 1.052 (0.020), g/cm^2, p= 0.030) with no significant change over the summer (1.050 (0.020) to 1.052 (0.020), g/cm^2, p=0.770). Minimal neck width increased in the group as a whole over the winter (28.4(0.3) to 28.7(0.3), mm p=0.039) with no significant change over the summer 28.8(0.3) 28.7(0.3), mm p=0.333). There were no significant seasonal variations in other bone parameters, and seasonal changes did not differ significantly between groups. EA increased trochanter BMD over the summer, and this was maintained over the winter. Conversely, AA lost femoral neck BMD over the winter and this was not recovered over the summer, although the increase in width of the femoral neck may have partly compensated BMD loss to maintain strength in bending.

The final prospective analysis was conducted in a separate sample of female athletes who were diagnosed with a stress fracture injury. The aim of this analysis was to determine the magnitude and time scale of bone loss following a stress fracture injury and subsequent regain following retaining. A group of 4 stress fracture cases and 3 controls were followed for a period of 6-8 months following a stress fracture injury. BMD and BMC (lumbar spine, femoral neck, and trochanter) and estimations of geometric properties CSA, Z and buckling ratio) were assessed using DXA. The mean difference of bone loss and bone regain was determined by BMD, BMC and geometric parameters from baseline to 6-8 weeks and 6-8 weeks to 6-8 months respectively. No significant bone loss was found in either cases or controls from baseline to 6-8 weeks at any of the bone parameters. A significant difference at the femoral neck was found in the injured leg of the stress fracture cases from 6-8weeks to 6-8months (mean (SE) 1.042(0.102) to 1.070(0.102) g/cm2, p=0.004) with no significant change in the contra-lateral case leg 1.036 (0.102) to 1.054(0.109) g/cm2). No significant bone regain was found in the control subjects (health or "injured legs"). Thus athletes do not seem to lose significant BMD during the recovery phase of training when partial weight bearing is required. Subsequent bone regain above the initial baseline value does seem to occur in the injured leg within 8 months following the stress fracture once training is resumed.

In conclusion the work within this thesis has not only reinforced previous stress fracture findings, showing that a history of stress fracture is increased in athletes with a history of amenorrhoea, but has identified novel results indicating a lower incidence of stress fracture in female endurance athletes than previously reported. Exercise cognitions have been identified as risk factors for stress fracture history independent of menstrual dysfunction. Furthermore and potentially the most novel finding of this research is the importance for the examination of bone geometric properties in amenorrhoeic athletes. Findings suggest that possible structural adaptations counteract the effects of low BMD and annual losses of BMD during seasonal training in amenorrhoeic endurance athletes. In light of these

findings this thesis highlights scope for further longitudinal research in the area of structural adaptation to bone in amenorrhoeic athletes.

Keywords: Stress fracture, bone mineral density, bone geometry, endurance athletes, menstrual dysfunction, eating and exercise cognitions.

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"A loving heart is the truest wisdom" (Charles Dickens)

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"Reach high, for the stars lie hidden in your soul. Dream deep, for every dream precedes the goal. "(Pamela Vaull Starr)

Publications

Conference Presentations and Posters

<u>Duckham R.L</u>, Peirce N,' Summers G.D, Cameron N, Brooke-Wavell K. "Seasonal bone changes in eumenorrhoeic and amenorrhoeic endurance runners, Medicine & Science in Sports & Exercise: *(In press, Thematic poster, (SSHB travel Award)*

<u>Duckham R.L</u>, Peirce N,' Summers G.D, Cameron N, Brooke-Wavell K. "Incidence of stress fracture in female endurance athletes is increased in athletes with no identifiable risk factors." United Kingdom Sport and Exercise Medicine annual conference, Nov 2010, (In press) (Poster)

<u>Duckham R.L</u>, Bailey C.A, Brooke-Wavell K. Bone Geometry and bone density in athletes and sedentary controls according to menstrual function, Medicine & Science in Sports & Exercise: May 2010 - Volume 42 - Issue 5 - p 104. (Oral) (*SSHB travel Award)* Doi:10.1249/01.MSS.0000385967.95330.4c

<u>Duckham R.L</u>, Peirce N, Summers G, Cameron N, Brooke-Wavell K. Effects of menstrual dysfunction on bone geometry and bone density in female endurance athletes, Annals of Human Biology, May 2010 (Oral Presentation)

<u>Duckham R</u>, Peirce N, Meyer C, Brooke-Wavell K. Stress fracture history, eating and exercise behaviours and menstrual dysfunction. BASEM Annual Congress 2009, (In press) (Oral presentation)

<u>Duckham R</u>, Peirce N, Summers G, Cameron N. Bone geometry and density according to menstrual function in endurance athletes, 2009 'Abstract' Journal of Sport Sciences, 27: 4, S38 To link to this Article: DOI: 10.1080/02640410903195819

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Chapter One:
Introduction

Chapter one gives an overview of the thesis, summarizing the past literature and highlighting the importance of the current research question.

Introduction

The growing interest in bone health stems from the increased public awareness of osteoporosis, "a systemic skeletal disease characterized by low bone mass and microarchitectual deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" (Kanis 2002; Who 1994). One in two women and one in five men over the age of 50 will be diagnosed with an osteoporotic fracture during their life time (Van Staa et al. 2001).

The aetiology of osteoporosis appears to be multi-factorial, influenced by an individual's genetic makeup, hormonal status, age, gender, nutritional habits, levels of physical activity and lifestyle in general (Heaney et al. 2000; Menard 1996; Nichols et al. 2007). Whilst osteoporosis most commonly affects older people, the prevention of osteoporosis should be considered from early in childhood and adolescence when the majority of peak bone mass is accrued (Heaney et al. 2000).

The gold standard for the assessment of bone health and fracture risk is BMD (g/cm^2). BMD has been used as a proxy for bone strength, suggesting that decreases in bone density is tantamount with declining strength (Cummings et al. 1993; Hui et al. 1988). However bone strength encompasses the bone architecture, geometry, cortical porosity and tissue mineralization which cannot be individually identified in BMD measurements (Bonnick 2007). The role therefore, of bone geometric properties in osteoporosis is still being elucidated. This is primarily due to the continued evolution of bone geometry research.

The bones response to mechanical loading is controlled by an intrinsic 'Mechanostat' which regulates the bones functional adaptation via mechanical thresholds (Frost 1987; Lanyon et al. 1982). The mechanical thresholds control whether bone is added or removed

to the skeleton. If the mechanical loads are below the optimal threshold bone will be resorbed, whereas, if the mechanical loads are above the optimal threshold bone will be added. Bone will adapt to mechanical loading in the concept of progression and overload which will increase bone formation therefore increasing bone strength (Hughes and Petit 2010). Mechanical loading through exercise can maximize peak bone mass during early growth and into the third decade, and retain bone mass into the fifth decade (Kelley et al. 2002; Khan et al. 2001). Exercise therefore seems to be a positive preventive measure of oseoporotic fractures. Increased impact loads applied to the bone during weight bearing exercise such as in gymnastics and running sports, can lead to increases in BMD which are not observed during participation in non weight bearing exercise (swimming and cycling) (Drinkwater 1994; Marcus et al. 1992; Mudd et al. 2007; Nikander et al. 2005; Taaffe et al. 1997). The current literature however is not sufficient to draw definite conclusions regarding the site-specific responses of bone to specialized sport training. Previous studies have used small sample sizes and often recruited multiple sports (Nichols et al. 2007). Weight-bearing sports such as running may be expected to be identified as one of the most osteogenic sports, but despite this endurance runners have been reported to have low lumbar spine BMD (Hind et al. 2006).

Even though it is well established that participation in weight bearing sport may be beneficial to BMD, due to the increased mechanical loading on the bone, the high prevalence of amenorrhoea (< 3 periods per year) (1-44%) (Bennell et al. 1997b) in athletes may implicate poor bone health, leading to increased risk of premature osteoporosis or stress fracture injury disabling an athlete's present and future career (Nattiv 2000).

The main skeletal function of circulating oestrogen in women is to preserve bone mass and bone geometry by suppressing bone turnover (Jarvinen et al. 2003a; Jarvinen et al.

2003b; Jurimae and Jurimaae 2008; Riggs et al. 2002). Evidence suggests that exercise and oestrogen are beneficial to BMD (Bass 2003; Frost 1999; Lanyon 1996; Saxon and Turner 2006) because exercise enhances bone accrual on the periosteal (outer) bone surface, which confers greater resistance to bending (Saxon et al. 2005). In the case of oestrogen, it may inhibit periosteal apposition, while increasing endocorticol contraction (Turner et al. 1990a) (Saxon and Turner 2006). It is unknown how bone geometric properties, hence strength in bending, are affected especially in amenorrhoeic (< 3 periods per year) athletes, where oestrogen deficiency may be present.

The beneficial effects of exercise in amenorrheoic athletes are often counteracted by oestrogen deficiency (Bass 2003; Saxon et al. 2005). Studies with rats have reported oestrogen deficiency not only decreases BMD but also causes structural adaptations. Whilst it is well established that amenorrhoeic athletes have substantially lower BMD at the spine and femoral neck than their eumenorrhoeic ($\geq$10 periods per year) peers (Drinkwater et al. 1984; Grimston et al. 1990; Marcus et al. 1985; Myburgh et al. 1993; Rencken et al. 1996), it is unknown whether bone geometric properties hence strength in bending, is affected in amenorrhoeic athletes.

Findings on the recovery of BMD following resumption of menses are conflicting with some reporting a full recovery (Hind 2008), and others indicating that BMD is not fully recovered (Keen and Drinkwater 1995; Keen and Drinkwater 1997). Thus, a full understanding of the effect of oestrogen deficiency on bone geometric properties may determine if low BMD which is not recovered is actually detrimental to amenorrhoeic athletes or whether bone geometry will compensate the decreased BMD, thus protecting the strength of the bone.

There is evidence that athletes display seasonal gains and losses in BMD with changes in training (McClanahan et al. 2002; Snow et al. 2001; Winters and Snow 2000), however in

amenorrhoeic athletes, it is possible that any seasonal losses may not be recovered thus contributing to lower BMD. Detraining studies in athletes have shown that BMD is lost following 3-4 months of detraining in team sports (McClanahan et al. 2002; Snow et al. 2001; Winters and Snow 2000). There is limited evidence to suggest whether seasonal training may result in bone changes in endurance runners.

Studies have reported incidence of stress fracture to range between 8.7 – 20.7% (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000) in athletes with female endurance athletes at the greatest risk possibly due to the aforementioned high prevalence of menstrual dysfunction and a demand for thinness (Nattiv et al. 2007; Nattiv et al. 1997). However prospective monitoring of stress fracture in female endurance athletes, the gold standard for measuring incidence, is limited with conflicting evidence of incidence and risk factors of stress fracture. Previously identified risk factors for stress fracture in female athletes are menstrual dysfunction, late age at menarche, energy restriction, low BMD and narrow calf girth (Bennell et al. 1999; Bennell et al. 1996a; Nattiv 2000; Snyder et al. 2006). However, possibly due to the complex interrelationship of stress factor risk factors, the lack of robust methodology and standard definition of stress fracture there is no compelling evidence to determine what factors are contributing to the incidence of stress fracture (Snyder et al. 2006).

There is evidence to suggest that the reoccurrence rate of stress fracture in athletes is high (12.6%) over the first 12-months following an initial stress fracture (Bennell et al. 1996a), this could be caused by retraining when the bone is at its weakest. Studies of other musculoskeletal injuries have shown bone loss following injury up to 12-months (Alfredson et al. 1998; Kannus et al. 1992; Petersen et al. 1997; Therbo et al. 2003), however there is no research to suggest how much bone may be lost following stress

fracture injury. Thus prospective studies are needed to determine the timescale and magnitude of bone loss and subsequent regain following a stress fracture injury.

As female participation in sport increases there is a need to fully understand the benefits and risks to bone health particularly in a sporting society that demands thinness. The following literature review (chapter 2) will show compelling evidence of the gaps in the present literature strengthening the need for further prospective studies in female endurance athletes. Chapter three will highlight the general methods used throughout this research giving justifications for the methodology used. The main aims of this study are two-fold with five specific objectives that will be explored in chapters 4-8. The final chapter will give an overall conclusion of the research highlighting the main findings and implications (chapter 9).

The following aims were generated to address some of the gaps highlighted in the literature:

Main Aims

- Determine predictors of bone health and stress fracture in female endurance athletes

- Determine whether bone geometry and bone density change following a stress fracture

Further objectives

- To determine the correlates of stress fracture history

- To compare bone density and geometry according to menstrual function

- To determine the incidence rates of stress fracture and identifiable risk factors

- To quantify the seasonal variation in parameters of bone health

- To determine magnitude and timescale of bone loss and subsequent regain

Chapter Two
Literature Review

Chapter two critically evaluates the past literature of bone health and stress fracture risk in female endurance athletes, identifying potential gaps, and highlighting the need for further research.

Literature Review

This chapter summarsises the past literature on bone health and stress fracture risk in female endurance athletes. It initially introduces bone morphology and physiology to give an understanding of the biological and mechanical concepts relevant to bone and exercise. This is followed by a critical review of the literature relating to bone and its adaptations through the endocrine system, mechanical loading, exercise, and nutrition. Furthermore, this literature review identifies potential gaps in the research in the understanding of stress fractures, an over use injury caused by micro damage to the bone. It highlights the incidence rates and potential risks of stress fracture in female athletes.

The studies presented in this literature review were published in English and identified through searches of the following computerized databases: Web of Science, Medline, Pubmed, Science Direct and Google Scholar. Furthermore, papers were found through examination of published reference lists and on recommendation of supervisors. Key words used in searches included: bone (bone mineral density, bone mineral content, bone geometry, section modulus, seasonal variations, bone loss), stress fracture (incidence, epidemiology, risk factors), menstrual function (amenorrh*ea, eumenorrhoea, oligomenorrhoea, menarche), female athlete triad (menstrual dysfunction, osteoporosis, energy availability, disordered eating, eating disorder), and athletes (endurance athletes, runners, triathletes). These key words included both UK and USA spellings and were used in combination with one another by adding words such as AND.

2.1   Bone morphology and physiology

Bone morphology and physiology must be comprehended when determining the mechanical adaptations of bone in the response to physical activity. Bone properties and the methods used to assess bone mass are identified in this section.

2.1.1   Bone morphology

The human skeletal system is constructed of approximately 206 individual bones and a network of connective tissue. Bone is unique in that it is self repairing, able to adapt to changes in mechanical requirements, and regulates extracellular matrix (Jee 2001). By structural design bone can be categorized into two distinct type's cortical (which makes up 80% of skeletal mass) and trabecular (20% of skeletal mass) (Borer 2005; Wynsberghe et al. 1995). Cortical bone is located in the diaphyses of long bones, and on the surface of flat and cubiod bones, deriving its strength from the geometric structure of the Haversian system (Figure 2.1)(Borer 2005; Jee 2001; Wynsberghe et al. 1995). Trabecular bone consisting of trabeculae is located in the epiphyseal and metaphysic ends of long bone at the periphery between the bone and the marrow cavity and within the interior of cuboid and flat bones. The trabecular bone merges with the intra-cortical haversian system. The periosteum, a layer of dense connective tissue covers the outer surface of the cortical and trabecular bone (Borer 2005; Rho et al. 1998). Constructed of two layers, the inner layer contains osteoblasts and few blood vessels and fibrous connective tissue, whereas the outer layer is extremely dense containing a vast network of blood vessels. The periosteum provides muscle, tendon and ligament attachment to bone (Borer 2005; Jee 2001; Rho et al. 1998; Wynsberghe et al. 1995).

The proportion of cortical and trabecular bone will vary within different individual bones, long bones such as the tibia will consist of approximately 95% cortical and 5% trabecular bone, whereas the vertebral bodies consists predominately of trabecular bone (62-70%),

(Borer 2005; Jee 2001; Snow-Harter and Marcus 1992). As the proportion of bone can vary within the same bone due to the structural location, it is important to consider the measurement location when assessing bone properties. In the hip, the ratio of cortical to trabecular bone is 75% to 25% at the femoral neck, 50% to 50% at the trochanter, and 57% to 43% at the femoral shaft (Riggs et al. 1982; Schlenker and Vonseggen 1976). Within the radius the greatest proportion of cortical bone (85-90%) can be found at the point one third of the distance from the styliod process to the olecranon, whereas trabecular bone (60-70%) is predominately found at the distal radius (Riggs et al. 1982; Schlenker and Vonseggen 1976).
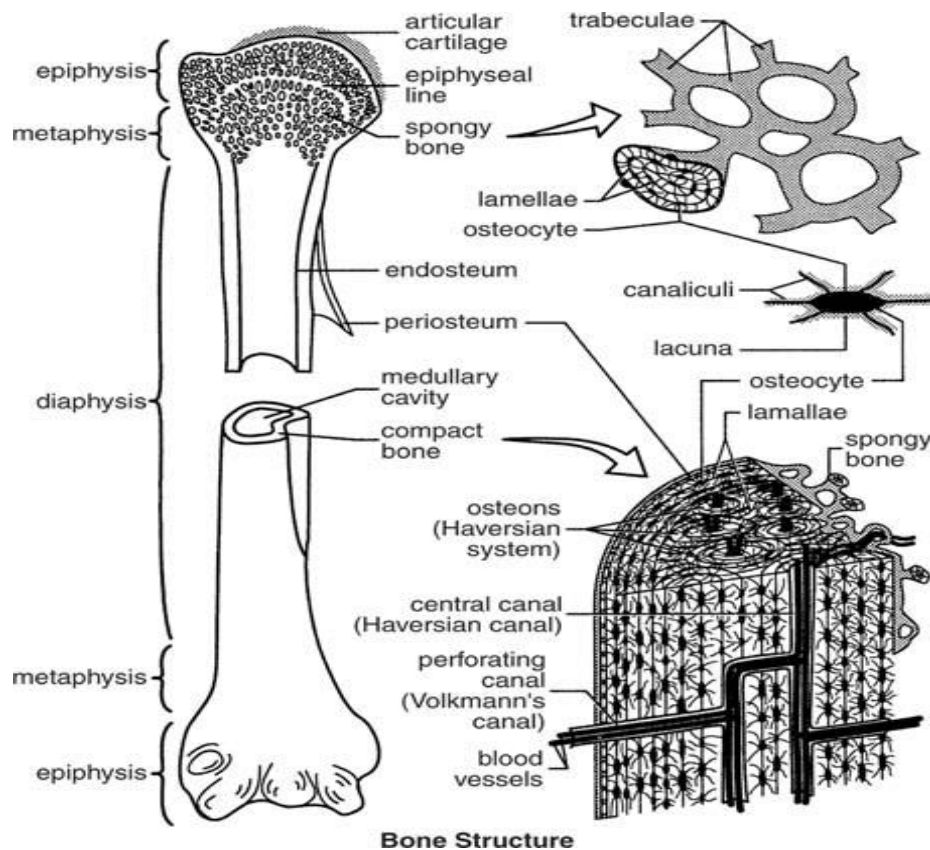
Figure 2.1: A diagram of the structure of bone identifying the architecture of the diaphysis, metaphysis and epiphysis regions, and distribution of cortical and trabecular bone and the periosteum. (Taken from "Bone structure cliffs notes.com)

Throughout skeletal development there is a continuous process of bone resorption and subsequent formation which is influenced by an individual's genetic make-up, hormonal status, dietary intake and physical activity over a lifespan (Nichols et al. 2007; Slemenda et al. 1996). Peak bone mass is attained at all bone sites by the later end of the third decade of life (Haapasalo et al. 1996; Heaney et al. 2000; Nichols et al. 2007; Recker et al. 1992), but the exact timing of attainment of peak bone mass will vary at each bone site. For-example bone gain at the spine is reported to continue well into the later stages of third decade, whereas at the regions of the hip it ends early in the third decade (Heaney et al. 2000; Hui et al. 1999; Recker et al. 1992). Once peak bone mass is attained and the ageing process begins, bone resorption will exceed the bone formation, thus a net bone loss results, and bone strength and integrity is compromised (Khan et al. 2001). The amount of peak bone mass a person can accrue during the early years of life can determine the extent of bone loss in the later years (Heaney et al. 2000) (figure 2. 2)
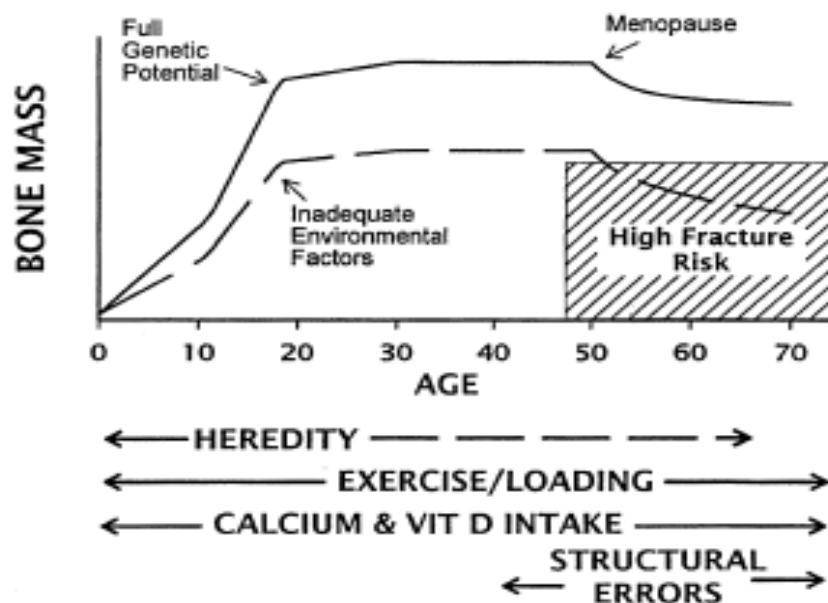
Figure 2.2: A diagrammatic presentation of the attainment of peak bone mass over a lifspan and the influences which can effect peak bone mass, (taken from (Heaney et al. 2000).

2.1.2 Bone remodeling

The modeling and remodeling process of bone is complex taking up to 6 months to complete. The modeling process leads to the formation of new bone; it controls the outer bone and marrow cavity diameter and the cortical thickness of bone, which is adjusted to the longitudinal bone and muscle growth. Macro-modeling of bone will increase the periosteal and endocortical diameter, thus increasing the moment of inertia, and hence the strength in bending of the bone. In contrast mini-modeling will not affect bone size but will change the orientation of bone at sites with high proportion of trabeculae such as at the femoral neck (Cordey et al. 1992; Frost 1989; Hughes and Petit 2010; Jee 2001; Khan et al. 2001).

The remodeling process involves coordinated activity of osteoclasts (cells which break down bone) and osteoblasts (bone formation cells) to remove bone volume and replace it with new bone (Khan et al. 2001; Parfitt 1993; Parfitt 1996; Wolman and Reeve 1995). The osteoblasts form a lineage of osteocytes and lining cells, which represent the end stage of the osteoblastic development. The osteocytes and lining cells are important in directing the osteoclasts towards sites of bone remodelling (Lanyon et al. 1982; Rodan and Matin 1981; Wolman and Reeve 1995). The osteocyctes erode the bone on the trabecular surfaces or drive tunnels into the bone of the cortex. Once the bone has been removed by the osteocyctes, the process reverses and the formation of bone becomes activated thus attracting osteoblasts to the surface. Bone formation will always succeed bone resorption, however not all bone will be replaced, thus remodeling spaces will be incompletely filled (Hughes and Petit 2010; Rodan and Matin 1981; Seibel 2002; Wolman and Reeve 1995). Under-mineralized bone will be found within the remodeling space thus measuring BMD may underestimate the bone tissue (Heaney et al. 2000).

The activation of remodeling is controlled by a number of nutrients and hormones such as calcium, parathyroid hormone (PTH), vitamin D, calcitonin and a spectrum of cytokines. Bone remodelling is essential for calcium homeostasis as the bone consists 98% of calcium. When calcium levels are increased the bone resorption is inhibited, thus the number of bone sites beginning the remodeling process will decrease. The equilibrium of calcium therefore is controlled by the calciotropic hormones which include PTH, vitamin D and calcitonin (Barr and McKay 1998).

2.1.3 Measurements of bone properties

In order to determine the extent of active bone formation bone size, strength and metabolic activity can be measured in humans using a variety of imaging techniques and biochemical markers of bone turnover. Predominantly these techniques are used by clinicians to determine the risk of fracture in older adults, however there are a vast number of researchers who have used the techniques to develop further understanding of bone behaviour.

The most commonly measured bone properties by clinicians and researchers are BMC and BMD. BMC refers to the total grams of bone mineral as hydroxyapatite within a measured bone region (g). BMD refers to the grams of bone mineral per unit of bone area scanned (g/cm^2). As BMD is a measure of bone density it cannot be used to determine bone volume. When comparing bones of different sizes it is important to distinguish the two because an individual who is larger will often have a greater BMC even if BMD is lower. Incorrect conclusions may therefore be drawn when only considering BMD (Watts 2004)

The most direct measure of BMD is the unadjusted score in g/cm^2, as it is useful for comparing subjects of the same age and sex. However as the absolute measurement

varies according to scanner make/model clinicians will diagnose osteoporosis by reporting the T-score, which is defined as the deviation of raw BMD (g/cm^2) compared with the mean BMD of young healthy adults, matched for sex (Who 1994). The T-score comparison was developed according to the WHO guidelines which suggest that a T-score of the hip or spine less than -2.5 indicates an osteoporotic region (Kanis et al. 1994a; Kanis et al. 1994b). The "Who" criteria used to diagnosis osteoporosis was developed based on a number of studies in postmenopausal women which showed a relationship between BMD and fracture risk. The relationship however, is not clearly identified in other populations. The Society of Clinical Densitometry (ISCD) has therefore posed that the T-score should not be used in children and premenopausal women; rather BMD should be expressed as a Z-score (the number of standard deviations from the mean, when matched for age and sex). A Z-score of -2 being identified as the most appropriate value for expressing "low bone density" (Leslie et al. 2006; Lewiecki et al. 2004; Nichols et al. 2007; Who 1994) Furthermore, in athletes the American College of Sports Medicine (ACSM) defines "low bone density" as a BMD Z-score between -1.0 and -2.0 in conjunction with a history of restrictive energy availability, estrogen deficiency and stress fracture. Osteoporosis in athletes is defined as a Z-score < -2.0 in combination with secondary clinical risk factors for fracture (Nattiv et al. 2007).

Bone imaging provides the measure of BMC and BMD. The most commonly used imaging techniques are Dual Energy X-ray Absorptiometry (DXA). Quantitative Ultrasound (QUS) is also used predominantly to reduce radiation exposure associated with DXA. It is believed that QUS reflects bone micro-architecture such as trabecular bone mass connectivity and orientation (Hans et al. 1997). Several studies have determined that QUS can predict fracture risk in postmenopausal women (Huang et al. 1998; Knapp et al. 2001; Thompson et al. 1998). However, due to unknown accuracy of QUS and the moderate correlation

between QUS and densitometry measures there is no agreement in how the QUS data should be interpreted in order to diagnoses fracture risk (Knapp et al. 2004).

DXA is the preferred method to measure BMD and BMC at clinically relevant regions including the lumbar spine (L1-L4), regions of the hip, and the radius. It is an accurate and precise measure of bone properties with reproducible results in humans (+/- 1% at the spine and +/- 1.5% at the femoral neck) and requires low levels of radiation (Gluer et al. 1995). There are limitations associated with DXA, the device will measure all the bone in a given area but will not assess spatial orientation or alignments in that area, or the architecture of bone. It will also only measure 2D imagine where as bone is a 3D structure.

In recent years there has been growing evidence to suggest that bone strength is not only determined by BMD but also by structural geometric properties (Faulkner et al. 2006), therefore it has become popular to measure bone geometric properties such as cross sectional area (CSA') which is highly correlated to BMC, cross sectional moment of inertia (CSMI) and section modulus (Z), a measure of the bones resistance to bending, and femoral strength index, the ratio of estimated compression yield strength of the femoral neck to the compressive stress of a fall on the greater trochanter (Faulkner et al. 2006) (fig 2.3). The measure of Z and hence the resistance to bending is highly dependent on the CSMI. It is important to note that CSMI can be preserved even when CSA and BMC are decreased due to a redistribution of mass (Bonnick 2007). It is believed that geometric properties such as CSA, CSMI, Z and femoral strength index could increase the understanding of the process leading to fracture risk and possibly predict fracture risk in older adults (Beck 2003; Bonnick 2007; Faulkner et al. 2006; Kaptoge et al. 2003; Szulc et al. 2006a). Studies in postmenopausal women over the age of 50 years have shown that individuals with a fracture will often have significantly lower CSMI, Z, CSA and femoral strength index at the femoral neck when compared to controls (Crabtree et al. 2002; Szulc

et al. 2006a). Faulkner et al (Faulkner et al. 2006) reported that femoral neck strength index predicted hip fracture independently of BMD, with those who fractured, having a significantly lower femoral neck index than controls.

There is also evidence to suggest that bone geometric properties can predict the risk of stress fracture in younger adults. Studies of military recruits have shown that recruits who stress fracture have significantly narrower tibial width, CSMI, CSA, and Z than controls (Beck et al. 1996; Milgrom et al. 1989).

The cross-sectional moment of inertia (I_x) for bending in the plane of the image is derived as:

$$I_x = \Sigma a_i d_i^2$$

where: a_i = the pixel thickness x pixel spacing along profile
d_i = distance of pixel from center of mass

For bending in the image plane, stress (σ_b) at a given point (pixel position) in the cross-section is given by:

$$\sigma_b = \frac{M d_i}{I_x}$$

where M = the bending moment. Bending strength is limited by the maximum stress. This occurs on the surface where d_i is a maximum. One can substitute:

$$Z = \frac{I_x}{d_{max}}$$

where Z is the section modulus, hence the maximum bending stress is:

$$\sigma_{max} = \frac{M}{Z}$$

Figure 2.3: The calculation of the cross sectional moment of inertia and section modulus of a bone (Taken from (Beck 2003)

Bone geometric measures are assessed primarily using magnetic resonance imaging (MRI) or quantitative computed tomography (QCT) which will derive a 3D image of bone. These techniques are often expensive, and QCT requires a higher degree of radiation dose. Alternatively peripheral quantitative computed tomography (pQCT) has been used to assess bone health at the forearm and tibia, although measurement is not possible at central sites such as the femoral neck and vertebrae especially in regards to physical activity where bone strength is influenced by the bones size, shape and type (Khan et al. 2001). Peripheral QCT involves substantially lower radiation doses, can measure BMD, BMC and CSA and assess cortical and trabecular bone separately. It has been suggested that QCT may even be more sensitive to bone change than DXA (Jarvinen et al. 1998). Therefore, more researchers are choosing to use pQCT (Khan et al. 2001).

Over the past decade DXA based techniques have been used to estimate the strength of the proximal femur using hip structural analysis (HSA) (Beck 2003; Beck 2007; Bonnick 2007; Martin and Burr 1984), due to the low radiation exposure and inexpensive operating costs. HSA with DXA enables the in-vivo measurement of CSA (a measure of axial compressive strength), CSMI, and Z, cortical thickness and buckling ratio. These geometric properties will vary with the radial distribution of bone tissue. As the periosteal envelope widens bending increases and bone mass is deposited further from the bone axis, thus increasing bone strength (Bonnick 2007). This process is often seen during exercise and early growth. To determine geometric measures using HSA assumptions are made regarding the shape and symmetry of the bone's cross-section (Bonnick 2007; Devlin et al. 2010) (Figure 2.4).
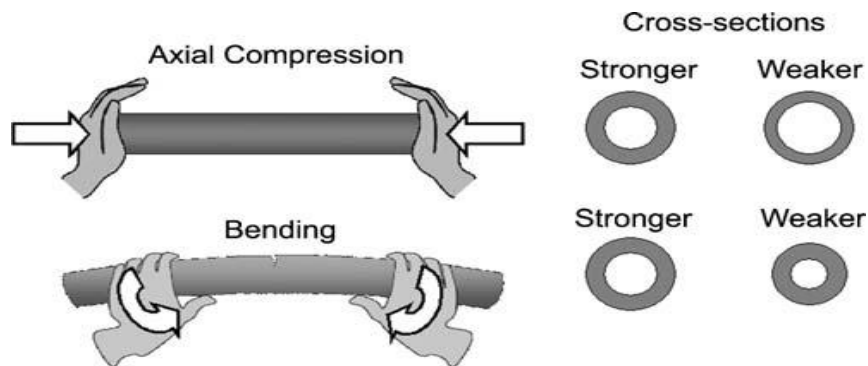
Figure 2.4: Cross sectional area of the bone will determine the axial compression strength, CSMI and Z will determine the bones resistance to bending loads (taken from (Devlin et al. 2010)

The Hologic HSA will traverse the proximal femur at three specific locations, determining the narrow neck (narrowest diameter of the femoral neck), the intertrochantanteric (bisector of the neck shaft angle) and the shaft (2cm distal to the midpoint of the lesser trochanter) regions. From the three regions the bone mass across the bone is determined and bone geometric properties are derived using specific formulae (figure 2.5) (Beck 2003; Khoo et al. 2005). Whereas the Lunar Prodigy HSA (or advanced hip analysis (AHA) will traverse only the narrow neck region of the femur to derive estimates of CSA, CSMI, Z and other geometric properties (fig 2.6) (Faulkner et al. 2006). It is important to acknowledge that DXA derived methods are not the optimal method to measure bone geometry as DXA was designed only to assess bone density and provides only 2D data in one plane. Estimations of bone geometry require precise positioning in order to obtain accurate results, thus caution should be taken when assessing bone geometry with DXA.
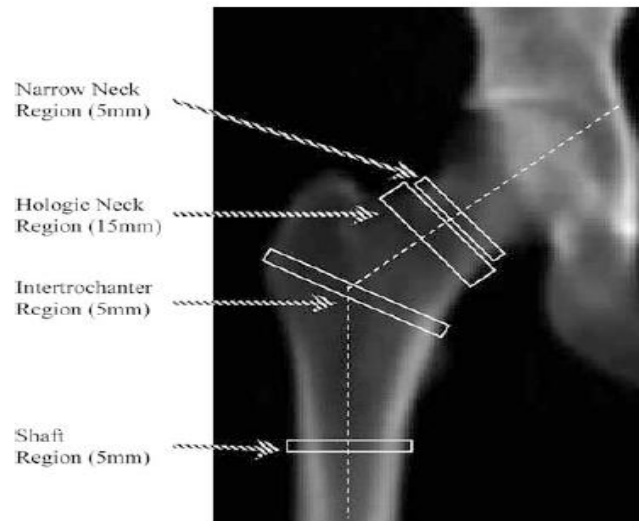
Figure 2.5: Illustrates the Hologic regions of the hip traversed to determine the narrow neck, intertrochanteric and shaft regions, which are used to determine bone mass profiles to derive geometric properties (taken from(Kaptoge et al. 2003) .
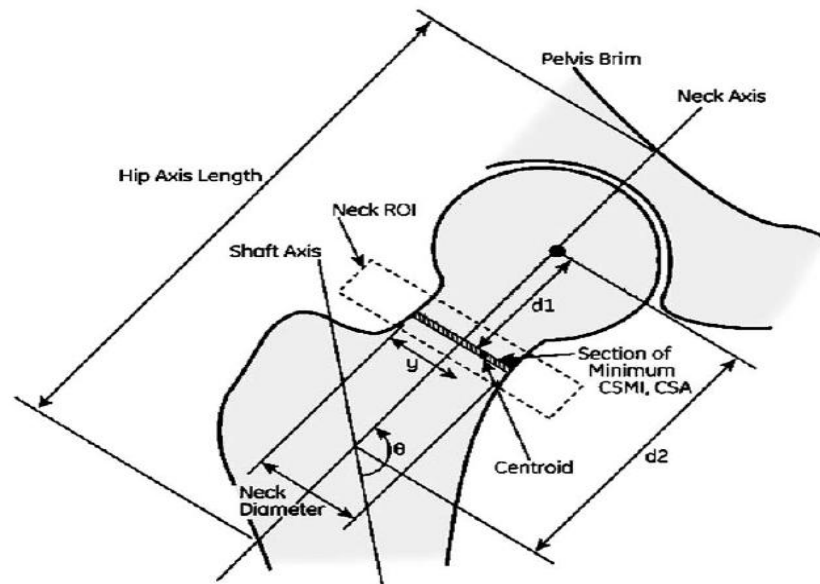
Figure 2.6: Illustrates the Lunar advanced hip analysis neck region of interest used to derive bone geometric properties CSA, CSMI, Z and other geometric properties (taken from (Faulkner et al. 2006).

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During adulthood the process of bone remodeling is constantly repeated to remove old bone and replace it with new. Information of this metabolic activity of bone remodeling can be examined using biochemical markers which give information about bone turnover, bone resorption and bone formation (Garnero 2009; Lindsay 1999). Biochemical markers may be divided into two categories: those which measure bone formation reflecting the activity of osteoblasts, these are often by products of collagen synthesis, matrix proteins or osteoblastic enzymes, and markers which measure resorption reflecting the activity of osteoclasts, which are most often the byproducts of degradation of type I collagen (Eastell and Hannon 2008). The most common bone markers used in postmenopausal osteoporosis studies are listed in table 2. 1.

Table 2.1: Most common biochemical markers used in postmenopausal osteoporosis (modified from (Eastell and Hannon 2008)

Bone formation	Bone Resorption
Osteoblast enzyme	*Osteoclast Enzymes*
Total alkaline phosphatase	Tartrate resistant acid phosphatase
Bone alkaline phosphatase	Cathepsin K
Matrix Protein	*Cross-Linked telopeptides of type I collagen*
Total Osteocalcin (OC)	N-terminal cross-linked telopeptide (NTx)
	C-terminal cross-linked telopeptide (CTx)
	C-terminal cross-linked telopeptide generated by matrix metalloproteinases
By-products of collagen synthesis	*Collagen degradation products*
Procollagen type I C-terminal propeptide (P1CP)	Hydroxyproline
Procollagen type I N-terminal propeptide (P1NP)	Pyridinoline (PYD)
	Deoxypyridinoline (DPD)

Bone formation markers (OC, P1CP, and P1NP) are predominantly measured in the serum where as resorption markers may be measured in both the serum and the urine (Eastell and Hannon 2008; Garnero 2009). Collagen molecules in the bone matrix are structured to form fibrils which are joined by cross-links. Breakdown of cross linked collagen produces the degradation products (PYD, DPD CTx and NTx), which are released into the circulation and cleared by the kidneys (Eastell and Hannon 2008).

Biochemical markers are clinically important to assess fracture risk and monitor treatment efficacy in postmenopausal women with osteoporosis (Garnero 2009). There is evidence to suggest that biochemical markers can predict individuals with increased bone loss and fracture risk (Garnero 2004; Garnero 2009; Stepan 2000; Szulc and Delmas 2008), predict patients who will benefit from treatment (Garnero 2009), to assess efficacy of treatment (Cremers and Garnero 2006; Szulc and Delmas 2008), and promote adherence to therapy (Szulc and Delmas 2008). In the athlete population biochemical markers have not been proven to be clinically useful in predicting the likelihood of stress fracture development (Bennell et al. 1998), however the assay used previously may not have been sensitive enough to detect change between the stress fracture group and the controls. Biochemical markers have shown that nutrition, not oestrogen is a possible factor of bone loss in athletes with exercise associated amenorrhoea (Zanker and Swaine 1998a). In athletes with amenorrhoea bone turnover seems reduced with bone formation and bone resorption markers lower than eumenorrhoeic controls (Camacho and Kleerekoper 2006; Christo et al. 2008; Misra 2008).

Even though biochemical markers are proving useful predominantly in postmenopausal women, there are considerations to consider when assessing bone markers which include: bone formation and resorption markers are sensitive to circadian rhythms and often peak in the early morning between 4 and 8am and are at their lowest point in the mid to late

afternoon (Eastell et al. 1992). It is also important to consider the large variability associated with bone markers measured in the urine (20-30%) and serum (10-15%, reduced to 3-5% with automated assays) due to the intra and inter assay, and individual patient biological variability (Camacho and Kleerekoper 2006; Eastell et al. 1992; Lindsay 1999).

2.1.4 Endocrinology and bone

The regulation of bone resorption and formation is intimately controlled by the endocrine system. Interactions between hormones can result in modifications of a number of tissue features of the skeleton, thus any abnormalities within the endocrine system may have a detrimental effect on bone development (Khan et al. 2001). The hormones that influence bone can be classed as either controlling hormones or influencing hormones for levels of bone related agents such as the regulation of serum calcium. The main controlling hormones of bone are the calciotropic hormones. As mentioned above, these hormones respond to changes in plasma calcium levels thus controlling bone formation and resorption (Barr and McKay 1998; Khan et al. 2001; Wolman and Reeve 1995). Researchers have also linked growth hormone, corticosteroid, thyroid and sex hormones with having either a controlling or influencing effect on bone, (Wolman and Reeve 1995). The sex hormones, in particular oestrogen, progesterone and testosterone have been strongly associated with BMD as they suppress bone remodelling and promote bone formation in both males and females (Ekblad et al. 2000; Greendale et al. 1997; Smith et al. 1994). However, oestrogen is believed to have the greatest association with bone, thus the main focus of this section will be to highlight the effect oestrogen has on bone maturation.

Oestrogen is believed to affect bone both directly and indirectly. Directly oestrogen inhibits bone resorption, decreasing bone turnover. Indirectly oestrogen affects bone formation via

the gut, kidneys and parathyroid gland to maintain calcium levels by changing the set point at which the parathyroid hormone responds to calcium (Pacifici 1996; Prince 1994). The main skeletal function of oestrogen in adulthood is to preserve the bone mass and geometry by suppressing bone remodeling in males and females (Jarvinen et al. 2003b; Riggs et al. 1998; Saxon and Turner 2005; Saxon and Turner 2006) . It has been well established that the development of bone during the pre-pubertal years is very similar in both males and females. However, during puberty when levels of oestrogen and androgens change to different extents in males and females, the location of bone accrual will also differ. During pubertal development in males it is believed that periosteal apposition and endocortical resorption continue, but in females' periosteal apposition decreases and endocortical apposition increases, causing narrow medullary cavities following puberty. In both males and females, however increases in cortical thickness during puberty will be observed (Saxon and Turner 2005). This theory can be supported by the findings of a number of animal studies (Kim et al. 2003; Turner et al. 1990b), which have often shown that in male rats androgens enhance periosteal bone formation, with no effect on the endocorticol surfaces, whereas in female rats oestrogen inhibits the bone formation on the periosteal surface but enhances the endocorticol contraction. The result is that males have a larger bone width and medullary cavity and a marked resistance to fracture compared to females (Bass 2003; Bass et al. 2002)

The reduction in the secretion of oestrogen in females after the menopause is associated with accelerated bone loss and an increased risk of fracture. When oestrogen levels are dramatically decreased after menopause periosteal bone formation is no longer inhibited, however the endocorticol contraction will continue, this in turn causes the cortical surface to move further away from the center axis of the bone increasing the CSMI and Z, thus possibly improving bones strength in bending, even through bone mass is decreased (Ahlborg et al. 2003; Cummings and Melton 2002; Saxon et al. 2007; Saxon and Turner

2005; Saxon and Turner 2006; Seeman 2008; Szulc et al. 2006b). Potentially, the greater risk in women for osteoporosis is a result of the geometric differences caused during pre and post-pubertal bone formation (Cummings and Melton 2002).

2.2 Bone adaptation to mechanical loading

Researchers have long tried to understand the principles of bone adaptation to mechanical loading, (Frost 1998; Frost 2003; Turner 1999; Wolff 1892). Wolff's law (Wolff 1892) states that bone will optimize structure to withstand functional loading and ensure metabolic efficiency in locomotion. The cells responsible for mechanical modeling and remodeling will thus adapt bone mass, bone geometry and material properties to withstand the loads applied. More recently it has been postulated that the response to loading is controlled by a mechanostat (Frost 1987; Lanyon et al. 1982).

2.2.1 The mechanostat

The mechanostat concept (Frost 1987; Lanyon et al. 1982), is that bone will adapt to mechanical loading via a homeostatic regulatory mechanism in which a minimum effective strain (MESr – remodeling threshold) is necessary for bone maintenance. In addition to strain magnitude (defined as the relative change in bone length) it appears that strain rate (rate of strain development determining bones adaptive response) and strain distribution (distribution of strain across a bone) are more responsible for stimulating bone loading than strain cycles (the number of bone repetitions which change bone dimensions)

Frost et al (Frost 1987), postulated that mechanical thresholds are controlled if bone is added or removed to the skeleton in response to loading. If bone is immobilized and strain magnitude is below the MESr threshold (< 50 -200 µε) there will be remodeling and bone loss (figure 2.7). This theory can be supported by bed rest and space flight studies, showing bone density is lost during periods of weightlessness (Lang et al. 2006; Laugier et

al. 2000; Rittweger et al. 2005). More recently, research has provided evidence that similar losses in bone occur following fracture (Eyres and Kanis 1995; Petersen et al. 1997), stroke (Bainbridge et al. 2006) and athletic detraining (Nordstrom et al. 2005b; Snow et al. 2001; Winters and Snow 2000). When strains are applied which are within the MESm (modeling) threshold or physiological loading zone (200-2000µε) bone strength is maintained as the remodeling is controlled at a steady state. During periods of bone overload (2000-3000µε) new bone will be formed thus bone mass and bone strength will increase, however if the load exceeds the overload threshold (> 4000 µε) the bone will enter a state of overuse causing micro-damage to occur (Frost 1987; Frost 2003; Lanyon 1987; Turner 1998).
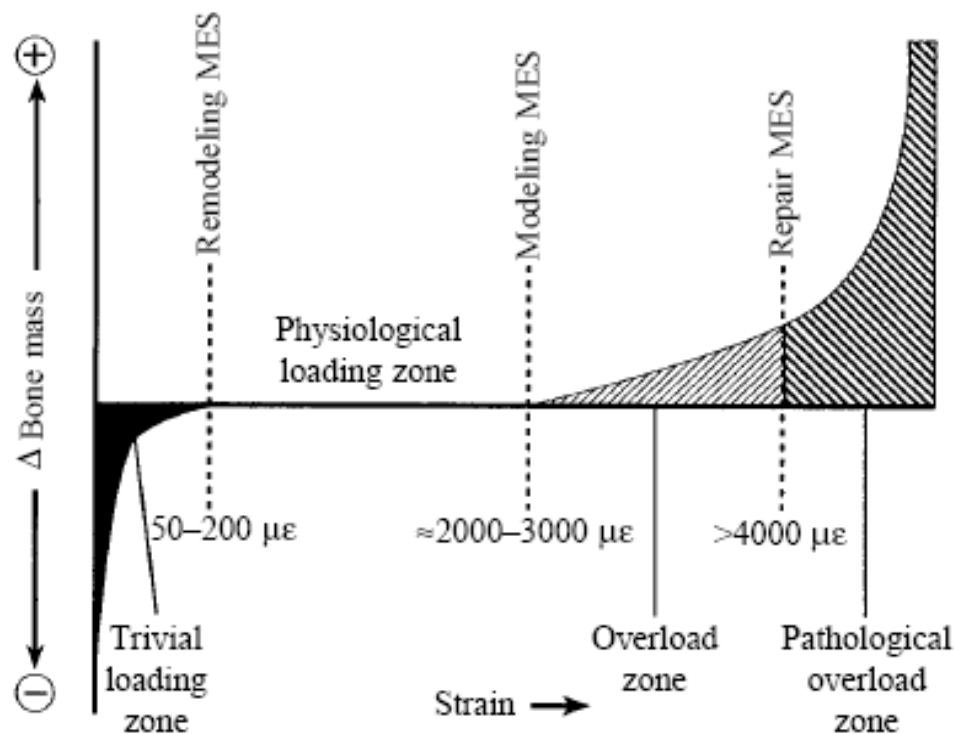
Figure 2.7: The Mechanostat theory (taken from (Hughes and Petit 2010)

As bone adapts to the mechanical loads it will strengthen such that the same load applied will evoke lower strains, returning loading to physiological loading zone. Bone will continue to adapt if mechanical loading is continuously increased consistent with the concepts of progression and overload (Kohrt et al. 2004; Turner et al. 1994).

In order for the bone mechanostat to be effective it must possess a number of independent components which are stimulated to control the system, returning it to a state of homeostasis. The independent components make up the mechanotransduction process and include a stimulus, a sensory mechanism and an effector mechanism (Hughes and Petit 2010; Khan et al. 2001; Turner et al. 1994).

The stimulus is the mechanical strain applied to the bone during loading; this can include the strain magnitude, rate, distribution and cycles (Hughes and Petit 2010; Khan et al. 2001; Robling et al. 2000a; Robling et al. 2000b; Turner 1998; Turner et al. 1994). The strain magnitude refers to the amount of change in bone length during loading. Animal studies have shown that bone can regulate strain magnitude by increasing bone formation (Turner 84). Moderate intensity running will therefore produce positive effects on bone mass in the cortical and trabecular regions in people not already adapted to running, whereas more intense cardiovascular physical activities may have negative responses with decreased bone mass, and trabecular thinning (Kohrt et al. 2004).

Strain rate determines the bones adaptive response to strain development and release therefore, a dynamic (e.g. jumping activity) rather than a static mechanical load (single sustained force) will result in an adaptive bone response (Kohrt et al. 2004; Martin and Burr 1989; Umemura et al. 1995). The strain distribution refers to the way in which strains are distributed across the bone (Khan et al. 2001). The response of bone to the strain distribution is greatest when the bone loading is unusual, variable, and dynamic deviating from normal daily activity (Lanyon 1996). Finally the strain cycle refers to the number of

load repetitions (Khan 2000). Animal studies have shown that bone response is improved with brief but intermittent exercise, identifying 36 strain cycles of loading to be optimal for a bone response to large loads in turkeys (Rubin and Lanyon 1984). Additional loading cycles did not provoke any greater response (Rubin and Lanyon 1984; Umemura et al. 1997). Skerry et al (Skerry 2006), identified that the various stimuli for bone adaptation can be incorporated into a unified concept termed the customary strain stimulus which is sex and site specific and can be modified genetically, biomechanically and pharmacologically. Studies identifying the optimal type intensity, duration and frequency of bone loading in association to physical activity are predominantly animal based.

Sensory mechanism or mechanocoupling is the term which signifies the process by which load is signaled to the cells. The cells which are often responsible for sensing a load are the mesenchymal stroma, the cell lineage, osteoblasts, osteoclasts and the osteocytes. The structures of the osteocytes allows for the local indication of changes in loading and, are critical for resorbing, forming and maintaining bone mass during alterations of mechanical loading (Hughes and Petit 2010; Turner et al. 1994).

The effector mechanism refers to a component which produces new or rearranged bone, thus in the response to bone loading the independent action of the osteoblast and osteoclasts will be responsible. Modeling as an effector mechanism is critical in growth as it is responsible for the size and shape of bone whereas remodeling is localized and requires the coupled action of both the osteoblasts and osteoclasts to produce new bone (Hughes and Petit 2010; Parfitt 1994).

The intensity of mechanical loading associated with physical activity is often approximated by measuring the ground reaction forces (GRF). The higher the impact of the physical activity the greater the GRF for example jumping will generate forces which exceed six times body weight whereas as running will induce force three times body weight (Bassey

et al. 1997; Heinonen et al. 1996). Therefore, high mechanical loading, and moderate cardiovascular activity will result in the greatest osteogenic response (Kohrt et al. 2004).

In women who experience amenorrhoea (lack of menstrual function), caused by low oestrogen levels, the sensitivity of bone mechanical loading is decreased. The adaptive response to mechanical loading requires the involvement of the oestrogen receptor (Lanyon et al. 2004) if the oestrogen receptor is blocked due to oestrogen deficiency this will result in an impaired bone formation response to mechanical loading (Borer 2005; Khan et al. 2001; Saxon and Turner 2005; Saxon and Turner 2006).

2.3   Bone health and exercise

In humans, exercise appears to play an important role in maximizing peak bone mass during early growth into the third decade, and retaining bone mass during the fifth decade, thus reducing bone loss with ageing. The majority of the research identifying the beneficial effects of exercise to BMD has been cross-sectional, comparing sedentary individuals with those who report to be physically active or comparing athletes with non-athletes (Kelley et al. 2002; Khan et al. 2001). Even though the information from cross-sectional studies have produced a valuable indication of exercise benefits it is important to be aware of the numerous confounding factors, and thus prospective studies are more beneficial. Bone strength is a measure of bone mass and bone size yet few studies have evaluated the effects of exercise on bone geometry, in athletes and sedentary individuals. Bone geometry may be a better indicator of osteoporosis risk than BMD alone.

2.3.1   Bone property responses to exercise

Both cross-sectional and prospective studies have shown strong evidence that BMD is often higher in physically active individuals than sedentary controls, with premenopausal athletes often having 10% higher BMD values than their sedentary counterparts

(Drinkwater et al. 1984; Haapasalo et al. 1996; Kannus et al. 1994a; Marcus et al. 1992; Snow 1996; Uusi-Rasi et al. 1998).

The increased BMD seen in athletes could be due to sports participation in the early growth years. There is substantial evidence indicating that the childhood and adolescent years are beneficial for optimizing peak bone mass, and increased mechanical loading through sports participation during the pubertal years will promote bone mineralization, thus increasing bone mass by as much as 2.2% every year until peak mass occurs (Kontulainen et al. 2001; Nichols et al. 2007; Taaffe et al. 1997). Selection bias may determine which individuals become athletes, for example it may be those who are predetermined to have high BMD that become active in sport and therefore resulting in high peak bone mass.

Researchers have identified that exercise interventions lasting 6 to 36 months can increase BMD between 1-5% in previously sedentary individuals (Bassey et al. 1998; Winters and Snow 2000) However BMD increases are site and exercise specific (Bassey et al. 1998; Winters-Stone and Snow 2006). In pre-menopausal women it has been shown that high impact and odd impact loading, with a high magnitude being effective at increasing BMD at the lumbar and femoral neck, whereas high impact loading alone only increased BMD at the femoral neck (Martyn-St James and Carroll 2010). In post-menopausal women exercise programs involving jogging and low impact loading and mixed impact activity with high magnitude such as resistance training reduced bone loss (Martyn-St James and Carroll 2010). These findings thus support the mechanical loading theory that bone will adapt to unusual patterns of mechanical strain.

Numerous cross-sectional studies in athletes have shown that BMD differs between sports with high impact sports such as gymnastics and weightlifting reporting higher BMD compared to low impact sports such as swimming and cycling (Bennell et al. 1997a;

Heinonen et al. 1994; Meyer et al. 2004; Mudd et al. 2007; Nikander et al. 2005; Taaffe et al. 1997; Taaffe et al. 1995). In swimmers the BMD values are reported to be similar to those in sedentary individuals indicating that non-weight bearing sports do not have beneficial gains in bone mass that can help prevent osteoporosis (Grimston and Zernicke 1993; Mudd et al. 2007; Taaffe et al. 1995).

High impact loading and exposure to high rates of bone strain are crucial for stimulating osteogenesis and heightened bone development (Egan et al. 2006; Mudd et al. 2007). Improved bone structural characteristics are found in athletes who participate in sports which require short bursts of high impact loading such as in volleyball and basketball (Egan et al. 2006; Nikander et al. 2005; Snow-Harter and Marcus 1992) This supports earlier animal mechanical loading studies which have shown that bone adapts to short bouts of high intensity strain cycles (Robling et al. 2000b; Rubin and Lanyon 1984; Umemura et al. 1997).

In high impact sports the type of training and body composition of athletes can affect the levels of BMD gain. Gymnasts have been reported to have BMD values 30-35% higher at the spine compared to runners despite similar menstrual patterns (Robinson et al. 1995). High impact, moderate cardiovascular intensity training has been identified as the most beneficial method of loading to promote an osteogenic response. Low repetitive impact and high cardiovascular intensity training on the other hand can result in detrimental bone health to the lumbar spine (Bilanin et al. 1989; Hetland et al. 1993; Hind et al. 2006; Macdougall et al. 1992). A negative correlation has been found between BMD of long distance runners and the distance run per week, with lumbar spine BMD 19% lower among runners who ran over 100km per week (Hetland et al. 1993).

There is evidence to suggest that the increased BMD shown in athletes is site-specific to the loaded bone. Cross-sectional comparisons of playing and control limb of racket sport

athletes with a within subject control limb have shown BMD higher in the dominant playing arm than the non-dominant control arm (Haapasalo 1998; Haapasalo et al. 2000; Kannus et al. 1994a). When compared with a non-athlete control, tennis players have 10-15% higher BMD at the lumbar spine and femoral neck, but have comparable BMD values of the non dominant radius. Thus, BMD is higher in sites which are loaded, but will remain similar at non-loaded sites when compared with sedentary controls (Calbet et al. 1997; Kannus et al. 1994a).

Past literature is not sufficient to draw definite conclusions regarding site-specific responses of bone to varying sports training. Sports which involve lower limb loading such as running are often associated with higher BMD in the legs (Morel et al. 2001; Nevill et al. 2004) (Duncan et al. 2002a; Mudd et al. 2007). Conversely, it has been reported that lower limb loading sports have produced positive osteogenic responses in the upper limbs (Nichols et al. 1995). The conflicting findings could possibly be as a result of differing methodology and recruitment criteria between studies. Some studies report regional BMD values from the total body scans, which are less accurate in obtaining BMD values than specific regional measures. Recruitment of athlete groups with differences in training type and intensity may result in comparative difficulties for example, in athletes in lower limb loading sports such as runners participation in resistance training may present with higher BMD at the spine and radius compared to a group of non-resistance trained runners (Nichols et al. 2007).

Specialized training differences could be responsible for the reported sport specific differences in the BMD of athletes. Duncan et al (Duncan et al. 2002a), compared a group of normal menstruating female runners, triathletes, cyclists, and swimmers and found that runners had site specific BMD 9-13% higher than swimmers and cyclists but that there was no difference in all BMD sites between runners and triathletes, possibly suggesting

that running 3 to 4 times per week can promote increases in BMD in triathletes. Even though athletic training cannot prevent bone loss during aging the higher peak may delay the onset of osteoporosis (Kudlac et al. 2004).

Bone geometry and exercise

Compared to the vast evidence for the association between BMD with exercise, there is limited understanding of the relationship between exercise and bone geometry, an important component of bone strength (Khan et al. 2001). The human body may adapt to mechanical loading through exercise by improving measures of bone geometry (CSMI, CSA, Z), hence resistance to bending without any marked change to bone mass (Adami et al. 1999; Jarvinen et al. 2005; Kimmel 1993). Animal studies have suggested that exercise evokes changes in bone shape which are disproportionately greater than BMD changes (Barengolts et al. 1993; Iwamoto et al. 1999; Joo et al. 2003; Notomi et al. 2001; Robling et al. 2002; Smock et al. 2009; Wallace et al. 2007).

The majority of studies investigating the association between bone geometry and exercise have been conducted in pre-pubertal children illustrating that CSMI, and Z, thus bone's resistance to bending is markedly improved (Bass et al. 2002; Ward et al. 2005). These findings are consistent with the suggestion that bone formation occurring on the periosteal surfaces during pre-pubertal years, resulting in periosteal apposition, will increase bone strength (Bass et al. 2002; Saxon et al. 2005).

Cross-sectionally it has been shown that bone geometric properties in prepubertal athlete groups are significantly higher than in controls (Bass et al. 2002; Daly 2007; Ward et al. 2005). Ward et al 2005 (Ward et al. 2005), reported significantly higher bone geometric properties in the radius in gymnasts than in non-active controls, although BMD values were not markedly different between groups. This study indicates that during the pre-pubertal years there is greater skeletal development in bone and muscle geometry than

changes in bone mass. However, as maturation develops into the later stages of puberty the bone response to mechanical loading through exercise changes, showing significant differences in medullary contraction rather than periosteal apposition. Therefore it could be suggested that the increases seen in bone geometry studies involving children are caused by maturation and not independently related to exercise (Bass et al. 2002).

In adult athletes, cross-sectional studies provide compelling evidence that bone geometry is adapted in response to exercise in the absence of a change in volumetric BMD (Ducher et al. 2005; Haapasalo et al. 2000; Heinonen 2001). In tennis players the CSA, and strength index of the humerus and radial shaft were significantly higher in the playing arm, (16 -21% and 23-27% respectively compared to the non-playing arm), with no significant differences in bone density between arms. Increased bone strength thus appeared to be due to increased bone size and not due to changes in volumetric BMD. The altered bone geometry in the playing arm of tennis players demonstrates the need to assess bone geometry rather than bone density alone to determine bone strength and possible risk for fracture (Haapasalo et al. 2000).

Recent research has shown that loading modality is a strong external determinant of structure, and associated with strength of the femoral neck. Athletes who participated in either high impact or odd impact loading sports showed higher CSA (22% and 27% respectively) and Z (22% and 26% respectively) than non-athlete controls after controlling for body size. No differences in bone geometric properties were reported between low impact sports and controls (Nikander et al. 2005). Accordingly, it seems the high strain rates from the dynamic and unusual directional movement are enhancing the osteogenic effect of loading on bone structure (Nikander et al. 2009; Vainionpaa et al. 2007).

It has been reported that participation in relatively high volumes of weight-bearing exercise such as running is associated with greater CSMI, bone strength indices, thicker cortices, denser trabecular and larger diaphyses compared to participants in non-weight bearing

activities (Duncan et al. 2002b; Greene et al. 2004; Greene et al. 2005; Nikander et al. 2005), thus enabling the bone to absorb more load energy per unit area increasing the resistance to fracture (Currey 2002). Thus, the outcome measure of CSMI or Z may be a more appropriate measure of strength than BMD which does not directly measure strength (Kaptoge et al. 2003).

Even though there are limited studies investigating the association between exercise and bone geometry it would appear that exercise can influence bone geometry in the absence of increased BMD. However further research is needed to fully understand the association between BMD and bone geometry. It is unknown whether geometric adaptation can counteract low BMD values, and therefore act to protect against fracture risk.

2.3.2 Effects of seasonal training and detraining on bone properties

It is well established that participation in sport during the pubertal years will result in a higher peak bone mass thus presumably reducing the risk of osteoporosis in later years (Heaney et al. 2000). Equally there is compelling evidence to show that athletes who participate in all modalities of sport will have increased BMD compared to their sedentary counterparts (Kannus et al. 1994a; Mudd et al. 2007). High impact and odd impact loading at moderate intensities are believed to have the greatest oestogenic response to bone. However, once the stimulus from the mechanical loading is reduced or removed the beneficial effects to bone are potentially lost. This is evident in studies of space flights which have shown detrimental losses in bone mass during periods of weightlessness (Lang et al. 2006; Laugier et al. 2000; Rittweger et al. 2005).

Similarly in exercise intervention studies researchers have identified that the beneficial gains in BMD following exercise programs can be lost within 12 months of mechanical disuse (Dalsky et al. 1988; Winters and Snow 2000). Winters et al (Winters and Snow 2000) found that exercise increased BMD by as much as 2.5% at specific loading sites in

previously sedentary individuals following a 6 month exercise intervention. However after 12 months of detraining the gains in BMD were returned to the baseline measures. No significant changes were observed in controls at any time point.

The effect of training and detraining could be explained by the principle of diminishing return. Adaptation of bone is curvilinear in nature in that it will respond to initial changes in mechanical stimulus quickly thus increasing BMD, but once adaption has occurred the beneficial gains will taper (Dalsky et al. 1988). Therefore in training and detraining studies the bone is first responding to the initial increase in mechanical stimuli by increasing bone formation and bone strength, however once the stimulus is removed and mechanical loading is below the threshold for remodeling, bone will be lost. Therefore, the mechanical strain that stimulates adaptation must be continuously applied or the adaptation will be lost (Hughes and Petit 2010; Lanyon et al. 1982; Turner 1999).

In athletes the magnitude of bone loss following detraining is relatively unknown and findings are often conflicting as many cross-sectional studies do not account for the BMD values before detraining occurs (Karlsson et al. 1996; Karlsson et al. 1995; Recker et al. 1992), similarly the age at which sporting participation commenced is not indicated. BMD is often higher in athletes who participated in sports prior to puberty therefore following detraining values will possibly remain above average. However, without determining BMD prior to detraining, and training years before puberty the rate of bone loss can not accurately be assessed.

In retired athletes, some studies suggest bone maintenance (Kontulainen et al. 1999; Kontulainen et al. 2001; Pollock et al. 2006), while others suggest that the benefits of bone gain are lost during detraining (Gustavsson et al. 2003; Kudlac et al. 2004; Nordstrom et al. 2005b). Kontulainen et al (Kontulainen et al. 1999) prospectively monitored young adult tennis players following retirement. BMD measures were taken during the final competitive

year of playing, with follow ups at least two years after retirement. It was found that BMD was not lost in retired players, and side-to-side differences in playing and non-playing arms were consistent with increased BMD in the playing arm. In contrast, bone loss has been reported at weight bearing bones of retired ice hockey players following five years of detraining (Nordstrom et al. 2005a; Nordstrom et al. 2005b). Differing physical activity levels following retirement and age at onset of training could be responsible for the conflicting findings in that athletes who remain physically active may maintain bone mass to a greater extent than those who do not.

Even though it is well established that athletes have an increased peak bone mass due to increased mechanical loading when compared to their sedentary counterparts, it is still unclear whether the rate of bone loss during ageing differs between the groups. Kudlac et al 2004(Kudlac et al. 2004), suggest the rate of bone loss will remain the same in individuals regardless of peak bone mass, but an increased peak bone mass will help to prevent osteoporosis later in life.

The evidence of seasonal detraining in current athletes conflicts with findings from studies of retired athletes revealing that significant bone loss can occur following short (3-4 months) periods of detraining (Carbuhn et al. 2010; Klesges et al. 1996; Snow et al. 2001; Winters and Snow 2000). Snow et al (Snow et al. 2001), illustrated a year on year seasonal trend in gymnasts with increases in BMD at the spine (3.7%), and total hip (2.3%), following an 8 month training season and decreases of 1.5% at the spine and 1.5% at the hip following 4 months of detraining. In basketball players offseason losses in BMD have been reported to be as high as 3.3% (Klesges et al. 1996). The seasonal decreases could increase the risk of stress fracture in sports with an identified season and offseason. Furthermore, the stress fracture risk may be increased if athletic training requires the bone to constantly adapt to high magnitudes of loading and unloading. This

could cause bone weakening as there will not be adequate time for the bone to adapt to change (Johnson et al. 1994; Myburgh et al. 1990; Snow et al. 2001).

Given that long distance running swimming and cycling may have a negative effect on BMD particularly in conjunction with amenorrhoea it would seem logical to expect negative changes in BMD over a 12 month competitive season in triathletes. However, in a recent study it was revealed that whole body BMD did not change in male and female triathletes over a season,(McClanahan et al. 2002). This study was limited to whole body BMD and did not report changes at weight bearing sites such as the hip or spine which may be directly affected by seasonal training.

Further studies are needed to establish a full understanding of the potential risks of seasonal detraining in athletes. The majorities of the studies to date, have small sample sizes, and as a result may be under powered to detect true bone loss. There is no compelling evidence showing the effects of seasonal training in athletes who train year on year with no identifiable offseason, but have seasonal changes in training which could affect the type of loading on the bone, possibly causing negative bone adaptations.

2.3.3 Factors affecting bone properties in athletes

It is well established that mechanical loading through exercise can be beneficial to bone health, with athletes having BMD values 10% higher than their sedentary counterparts. However, exercise may also have a detrimental effect on bone in some athletes. Endurance athletes often conduct high volumes of training at high cardiovascular intensity which can result in a negative association with bone health. It has been reported that approximately 3 to 8% of athletes will be diagnosed with low BMD, and female athletes in particular are at a greater risk due to the components of the female athlete triad (Nattiv et al. 2007; Torstveit and Sundgot-Borgen 2005a; Torstveit and Sundgot-Borgen 2005b;

Warren et al. 1986). The female athlete triad refers to the interrelationship between menstrual dysfunction, low energy availability and low BMD (Nattiv et al. 2007).

2.3.4 Menstrual dysfunction

An athlete diagnosed with menstrual dysfunction will either have primary amenorrhoea (defined as a delayed age at menarche resulting in no menstrual flow), secondary amenorrhoea (absence of menstrual function lasting 3 months or more starting after the start of menarche), or oligomenorrhoea (having 4-9 menses per year). Even though amenorrhoea and oligomenorrhoea are two forms of menstrual dysfunction the symptoms may reflect two hormonally distinct conditions. Primary amenorrhoea with delayed age at menarche may be associated with hyperandrogenism. Secondary amenorrhoeic athletes tend to display a hormonal pattern in agreement with hypothalamic inhibition due to energy deficiency, whereas oligomenorrhoeic often exhibit hyperandrogenism due to increased diurnal secretion of testosterone and late menarche (Rickenlund et al. 2004) . Eumenorrhoeic athletes are normally menstruating with >10 menses per year (Bennell et al. 1997b; Nattiv et al. 2007).

The prevalence of menstrual dysfunction in female athletes has been reported to range between 1 to 44% compared to only 2 to 5% in the general population (Bennell et al. 1997b). The variation in reported prevalence in athletes is due to the definition of menstrual dysfunction, the sporting population, age, nutritional status and the activity level of the individuals (Bennell et al. 1997b). It appears that athletes who conduct high volumes of training at high cardiovascular intensity in sports emphasizing leanness such as gymnastics, and long distance running are at an increased risk of menstrual dysfunction (Bennell et al. 1997b)

Amenorrhoea and Oligomenorrhoea are normally associated with low concentrations of circulating oestrogen, which can result in increased resorption (Ducher et al. 2009). Evidence suggests that amenorrhoea is caused by an alteration of the pulsatile release of gonadotrophin releasing hormone (GnRH) during exercise, leading to a reduction in the pulsatile release of luteinizing hormone (LH) which affects ovarian function. Short luteal phases and anovulation along with a decrease in progesterone production has been reported to result in a loss of 2-4% per year of BMD at the lumbar spine (Bennell et al. 1997b; Prior et al. 1990; Snead et al. 1992; Warren 1999).

Delayed menarche and the duration of amenorrhoea are believed to be associated with decreased BMD, however the findings are conflicting (Bennell et al. 1997b; Drinkwater et al. 1984; Malina 1983; Rutherford 1993; Stager and Hatler 1988; Young et al. 1995). Menarche is often attained later in athletes compared to non-athletes (Malina 1983; Stager and Hatler 1988). If menarche is delayed resulting in primary amenorrhoea, the rate of bone accrual may be lower during adolescence, thus resulting in decreased BMD (Young et al. 1995). However, the findings in cross-sectional studies of athletes are conflicting with some showing delayed menarche to result in lower BMD (Robinson et al. 1995; Warren 1992), and others having showing delayed menarche to have no correlation with BMD (Myburgh et al. 1993; Rutherford 1993). The sample sizes in these studies however were small and designs do not allow for confounding factors which may influence BMD, thus clarification of this association in a larger sample of athletes is required.

The duration of menstrual dysfunction may be a better determinant of bone mass than current menstrual status, and delayed menarche. It is often difficult to determine bone loss in current amenorrhoeic athletes without an indication of menstrual history as bone loss is often greater in the first year of amenorrhoea, and levels out after this point, following the principle of diminished return (Cann et al. 1984; Drinkwater et al. 1990; Drinkwater et al.

1986; Myburgh et al. 1993). In athletes with a longer history of amenorrhoea annual bone loss may be markedly lower, but cumulative bone loss substantially higher thus determining a cumulative measure of menstrual dysfunction such as the menstrual index may give a better indication of bone loss (Bennell et al. 1997b; Grimston et al. 1990).

The cross-sectional studies in the literature examining the effects of menstrual dysfunction on bone health have suffered from a number of methodological inconsistencies and limitations, including small sample sizes, poor definitions of menstrual dysfunction, varying densitometry techniques and differing sporting activities, making it difficult to synthesize findings between studies. These inconsistencies and limitations result in large variations of reported BMD (between 0.9 to 20% lower) when comparing a/oligomenorrhoeic and eumenorrhoeic athletes (Carbon et al. 1990; Carbon 1992; Myburgh et al. 1993; Nelson et al. 1986; Rencken et al. 1996; Warren 1992; Warren 1999). Regardless of these limitations, the consensus of these studies is that low BMD is evident at the lumbar spine in athletes with menstrual dysfunction when compared to eumenorrhoeic athletes (Marcus et al. 1985). It is understood that the lumbar spine is largely controlled by menstrual and hormonal alterations due to the higher proportion of trabecular bone, thus counteracting the beneficial effects of exercise at this site despite the high level of mechanical loading compared to sedentary controls (Bennell et al. 1997b; Hind 2008; Marcus et al. 1985; Pettersson et al. 1999). Runners seem to be more prone to lower BMD at the lumbar spine than other athletes such as gymnasts or rowers which may be a result of the insufficient mechanical loads at the lumbar spine to maintain bone mass in runners (Drinkwater et al. 1984; Warren 1992).

The effect of menstrual dysfunction at other loading sites such as the femoral neck, and trochanter are still unclear with some researchers reporting no significant decreases at the lower limbs in a/oligomenorrhic athletes compared to eumenorrhoeic controls (Bennell et

al. 1997b), and others stating that menstrual dysfunction affects multiple sites in amenorrhoeic athletes (Pettersson et al. 1999; Rencken et al. 1996). There is no compelling evidence to suggest that bone loss due to menstrual dysfunction will be fully recovered after the independent resumption of menses in athletes (Hind 2008; Keen and Drinkwater 1995; Keen and Drinkwater 1997; Warren et al. 2002). Hind et al (Hind 2008), did report an increase in BMD at the lumbar spine of 18.6% over a six year period which resulted in a normal BMD value when menses was returned to normal. However, this result was reflection from a case study and may not be evident in all amenorrhoeic athletes. Drinkwater et al. (Drinkwater et al. 1990), reported a rapid increase of 6.3% BMD in the first year of resumption of menses, to 3% in the second year with BMD at the lumbar spine, and plateauing in the following years and remaining low. The length of history of amenorrhoea, therefore may result in irreversible effects on BMD, thus compromising exercise induced benefits on cortical and trabecular bone, by inhibiting oestrogen.

Even though it seems evident that menstrual dysfunction is associated with low BMD we do not know what effect amenorrhoea has on bone geometry, thus bone strength in athletes. Lower oestrogen levels are believed to favour periosteal expansion, and reduce endocorticol contraction (Bass 2003; Saxon and Turner 2006). Ducher et al. (Ducher et al. 2009), reported that there were no significant differences in retired gymnasts with a history of amenorrhoea in bone density and bone strength at the distal radius, tibia and lumbar spine when compared with controls. Eumenorrhoeic retired gymnasts had approximately 10-20% increases in density and strength. The cortical thickness, thus resistance to bending of the radius and tibia was lower in the amenorrhoeic retired gymnasts but not enough to compensate for the comprised bone density. In a bone geometric study completed in anorexic women who presented with menstrual dysfunction and were physically inactive it was reported that CSA (-11%) and Z (-35%) of the hip were

significantly lower in anorexic women compared to healthy controls even after adjusting for lean mass, even though the outer diameter was the same between groups (DiVasta et al. 2007). This may indicate that menstrual dysfunction in athletes may negatively affect BMD, which could possibly be accompanied with structural adaptations.

Not all athletes with menstrual dysfunction will present with osteopaenia (t-score -1 to -2) as other factors such as the individuals genetic background, body composition, nutritional intake and sporting activity will each act to mediate the effect of menstrual dysfunction on BMD (Drinkwater et al. 1984; Zanker and Cooke 2004).

2.3.5 Energy availability

According to the energy deficiency theory, menstrual dysfunction manifests from restriction of energy availability which causes low body weight and lean mass, resulting in decreased BMD particularly at the lumbar spine (Nattiv et al. 2007; Nelson et al. 1986; Zanker and Cooke 2004). The energy availability of an athlete is defined as the amount of nutritional energy available for other metabolic processes after exercise training, and is estimated by dietary energy intake minus exercise energy expenditure. Excessively low levels of energy availability will lead to impaired skeletal and reproductive health (Loucks 2007; Nattiv et al. 2007). In athletes who train at high volumes and high cardiovascular intensity and do not increase nutritional intake accordingly energy availability may be reduced to < 30 kcal/kg FFM/day, resulting in the failure of metabolic processes such as bone remodelling (Loucks 2007). By reducing energy intake by >30% there is increased evidence of infertility. It is thus hypothesized that the reduced rate of LH release known to cause menstrual dysfunction is primarily caused by a continually disrupted energy intake. Therefore, it is possible that the decreased BMD in amenorrhoeic athletes is a consequence of energy deficiency rather than menstrual dysfunction alone (Nattiv et al. 2007). Loucks et al

(Loucks 2007) state that energy availability should be around 45 Kcal/kg FFM/day in order for energy balance to occur.

Some athletes will reduce energy availability by practicing abnormal eating behaviours such as fasting, binge eating and purging (Beals 2002; Beals and Hill 2006; Beals and Manore 2002; Sundgot-Borgen and Torstveit 2004). The prevalence of disordered eating (disturbance in eating behaviours) or eating disorders (clinically diagnosed anorexia and bulimia) in athletes' ranges from 1 to 78% (Sundgot-Borgen and Torstveit 2007) with variations due to the sensitivity of techniques used. Most athletes with eating disorders will not reach the threshold for clinical diagnosis (Beals and Hill 2006; Beals and Manore 2002; Sundgot-Borgen and Torstveit 2007). Cobb et al (Cobb et al. 2003), found that eating disorders in runners are correlated with menstrual dysfunction. Of 91 athletes examined, those scoring higher on eating disorder questionnaires often reported a lower total intake of fat than athletes who scored lower on the eating disorder questionnaires (Cobb et al. 2003). This supports earlier findings associating energy imbalance to depressed oestrogen levels, metabolic disturbances, and amenorrhoea (Marcus et al. 1985; Nelson et al. 1986; Zanker and Swaine 1998b).

Evidence has suggested that improvements in BMD can occur despite multiple years of restrictive eating and menstrual dysfunction simply by increasing energy intake in athletes. (Cobb et al. 2007; Hind 2008).

2.3.6 Interrelationships of the female athlete triad

There are only a few studies which have identified the presence of all three components of the female athlete triad in athletes, and the prevalence is low ranging from 1.2 to 3.4% (Beals and Hill 2006; Cobb et al. 2003; Nichols et al. 2006; Pollock et al. 2010; Torstveit and Sundgot-Borgen 2005b).

Athletes usually present with either one or two of the components and rarely all three (Beals and Hill 2006; Cobb et al. 2003; Torstveit and Sundgot-Borgen 2005b). However, regardless of the number of the components identified, the syndrome may have a negative effect on health. Eating disorders have been identified to promote menstrual dysfunction despite normal BMD (Beals and Manore 2002). Menstrual Dysfunction in the absence of disordered eating was associated with lower BMD, particularly at the spine (Cobb et al. 2003; Drinkwater et al. 1984; Robinson et al. 1995; Zanker and Swaine 1998b) and finally athletes with eating disorders with no menstrual dysfunction had lower BMD (Cobb et al. 2003; Zanker and Swaine 1998b).

DeSouza et al. (De Souza and Williams 2004), suggested that the low prevalence of the female athlete triad is due to the syndrome developing on a continuum with energy availability and menstrual function being affected long before reductions in BMD are observed. Therefore the prevalence of osteoporosis in athletes (10-13%) is substantially lower than that of menstrual dysfunction and disordered eating (Cobb et al. 2003; Pettersson et al. 1999). Treatment of the syndrome is advised early and should be administered to prevent possible detrimental effects of bone health such as increased risk of stress fracture (Nattiv et al. 2007)

2.4 Stress fractures

As previously mentioned, mechanical loading can be associated with a positive adaptation to bone. However, if loading is excessive structural damage may occur to the bone resulting in accumulated damage to the skeletal system (Bennell et al. 1996c; Grimston and Zernicke 1993). Stress fracture injury represents one form of skeletal breakdown, and has been reported as the most common over use injury in athletes (Bennell et al. 1999; Khan et al. 2001; Snyder et al. 2006). The first reported stress fractures were in military recruits in the 19th century (Khan et al. 2001; Shaffer 2001), but over the past 20-25 years

reports of stress fracture incidence in the non-military population has increased. The higher incidence rates are primarily linked to dramatic increases in sport and exercise participation, a better understanding of stress fractures, and advanced diagnostic procedures for detecting the injury (Brukner et al. 1998; Khan et al. 2001; Shaffer 2001).

2.4.1 Stress fracture pathology

Stress fractures are classified as either fatigue or insufficiency fractures (Datir et al. 2007; Peris 2003; Romani et al. 2003). Fatigue fractures are common in younger adults as they are often caused by excessive loading whereas insufficiency factures occur predominantly in adults over the age of 50 years as a result of weakened bones due to conditions such as osteoporosis, rheumatoid arthritis, osteomalacia, Pagets bone disease, or corticosteroid use (Datir et al. 2007; Khan et al. 2001; Peris 2003). The focus of this section will predominantly be on stress fractures which are classified as fatigue fractures as these are commonly diagnosed in athletes and military recruits and are caused by the changes in regularity and intensity of training (Bennell et al. 1996c; Milgrom et al. 1985a; Peris 2003; Romani et al. 2003).

A fatigue stress fracture is a micro fracture that develops in bone which has been subjected to excessive and repetitive mechanical loading or trauma (Khan et al. 2001; Romani et al. 2003). If the healthy bone is unable to withstand the repetitive mechanical loading or the application of a heavy load which exceeds the biological capacity of the bone, then a stress fracture will become imminent (Khan et al. 2001; Shaffer 2001).

Although athletic training involves bone loading, the association between the two is not linear as volume of training will include both the total number of strain cycles as well as the intensity, thus affecting the magnitude of strain cycles applied to the bone (Khan et al. 2001; Shaffer 2001). If the strain magnitude exceeds the threshold level of the bone's

biological capacity microdamage accumulates (Khan et al. 2001; Schaffler et al. 1990; Yerby and Carter 2001). The threshold level of bone capacity is approximately 2000 microstrains (Khan et al. 2001), which is the upper range of the physiological value. The accumulation of continued micro-damage will trigger a remodeling response aimed to repair the damaged bone (Khan et al. 2001). If the damage accumulates faster than the bone can be remodeled a fatigue stress fracture will develop (Bennell and Brukner 2005a; Khan et al. 2001; Yerby and Carter 2001). Fatigue fractures have been diagnosed in a number of locations such as the lower extremities, sacrum, vertebrae and ribs (Romani et al. 2003; Yerby and Carter 2001). Even though it is well established that stress fractures are caused by accumulation of micro-damage there are still gaps in the knowledge necessary to fully explain the relationship between strain, strain rates and occurrence of stress fracture (Milgrom 2001). One approach to understanding the factors which compromise bone thresholds is to understand the mechanostat and apply it as a therapeutic treatment (Frost 2003; Martin et al. 1998). Learning how to change the strain threshold possibly through understanding the intensity, frequency and duration of bone loading in humans could reduce the repetitive strains causing microdamage thus reducing fracture risk (Milgrom 2001). However in order to do this further invasive research requiring the use of strain gauges in humans would be required (Milgrom 2001; Milgrom et al. 2007).

Unlike a muscle contraction, a fatigue stress fracture is not a sudden, all or none phenomenon but occurs on a continuum, which can be intervened prior to the development of a full stress fracture (figure 2.8) (Bennell et al. 1996c; Khan et al. 2001).

Due to the continuum of the overuse injury the symptoms and treatment will vary, thus clinicians classify the bone stress injury into stages of severity ranging from grade 0 (the least degree of injury) to grade 4 (full stress fracture) (Bennell and Brukner 2005a; Khan et al. 2001). The stages of the stress fracture injury can be detected by radiological methods

such as radioisotopic imaging (bone scan), magnetic resonance imaging (MRI), computed

tomographic (CT) scan or x-ray. (table 2.2) (Puddu et al. 1996).
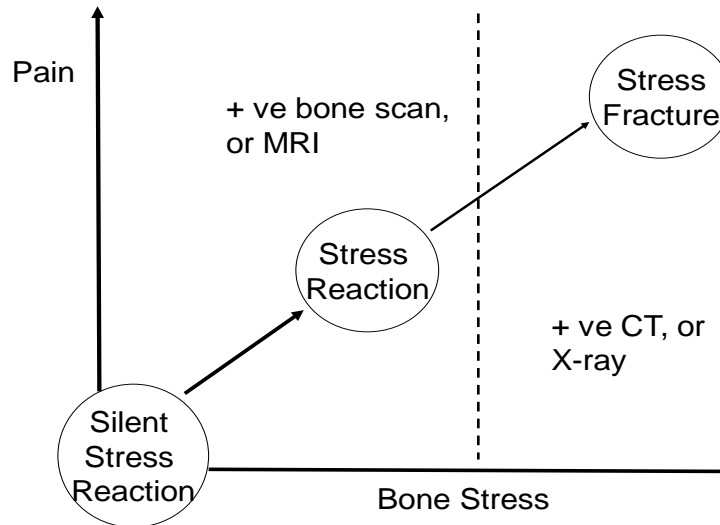
Figure 2.8: Continuum of stress fracture injury in bone and the relationship between the continuum and the stress fracture diagnosis. (Modified form (Bennell and Brukner 2005a)

Table 2.2: Stage of clinical stress fracture diagnosis

Bone Injury	Reaction	Symptom	Clinical Observation	Radiological diagnosis
Grade 0	Silent Stress Reaction	Pain Free	Normal bone modeling, but periosteal thinning	Bone Scan
Grade 1	Mild Stress Reaction	Local pain exacerbated by physical activity,	Cortical reabsorption on the periosteal surface.	Bone Scan
Grade 2	Moderate Stress Reaction	Bony tenderness	Extensive periosteal and cortical tunneling.	Bone scan, MRI
Grade 3	Severe Stress Reaction		Extensive periosteal and cortical tunneling.	CT, Bone Scan MRI
Grade 4	Stress Fracture	Significant pain and localized tenderness.	Micro crack	CT, MRI, Bone scan and x-ray.

2.4.2 Stress fracture epidemiology

Epidemiology is the study of disease in a population, including the relationship between the exposure and the outcome. The major challenge with stress fracture injury is defining and collecting meaningful exposure data (Snyder et al. 2006). Methodology for establishing rates varies between individuals and sporting events, thus it is important to use incidence and prevalence rates of stress fracture with caution.

2.4.3 Problems defining stress fracture incidence and prevalence

Fundamentally it is difficult to apply epidemiological methods to determine the incidence rate (number of cases/ number exposed x follow-up time) and prevalence (existing cases at a point in time) of stress fracture injuries (Shaffer 2001). The most common problem with incidence and prevalence of stress fracture is the definition of the injury. Clinicians do not uniformly accept the term 'stress fracture' to apply to a broad range of osseous reaction, resulting from excessive loading. This leads to a number of interchangeable terms which include stress fracture, stress reaction, fatigue fracture, pathological fracture, and periostitis (Shaffer 2001). The definition of stress fracture also varies widely in the research literature with differences in case denominators that result in rates of stress fractures which are hard to compare (Bennell and Brukner 1997; Shaffer 2001; Snyder et al. 2006).

As a stress fracture injury develops over stages case definition for researchers and medical physicians becomes difficult. It is questionable at which stage of development an individual should be counted as having a stress fracture. Fortunately with an increased use of radiographic measurements to assess injury, the consistency of case definition for stress fracture has improved (Bennell and Brukner 1997; Shaffer 2001; Snyder et al. 2006). Difficulty also arises in whether to count the individuals with at least one stress fracture or the number of stress fracture sites. Individuals with a stress fracture will often

have an occurrence of multiple stress fractures at varying anatomical sites in close succession. Past research has indicated that 11-50% of military and athletic populations will present with more than one stress fracture within the same period (Beck et al. 1996; Bennell et al. 1995; Bennell et al. 1996a; Giladi et al. 1991; Matheson et al. 1987). Researchers therefore have indicated that it is appropriate to report the number of individuals with at least one stress fracture when determining the risk factors of stress fracture, and that multiple sites of stress fracture rates should be counted when site disruption is investigated. However, the most desirable solution for this situation would be to report both outcomes (Shaffer 2001).

Selecting the appropriate reference study population is critical in order to compare the rates among differing groups such as military recruits and athletes. Arguably the best comparison of incidence between populations is to report a measure of accumulated exposure of physical activity or training in a population (Bennell et al. 1996a). However, the most commonly used method is to report the number of individuals making up a cohort in which stress fracture was assessed providing a general percentage of the population with a stress fracture (Shaffer 2001).

Studies can be designed to ascertain case number either actively (monitored by researchers) or passively (relies on patient seeking medical care), which will result in varied rates of stress fracture in the same population. In most reports stress fractures are monitored passively which could, in military and athletic populations, lead to the under reporting of stress fracture incidence (Shaffer 2001; Snyder et al. 2006), as these individuals are often highly motivated and able to train through pain to prevent training setbacks. When incidence rates are assessed actively and diagnostic procedures are used to confirm stress fracture injury in athletes and military recruits the incidence rate is often greater as this method will identify individuals who would not necessarily passively report

symptoms. Furthermore gender contributes to differences in symptom reports, with females more likely to report all injury symptoms. In military recruits males have been reported to underestimate injuries by more than 25%, whereas females reported all symptoms (Almeida et al. 1999). In a recent study of female athletes it was indicated that self reporting of stress fractures in females had a low validity, over-estimating stress fracture cases by approximately 7% (Oyen et al. 2009). However, both passive and active designs for case ascertainment have positive contributions to the literature. The active method is important to minimize misclassification of stress fracture especially when determining potential risk factors, whereas, passive methods are important when impact of injury on a population is investigated (Shaffer 2001).

The rates of stress fracture identified in the literature are derived from a variety of study designs that can potentially affect the true representation of stress fracture injury within a population. The three most commonly used designs include medical case studies, retrospective data collection and prospective monitoring of stress fractures. Medical case studies require the collection of various stress fracture cases seen within a medical setting, this method does not therefore, represent a general population and should not be used to determine incidence of stress fracture (Bennell and Brukner 1997; Shaffer 2001; Snyder et al. 2006). Retrospective data collection is the most popular method used to determine the incidence of stress fracture; this method surveys a group of individuals and determines rates from a history of stress fracture. Even though this method can determine rates it often results in sampling bias, and recall error. A more accurate method, and often considered the gold standard for determining incidence rate, is to prospectively monitor a cohort of uninjured individuals over a set period of time, and document the number of new cases. The advantage of knowing the time period of follow-up and the number exposed allows for a more accurate calculation of incidence rate. This method also allows for the

comparison between injured and non injured groups (Bennell and Brukner 1997; Shaffer 2001; Snyder et al. 2006).

A number of prospective studies have been conducted in military populations (Beck et al. 2000; Giladi et al. 1985b; Milgrom et al. 1985a; Milgrom et al. 1988; Valimaki et al. 2005), but there are few in the athletic population, with only three identified to date (Bennell et al. 1996a; Johnson et al. 1994; Nattiv et al. 2000). The incidence of stress fracture reported from prospective studies will vary according to the attributes of the individuals recruited within the study and the length of time over which incidence is calculated. As an individual adapts to their physical stressors the risk of stress fracture will decline. Highly trained individuals will often experience a higher risk of stress fracture corresponding to a consistent change in training (mileage, and frequency) (Macera 1992). The time when an incidence is calculated can determine the magnitude of injury, for example the incidence rate of stress fracture will be greater in recruits if the observation time is within the first three weeks of basic training, whereas if incidence was observed in the second three weeks it would be lower (Shaffer 2001). Thus the most desirable method of stress fracture incidence to reduce risk variation would be to observe a comparable population over a 12-month period or entire athletic season.

2.4.4 Incidence of stress fracture

The incidence of stress fracture in the military population varies due to a number of factors such as the type of training programs reported, and the method of case ascertainment (Bennell and Brukner 1997; Jones et al. 1993; Jones et al. 1989a). In the United States the incidence of stress fracture in military recruits is reported to range from 0.2 to 4.0% (Almeida et al. 1999; Beck et al. 1996; Jones et al. 1989b; Kelly et al. 2000; Shaffer et al. 1999), with rates higher in females (1 to 7%) than in males (Almeida et al. 1999; Kelly et al. 2000). The studies from Israeli recruits demonstrate much higher incidence rates than

that within the United States, with reports as high as 50% during a 14 week period. This contrast in findings is potentially due to a difference in methods for identifying cases, with studies from Israel adopting an active surveillance method compared to the a passive method within the United States (Giladi et al. 1991; Milgrom et al. 1985b). However, even with these differences in rates of stress fracture the military findings benefit from the prospective designs and recruitment of large cohorts.

Estimates of incidence rates of stress fracture within the athletic population are limited. In a recent review of stress fracture epidemiology in athletes only fourteen studies were identified as measuring the proportion or cumulative incidence of stress fracture among athletes. Only three of these were prospective designs (Snyder et al. 2006), the remaining 11 studies were retrospective collected from medical records. An earlier review (Bennell and Brukner 1997), included retrospective studies with self administrated questionnaires identifying only a total of twelve studies. To our knowledge only one other prospective study in runners was identified between 2006 and 2009 when searching the key words epidemiology, athletes, stress fracture and incidence.

Incidence rates in retrospective studies collected from medical records of athletes range from 1 to 21 % (Arendt et al. 2003; Dixon and Fricker 1993; Goldberg and Pecora 1994; Hame et al. 2004; Iwamoto and Takeda 2003; Johnson et al. 1994; Matheson et al. 1987; Nattiv et al. 2000), with differences largely due to the study methodology, classification of stress fracture, and the sporting population studied.

Prospective studies, have reported incidence of stress fracture between 3.7 and 21.7% (Bennell et al. 1996a; Johnson et al. 1994; Kelsey et al. 2007; Nattiv et al. 2000). Johnson et al (Johnson et al. 1994), reported an incidence of 3.7% per year in a cohort of 914 male and female athletes, aged 19.8 years, over a two year study period. Individuals were recruited for the study from a variety of sporting events ranging from track and field to

lacrosse. The highest incidence of stress fracture was reported in female track athletes with 14 (31.1%) out of 45 athletes stress fracturing over a year, male track athletes reported 6 (9.7%) stress fractures out of 62 athletes. Nattiv et al (Nattiv et al. 2000), prospectively monitored 87 (male and female) track and field athletes, reporting a 8.7% total incidence of stress fracture over a 3 year period. Kelsey et al (Kelsey et al. 2007), in possibly the largest prospective study to date in female runners identified risk factors for stress fracture in 127 female cross country runners, aged between 18-26 years, reporting a 14.1% incidence of stress fracture over a 1.85yr follow-up. The data used for this study was originally collected for a randomized trial of the effect of oral contraceptives on bone health. Athletes were recruited to this study if they ran over 40 miles/week and had not taken oral contraceptives over the past six months (Cobb et al. 2003), recruitment in this manner may have lead to selection bias thus increasing the incidence of stress fracture in this study. Bennell et al (Bennell et al. 1996a), to our knowledge is the only prospective study which has expressed stress fracture incidence per training exposure over 12 months in a cohort of 49 female and 46 male track and field athletes, aged 17 to 26 years. Over the 12-month period 10 males and 10 females sustained at least one stress fracture giving an annual incidence of stress fracture as 21.1%. The reported incidence is markedly higher than other prospective studies in athletes (Johnson et al. 1994; Kelsey et al. 2007; Nattiv et al. 2000). Bennell et al (Bennell et al. 1996a) clearly state that athletes were closely monitored for the signs and symptoms of a stress fracture over the 12 month period, which would be identified as active monitoring which has been reported to promote a higher incidence of stress fracture.

The variation in incidence of stress fracture in athletes is largely due to the lack of comparable methodology between studies. Comparisons of incidence occur in athletes from all sports rather than within particular events. With no clear definition of stress

fracture case, some studies are reporting the number of individuals with at least one stress fracture whereas others are reporting the number of stress fractures. The difference in classification of stress fracture can lead to a marked difference, for example when Bennell et al (Bennell et al. 1996a) reported the incidence of stress fracture as the number of individuals with a stress fracture the reported rate was 21.1%, however when the incidence was expressed as the number of stress fractures the rate increased to 27.0%. Differences in incidence during prospective monitoring could also be due to selection bias resulting in a sample of athletes, who were susceptible to stress fracture injury, or due to the definition used to determine stress fractures.

The inconsistencies in estimating stress fracture incidence reported in the athletic population highlights the need for further prospective studies to establish the risk of stress fracture in specific sports. Future studies require larger cohorts of athletes from specific sporting events to allow a better delineation of injury risk thus allowing the foundation for future preventative strategies.

2.4.5   Stress fracture distribution

Fatigue stress fracture can occur in virtually any anatomical bone in the body, however the most commonly reported stress fractures occur within the lower extremities, such as the metatarsals, tibia, fibula, femur and pelvis (Bennell et al. 1996b; Brukner et al. 1998; Giladi et al. 1985a; Iwamoto and Takeda 2003; Khan et al. 2001; Matheson et al. 1987; Milgrom et al. 1985a; Rauh et al. 2006; Snyder et al. 2006). The common occurrence of stress fractures in the lower extremities could be explained by the vast body of literature reporting stress fractures in sports that require lower extremity loading such as running, soccer and basketball. There are limited reports of stress fracture incidence in predominant upper extremity loading sports such as rowing, and racquet sports, therefore the incidence and distribution of stress fracture in the upper extremities will naturally be lower (Snyder et al.

2006). The distribution of stress fracture injuries are associated with the loading activity of an individual, a stress fracture will only develop at sites which are subjected to excessive repetitive loading (Bennell and Brukner 1997).

Literature on the military suggests that the distribution of stress fractures has changed over the years, possibly due to changes in training methods, initial fitness levels and/or footwear worn during physical activity (Bennell and Brukner 1997; Jones et al. 1989a). Early reports identified the metatarsals to be the most common site for stress fracture injury, associating the site location with excessive periods of marching in inadequate footwear (Bennell and Brukner 1997). Over the past 30 years the tibia (41-58%) has been identified as the most common site followed by the metatarsals (8-26%) and the fibula (6-12%) (Beck 1998; Giladi et al. 1985a; Jones et al. 1989a; Milgrom et al. 1985b; Rauh et al. 2006). These findings are closely associated with those found in athletes (Bennell et al. 1996b; Matheson et al. 1987).

In athletes the majority of the literature is consistent in identifying the tibia to be the most common site for stress fracture injury followed closely by the metatarsals, and fibula (Bennell and Brukner 1997; Bennell et al. 1996b; Matheson et al. 1987; Snyder et al. 2006) (Iwamoto and Takeda 2003; Nattiv et al. 2000). Goldberg et al (Goldberg and Pecora 1994) were the only study to our knowledge in athletes who have reported a greater distribution of stress fracture at the metatarsal (26%) than the tibia (19%).

Whilst tibia stress fracture is predominantly associated with endurance running, power athletes such as sprinters sustain more foot injuries (Bennell and Brukner 1997; Bennell et al. 1996b; Matheson et al. 1987; Nattiv et al. 2000). Thus the type of sporting population studied will typically determine the distribution and patterns of stress fracture, for example stress fractures of the ribs are commonly reported in rowers, and golfers, humeral stress

fracture in racquet sports, foot fractures in dancers and pars fractures in gymnasts (Bennell and Brukner 1997; Iwamoto and Takeda 2003).

Levels of conditioning, age, and gender may also affect stress fracture distribution. It is believed that an athlete who is highly conditioned may experience a different pattern of stress fracture distribution than a recreational athlete, with highly trained competitive athletes more likely to develop tibia stress fractures, whereas recreational athletes present with more metatarsal or pelvic fractures (Bennell and Brukner 1997). Age is believed to play a part in the location of stress fracture, with older athletes presenting with more femoral and tarsal fractures and younger athletes with greater tibia stress fractures in one study (Matheson et al. 1987), although no such associations were reported in another(Hulkko and Orava 1987). Finally gender differences have been reported with female athletes presenting with more metatarsal fractures than males (Nattiv 2000).

2.4.6 Risk factors for stress fractures

Risk factors for stress fracture have primarily been identified in large cohort military studies which have found stress fracture incidence to be associated with lower BMD, narrower tibia width, menstrual irregularity, lower lean mass, gender, variations in training regimes and low level of physical fitness (Beck et al. 1996; Bennell and Grimston 2001; Giladi et al. 1987b; Jones et al. 1993; Jones et al. 1989b; Jones et al. 2002) The growing interest from researchers and sports medical physicians in the prevention of stress fracture injury in athletes has lead to an increased attention being paid to the risk factors associated with the injury. The knowledge gained by military studies has developed a base for this research however the findings are not easily translated to the athletic population, as the primary risk factor associated with stress fracture in military recruits is low levels of physical fitness prior to training. Athletes are usually highly trained therefore primarily risk factors associated with stress fracture in athletes must differ to military recruits.

In order to prevent the occurrence of a stress fracture injury a clear understanding of the factors associated with the injury are required, thus allowing the development of preventive strategies. However the study of risk factors is difficult to fully understand due to the possible curvilinear relationship between the various factors and the stress fracture injury (Bennell et al. 1999). Grimston et al (Grimston 1993), as described in Burr et al (Bennell and Grimston 2001) constructed a five-level research model (figure 2.7) to conceptualize the possible risk factors for stress fracture based on the mechanostat (Frost 2003). This model considers the risk factors in the context of the influence they have on the adaptation process, as the weakening of the bone causes stress fractures. Thus this section of the literature will focus on the five level-model and the risk factors identified predominantly discussing the risk factors associated with the athletic population.

2.4.7   Five-Level research model

As identified in figure 2.9, the five-level research model described by Grimston et al (Grimston 1993) is developed on the primary outcome of stress fracture occurrence (Level 1), where repetitive mechanical loading has exceeded the biological capacity of the bone. Level two discusses the association with bone properties, level three the controlling factors of bone properties, level four, the functional stimuli associated with the injury and finally level 5 discusses the constraints and environmental factors associated with stress fracture.
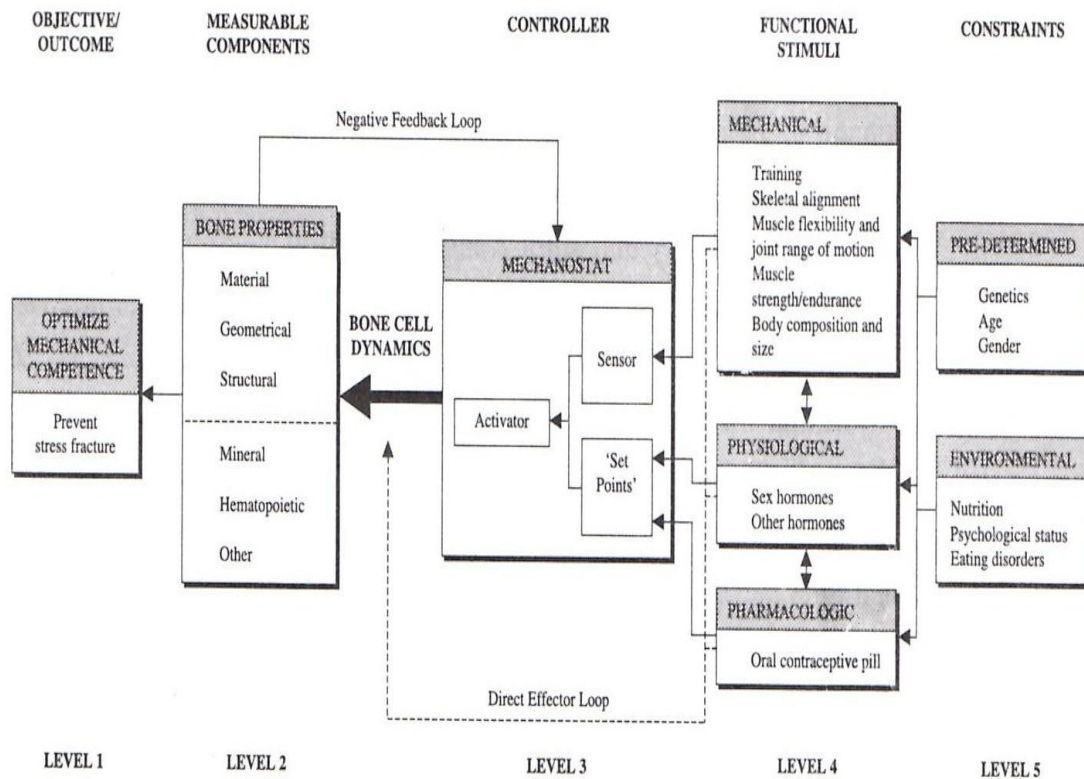
Figure 2.9: The five level model for stress fracture risk factors (Taken from (Bennell and Grimston 2001)

Level Two: Bone properties (BMD, BMD, geometric properties)

The bone properties that have been identified as influencing mechanical adaptation and considered as possible risk factor for stress fracture are BMD and bone geometry. However the current research in this area has been largely contradictory with no strong evidence to suggest a true independent association with stress fracture injury.

Bone Density: Past research has suggested that low BMD could contribute to an increased risk of stress fracture as it contributes to the decrease of bone strength thus increasing the

likelihood of micro-damage accumulation (Bennell et al. 1996a; Kelsey et al. 2007; Khan et al. 2001; Nattiv 2000). However, in the athletic population BMD is often higher than their sedentary counterparts due to the increased loads to the bone through exercise (Bass 2003; Bennell et al. 1997a). It is feasible to suggest that the load at which microdamage develops in athletes may be higher than in a sedentary individual. BMD values required by athletes, to withstand the repetitive strains applied to the bone, thus resisting the development of a stress fracture may be greater in athletes than in the sedentary individuals (Cummings et al. 1993; Melton et al. 1993).

There is very limited evidence from prospective studies in athletes to clearly support a relationship between BMD and stress fracture risk (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000). In prospective studies of female athletes BMD was often lower in the stress fracture group compared to the non-stress fracture group (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000), possibly indicating lower BMD predisposes athletes to stress fracture (Bennell et al. 1996a). Nevertheless, BMD remains significantly higher in the stress fracture athletes compared to the sedentary controls and would not be diagnostically identified as a potential health risk (Bennell et al. 1996a). In male athletes the results are contradictory (Bennell et al. 1996a; Nattiv et al. 2000), with some researchers indicating that there are no significant differences in BMD between cases and controls and that BMD does not predict stress fracture incidence (Bennell et al. 1996a) and others suggesting BMD is compromised in male athlete stress fracture cases in a similar nature to females (Nattiv 2000). This same contradictory evidence is found in cross-sectional studies with stress fracture cases reported to have higher (Crossley et al. 1999; Grimston et al. 1991) and lower (Bennell et al. 2004; Bennell et al. 1995; Myburgh et al. 1990), BMD. Evidence to suggest there is an association between BMD and stress fracture is conflicting and could indicate that there are other factors independent of bone mass contributing to stress fracture risk. Discrepancies could be due to factors

independent of BMD, small sample sizes, inadequately matched samples, measurement techniques and measurement period after the stress fracture injury.

Bone geometry: Recent findings in military studies have indicated that differences in bone geometric properties may partly explain the predisposition for stress fracture (Beck et al. 1996; Giladi et al. 1987a; Milgrom et al. 1988; Milgrom et al. 1989). Studies have shown that military recruits with a stress fracture have significantly narrower (4.4-10.6%) tibia width, CSA, CSMI and Z than non stress fracture controls (Beck et al. 1996; Milgrom et al. 1988), possibly suggesting that bone geometric properties could be a better indicator of stress fracture than BMD values. There is less evidence in female recruits to suggest a correlation with stress fracture (Beck et al. 2000; Bennell and Grimston 2001).

In athletes, the evidence for the association of bone geometry with stress fracture is limited (Bennell et al. 2004; Crossley et al. 1999). In male runners, stress fracture cases had significantly narrower tibial CSA than controls (Crossley et al. 1999), although in female runners bone geometric properties did not differ (Bennell et al. 2004).

Further prospective studies in athletes are needed to fully understand the potential association between bone geometry and stress fracture. The studies to date have a number of limitations as they rely on cross sectional data obtained from small sample sizes, bone geometry measurements were not taken at the time of stress fracture which could have resulted in misleading findings and measurements have only occurred at the tibia. It has been suggested that the femur determines the size of the tibia therefore it may be beneficial to examine bone geometry at regions of the hip (Giladi et al. 1987b).

Level Three: Controller (mechanostat)

Changes in cell dynamics may influence the risk of stress fracture. An individual who is susceptible to stress fracture injury may not have the ability to successfully repair the

accumulation of microdamage through bone remodeling. As the direct measure of bone remodeling is invasive in humans, researchers have attempted to examine the remodeling phase of bone injury using biochemical markers of bone turnover (Bennell and Grimston 2001). Limited studies and inadequate biomechical markers for bone turnover have resulted in conflicting findings in military and athletic populations.

In military recruits who sustained a stress fracture, plasma hydroxyproline levels were reported to be elevated when compared to non-injured controls. However, plasma hydroxyproline is a non-specific indicator of bone remodeling, thus the elevated levels could have been a result of other non skeletal factors. Studies in the athletic population show a negative association between bone turnover and stress fracture risk, indicating that bone turnover is not a significant predictor of stress fracture as levels of bone turnover do not differ in injured versus non-injured individuals (Bennell et al. 1998; Carbon et al. 1990).

It is suggested that the control of stress fracture risk in females not only includes the dynamics of bone remodeling but is controlled by hormonal levels. Low oestrogen levels could increase the set point for mechanical strains such that a higher mechanical load will be required to maintain bone mass when oestrogen is deficient, if the mechanical load is not sufficient bone will be lost (Bass et al. 2002; Bennell and Grimston 2001; Saxon et al. 2005). Thus in women the risk of stress fracture could be increased due to the interaction of the controller (level 3- mechanostat) and the controlling mechanism for bone adaptation (level4- mechanical and physiological functional stimuli)(Bennell and Grimston 2001). Further evidence is needed to determine the influence and possible interactions bone turnover and hormone levels have on stress fracture risk in both male and female athletes. Prospective studies are needed to monitor bone turnover and hormonal levels following a stress fracture diagnosis to determine change in controllers (bone turnover and hormones) during management and retraining.

Level 4: Functional stimuli (mechanical, and physiological)

Functional stimuli include factors which influence risk of stress fracture through mechanical (training, gait, impact attenuation) or physiological processes. In men and women the functional stimuli will vary. The physiological factors will affect female athletes to a greater extent than males, thus physiological risk will predominately focus on the female athlete.

Mechanical: It is known that exercise training can benefit BMD, but it is unclear what factors of training (type, intensity, volume, frequency) contribute to stress fracture risk (Bennell et al. 1999). Poor physical fitness has been attributed to increased stress fracture risk in military recruits (Shaffer et al. 1999), however in athletes the incidence of stress fracture is greater in the highly trained athlete who has often been competing for a number of years (Bennell and Grimston 2001; Bennell et al. 1999).

The surface on which an individual trains has been associated with stress fracture in military and athlete studies (Bennell et al. 1999; Greaney et al. 1983; Milgrom et al. 2003). Milgrom et al (Milgrom et al. 2003), found runners who trained on a treadmill were less likely to develop a stress fracture than those who trained over ground. Strain measured by strain gauges on the bone during treadmill running were markedly less, however decreased strains in the bone resulted in decreases in bone strength. The sample size of this study was small due to the invasive nature of the strain gauges thus caution must be taken, with large cohorts the results may be different.

The ground reaction force (GRF) may be the most informative measure of loading magnitude during training. However, there are no consistent findings between studies, with some cross-sectional studies in athletes reporting no significant differences (Bennell et al. 2004; Crossley et al. 1999), one reporting higher GRF (Grimston et al. 1994) and one lower GRF (Grimston et al. 1991) in athletes with a history of stress fracture compared to

non stress fracture groups. There is an indication that the degree of force could be influenced by muscle fatigue. Grimston et al (Grimston et al. 1994), found that athletes with a history of stress fracture had significantly greater GRF at the latter stages of a 45 minute run compared to athletes with no stress fracture history. Some researchers have postulated that muscle will act to protect the skeleton against increased forces by contracting to reduce strains on the bone (Bennell and Grimston 2001; Khan et al. 2001; Schoenau et al. 1996). Others have suggested that muscle acts dynamically to increase strains at muscle attachments thus causing stress fracture injuries (Meyer et al. 1993). However, there is limited evidence in athletes to support either theory of muscle involvement in stress fracture development (Bennell et al. 2004; Bennell et al. 1999; Crossley et al. 1999; Grimston et al. 1994).

The measurement of muscle size in past research has been used to determine if the size of the muscle is indicative of stress fracture risk (Bennell et al. 1996a; Milgrom et al. 1989), and have indicated that as the size of the muscle increases the risk of stress fracture decreases. Bennell et al (Bennell et al. 1996a) showed evidence that calf girth circumference is an independent predictor of stress fracture in female track and field athletes, with increased incidence of stress fracture in females with decreased calf girth. Similarly, decreased body size (height, body mass, fat percentage) has been postulated as a risk factor for stress fracture in a number of studies (Barrow and Saha 1988; Bennell et al. 1996a; Kelsey et al. 2007), however, no study has found an association between body size and stress fracture incidence. The lack of association may be due to the homogenous nature of athletes or possibly due to a curvilinear relationship of stress fracture risk factors.

Physiological: Sex hormones are essential in bone health, thus are potential risk factors for stress fracture. The key gender difference which is associated with increased risk for stress fracture is the prevalence of menstrual dysfunction in highly active female athletes.

The low circulating oestradiol levels in amenorrhoeic athletes possibly counteract the beneficial effects of exercise on the bone thus increasing the risk for microdamage accumulation. Retrospective studies have shown that athletes with a high prevalence of menstrual dysfunction have a relative risk for stress fracture two to four times greater than eumenorrhoeic athletes (Barrow and Saha 1988; Bennell et al. 1995; Bennell et al. 1996a; Carbon et al. 1990; Kelsey et al. 2007; Marcus et al. 1985; Myburgh et al. 1990; Nattiv 2000; Nattiv et al. 2000; Nelson et al. 1986) (Barrow and Saha 1988; Grimston et al. 1991; Nelson et al. 1986). Many of these studies had small sample size and poor questionnaire response. The studies examining the relationship between menstrual dysfunction and stress fracture risk are often specifically recruited to have either a history of stress fracture or current menstrual dysfunction, thus biasing the sample and possibly resulting in increased relative risk of stress fracture according to menstrual dysfunction (Carbon et al. 1990; Grimston et al. 1991; Marcus et al. 1985; Myburgh et al. 1990).

There are conflicting findings in the literature regarding the association of stress fracture and exposure length of amenorrhoea. Grimston et al (Grimston et al. 1990),developed the menstrual index to quantify the average number of menses per year from the age of menarche to determine if athletes who had extended periods of amenorrhoea had a greater risk for stress fracture. No association between the index and the incidence of stress fracture was found in runners. Conversely, Bennell et al (Bennell et al. 1996a), found using the menstrual index that female track and field athletes who were classified as having a low menstrual index, were at a greater risk for stress fracture than those with a high menstrual index. Similarly Barrow et al (Barrow and Saha 1988), reported lifetime menstrual dysfunction to increase incidence of stress fracture.

Age of menarche often occurs later in athletes who participate in sports such as running, ballet dancing and gymnastics (Bennell et al. 1999; Malina 1983; Nattiv 2000). The

relationship between age at menarche and stress fracture risk is unclear with some researchers finding no association with stress fracture risk (Kelsey et al. 2007; Myburgh et al. 1990) and others finding that late age at menarche increase relative risk (Bennell et al. 1996a; Carbon et al. 1990; Warren et al. 1986). In one of the only prospective studies of stress fracture risk factors, Bennell et al, (Bennell et al. 1996a) found that late age at menarche was an independent predictor for stress fracture risk. For every additional year prior to menarche there was a 4.1% increased risk for stress fracture. The possible association between age at menarche and increased stress fracture risk could be due to a lower rate of BMD accrual during adolescence thus decreasing the peak bone mass (Bennell and Grimston 2001; Bennell et al. 1999).

Despite the limitations there does seem to be clear evidence to suggest that menstrual dysfunction does increase the risk of stress fracture in athletes and that individuals with a late onset of menarche could be at a greater risk (Bennell et al. 1995; Bennell et al. 1996b; Carbon et al. 1990; Grimston et al. 1991; Marcus et al. 1985; Warren et al. 1986). However the mechanism by which menstrual dysfunction increases stress fracture is unknown. It appears that the effect of menstrual dysfunction may co-exist with other factors such as osteoporosis, and nutritional deficiency thus making it difficult to ascertain what factors are contributing to the cause of stress fracture risk.

Level Five: Constraints (predetermined, and environmental)

Factors which are predetermined (age, gender, genetic) or those influenced by the environment (nutrition, psychological traits), often have an indirect effect on stress fracture risk. Constraints (predetermined and environmental) are often associated with increased risks for stress fracture in female athletes rather than males due to the influence of the environment for women having increased concerns with weight and shape. High volumes and intensity of exercise and dietary restriction may be strategies employed by female

athletes to achieve an ideal body size however, there is limited reported evidence in athletes associating nutritional and psychological traits with stress fracture risk (Gerlach et al. 2008; Loucks 2007).

Nutrition: Dietary and energy deficiencies may be associated with increased stress fracture risk however, perhaps due to the difficulty obtaining adequate evidence of energy intake immediately prior to a stress fracture, the past research is conflicting (Bennell et al. 1999; Bennell et al. 1996a; Loucks 2007). It is possible that nutritional factors contribute indirectly rather than directly to stress fracture development.

Dietary questionnaires administered retrospectively have revealed that female athletes have inadequate intakes of macro and micro nutrients, resulting in insufficient energy intake to withstand the demands of the training regimes (Bennell et al. 1995; Loucks 2007; Sundgot-Borgen and Torstveit 2007; Sundgot-Borgen and Torstveit 2004). The low calorie intake in athletes has been hypothesized as a mechanism for amenorrhoea, which has been reported as a direct predictor of stress fracture risk (Carbon 1992; Cobb et al. 2007; Loucks 2007; Nattiv et al. 2007). The interrelationship between low energy intake, amenorrhoea, and low bone density, classified as the female athlete triad is recognized in guidelines for prevention and treatment of stress fracture injury (Nattiv et al. 2007; Torstveit and Sundgot-Borgen 2005b). Recent findings have shown that the female athlete triad can manifest itself in athletes who would not necessarily be considered lean. It is important to consider all female athletes at risk of the triad and not necessarily concentrate attention on individuals with apparent amenorrhoea (Cobb et al. 2007; Torstveit and Sundgot-Borgen 2005a).

The restrictive dietary intake and patterns of eating disorders in athletes have been reported to influence stress fracture incidence, due to alterations in nutritional intake, menstruation and body composition. Bennell et al (Bennell et al. 1995) found that female

athletes who had a history of stress fracture reported higher scores on the EAT 40 (eating psychopathology questionnaire) than those with no stress fracture history. However, no athletes within this study were identified as having an eating disorder and scores were on a par with the general population (Warren et al. 1990). Conversely, other studies have reported no significant difference in eating disorder patterns in athletes with and without a stress fracture history (Kelsey et al. 2007; Warren et al. 1986). An eating disorder questionnaire administered at the time of stress fracture as well as a more robust measure of eating concerns may have detected different findings.

Prospective monitoring of stress fracture risk factors have reported athletes who sustain a stress fracture have significantly lower intakes of fat in their diet compared to non stress fracture athletes (Bennell et al. 1996a). This suggests that low fat intake may possibly be a better predictor of stress fracture than total energy expenditure. However, the interrelationship between diet, menstruation and bone density seems to imply that other confounding factors may contribute to increased stress fracture risk independent of nutritional behaviours.

Inadequate caloric intake in athletes could result in calcium deficiency. Calcium is an essential nutrient for bone. If the calcium intake is deficient the body will obtain the nutrient from the body's calcium reservoir resulting in decreased bone strength. In cross-sectional and prospective studies it is difficult to determine if calcium is a risk factor of stress fracture in athletes as calcium is not assessed at the time of stress fracture diagnosis. Therefore findings are conflicting with some studies reporting calcium levels in athletes with a stress fracture to be over the recommended values (Bennell et al. 1996a), one reporting no differences (Grimston 1990) and one reporting lower levels (Myburgh et al. 1990) when comparing stress fracture groups with controls. The conflicting finding could also be due to differences in the definition of stress fracture injury, inadequate methods used to obtain

calcium levels, and the possibility that stress fracture injuries are influenced by different factors in each individual.

Further research is needed to determine the importance of nutritional factors such as fat and calcium intake as a risk factor for stress fracture and to determine if the prescription of calcium tablets helps to prevent the risk of stress fracture in amenorrhoeic athletes, although the evidence from prospective studies seems to suggest those athletes who develop stress fracture have calcium intake within the recommend range (Bennell et al. 1995).

Psychological traits: The research on the association of psychological traits and stress fracture in athletes is limited. Female athletes are reported to have a greater incidence of eating disorders than males, however it is relatively unknown what triggers the eating disorder behaviour, and whether there are specific cognitions which result in an increased risk of stress fracture (Bennell and Grimston 2001). In earlier, military studies associations were found between high obedience and low achievement and increased stress fracture risk, which results from increased training volume and intensity and restrictive eating behaviours (Bennell and Grimston 2001). These associations have not been extensively evaluated in stress fracture risk in athletes. Nattiv et al, (Nattiv et al. 1997) did however find in a large athlete cohort that pathological weight control behaviours are associated with a two-fold increase in stress fracture risk. Further investigation is required to determine the association of eating psychopathology and exercise cognitions in athletes as a potential risk factor of stress fracture.

In summary the five-level research model has summarized factors which could directly and indirectly influence stress fracture risk in athletes. However, due to the complexity of the interrelationships a number of these factors have not been proven. Retrospective studies have demonstrated the presence of some factors in individuals with a history of stress

fracture. However there are a number of limitations associated with retrospective study designs which could bias the findings, thus over estimating the importance of the factor to stress fracture risk. Limited prospective studies have identified possible independent risk factors which include low BMD, lower bone geometry, calf girth circumference, age at menarche, and menstrual dysfunction, however clear conclusions are conflicting. Thus further prospective monitoring with multivariate analysis is required to determine current independent predictors of stress fracture. If such factors are confirmed they may be targets for modifications in preventative and treatment strategies.

2.4.8 Stress fracture management

The treatment of a stress fracture is often determined by the location and severity of the injury. The majority of stress fractures will require avoidance of physical activity for a period of 6 to 8 weeks on average. The return to sport is usually gradual as a healing bone is often weak, thus progressive increases in bone loading through weight bearing activity is needed (Bennell and Brukner 2005a; Carmont et al. 2009). The healing of the bone is determined by the absence of localized pain and tenderness during physical activity and does not require a CT scan for confirmation (Bennell and Brukner 2005a; Khan et al. 2001; Romani et al. 2003).

It is well established in the research that there are a number of interrelating risk factors associated with stress fracture (Bennell and Grimston 2001; Bennell et al. 1996a; Kelsey et al. 2007; Nattiv 2000), however there is limited knowledge to explain the reoccurrence rates of stress fracture in athletes. Literature has indicated that the reoccurrence rate of stress fracture in male and female track athletes over a 12 month period is as high as 23% (Bennell et al. 1996a; Bennell et al. 1996b). One possible explanation for stress fracture reoccurrence is the inadequate repair of bone micro-damage which could result in decreased bone strength during the retraining phase of stress fracture injury. However, to

our knowledge there is no evidence to suggest bone is lost during the initial period following stress fracture injury.

The 6 to 8 week period of physical activity avoidance during stress fracture healing could initiate a similar pattern of bone loss as evident in detraining studies (Dalsky et al. 1988; Snow et al. 2001; Winters and Snow 2000). However, it could be argued that bone loss during stress fracture injury may not occur as the avoidance period is particularly short in comparison to detraining studies.

Following fractures and musculoskeletal injuries it is believed that non-weight bearing or immobilization leads to a rapid loss of trabecular and cortical bone (Petersen et al. 1997; Therbo et al. 2003). The recovery periods for these injuries may be similar to those experienced during stress fracture healing. Furthermore, both cross-sectional (Clement et al. 1999; Kannus et al. 1994b; Kannus et al. 1992) and prospective studies (Alfredson et al. 1998; Karlsson et al. 1993; Petersen et al. 1997; Therbo et al. 2003) have shown the loss of bone density following post-traumatic injury is not always reversible. Bone loss can be detected in bones which are more proximal to the injured area as well as in the contra-lateral limb (Kannus et al. 1994b; Kannus et al. 1992; Therbo et al. 2003). It is thus possible that stress fracture reoccurrence is due to bone loss.

Veitch et al (Veitch et al. 2006) reported persistent regional loss of bone following a tibial shaft fracture, accompanied by losses of BMD at the trochanter (12.5%) one year following fracture, thus increasing risk of re-fracture by four fold. Whereas, Cattermole et al (Cattermole et al. 1997) identified loss in BMD following fracture to return to the baseline level at the point of fracture within five months, however at sites proximal to the fracture persistent bone loss was evident increasing the risk of post-traumatic osteoporosis. Therbo et al (Therbo et al. 2003), reported decreased BMD at the tibia (6.4%), femoral neck (2.3%) and greater trochanter (6.8%) one year following an Achilles tendon rupture in

an uncontrolled study. It is believed that early weight bearing after an injury can limit the degree of bone loss (Emami et al. 2001) however, Petersen et al (Petersen et al. 1997) reported bone loss in patients, who after anterior cruciate ligament (ACL) surgery were treated with partial weight-bearing. Following a stress fracture injury, athletes are required not to take part in weight-bearing activities for at least 6 to 8 weeks; thus potentially promoting bone loss. Evidence in musculoskeletal injuries indicates that the greatest point of bone loss occurs 3-4 months following the initial detection of injury. A point at which weight bearing activities would have already been introduced (Therbo et al. 2003).

In athletes there is no previous literature explaining the possible magnitude of bone loss and subsequent regain following stress fracture and retraining, but it is believed that the bone loss may be less in comparison to other musculoskeletal injuries due to decreased periods of non weight bearing. Future prospective studies investigating the magnitude potential bone loss following stress fracture could benefit effectiveness of injury management, thus reducing the possible reoccurrence rates of injury.

2.5   Summary

The review of the relevant literature has clearly highlighted the need for further investigation into the predictors of bone health and stress fracture risk in female endurance athletes. There is compelling evidence that BMD is higher in athletes compared to their sedentary counterparts due to increased mechanical loading during young age and into the third decade. However, in female endurance athletes, in particular, the demand for thinness within the sporting society has lead to factors such as high intensity seasonal training regimes, menstrual dysfunction and energy restriction which may have a detrimental effect to bone. There is consensus in the literature that the benefits of exercise in amenorrhoeic athletes will be counteracted by oestrogen deficiency resulting in lower BMD compared to their eumenorrhoeic counterparts. There is, however, no indication of

the menstrual dysfunction association with bone geometry and the effects seasonal training may have on bone health in female endurance athletes.

Risk factors for stress fracture are complex due to the interrelationship between factors which has lead to conflicting evidence in the literature. There are limited prospective studies in female endurance athletes, with no studies to date identifying incidence rates in athletes based in the United Kingdom. There is no evidence to explain fully why stress fractures reoccur within 12-months of the initial injury. The evidence of bone loss following musculoskeletal injuries identify the need to fully understand the possible timescale and magnitude of bone loss following a stress fracture injury to determine if reoccurrence of stress fractures are due to bone weakening following injury and periods of disuse.

In light of this the following aims will be addressed in this thesis throughout the following chapters:

The main aims of this study are two-fold with five specific objectives that will be explored in chapters 4-8:

Main Aims

- Determine predictors of bone health and stress fracture in female endurance athletes.

- Determine whether bone geometry and density change following a stress fracture.

Further objectives

- To determine the correlates of stress fracture history. This objective will be addressed in chapter 4

- To compare bone density and bone geometry according to menstrual function. This objective will be addressed in chapter 5

- To determine the incidence rates of stress fracture and identifiable risk factors. This objective will be addressed in chapter 6

- To quantify the seasonal variation in parameters of bone health. This objective will be addressed in chapter 7.

- To determine magnitude and timescale of bone loss and subsequent regain. This objective will be addressed in chapter 8.

Chapter Three:
Methods

Chapter three provides a summary of the general methods used in the current study, describes in detail the procedures, gives justification for the materials and methods used, describes reproducibility and its assessment to determine coefficients of variance (CV). A detailed description of the methods and statistical analysis for each separate study are presented in the relevant results chapters.

Methods

The following chapter will highlight the key methods used in this research to determine bone health and stress fracture risk in female endurance athletes. The research design and recruitment criteria will be given for all the research studies in this thesis, with specific research designs and recruitment criteria presented in each relevant chapter. Detailed procedures and justifications will be given for all outcome measurements and an assessment of reproducibility will be reported to highlight the investigators coefficients of variance (CV).

3.1　Research design

To determine bone health and stress fracture risk in female endurance athletes a prospective monitoring design was employed. Athletes were recruited between April 2008 and April 2009 and prospectively monitored with regular email contact and face to face visits at three time points 6 months apart (figure 3.1). BMD BMC and geometric parameters and body composition were measured using dual energy x-ray absorptiometry (DXA). History of menstrual function, dietary behaviours, training and injury were assessed using questionnaires and training was prospectively monitored via questionnaire and training diaries. Anthropometric measures were taken and isometric knee extensor force was measured.

* Outcome measures
- •Bone properties: Bone mineral density, bone mineral content, bone geometry
- •Diet/eating behaviours
- •Muscle Function
- •History of menstrual function, training and injury.
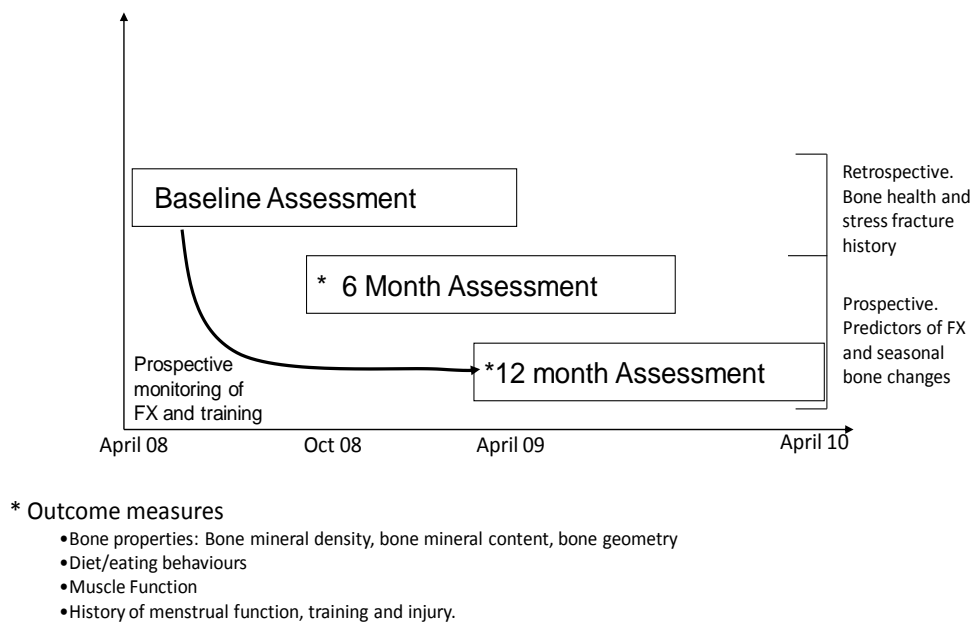
Figure 3.1: Flow diagram of study design illustrating the recruitment period, the assessment points, and outcome measures

3.1.1 Ethical Approval

Ethical approval for all the studies described in this thesis were obtained and approved

from both the National Research Ethics Service (NRES) (Appendix 1), and the

Loughborough University Ethical Advisory Committee (LUEAC).

Approval Reference:

Predictors of bone health and stress fracture in female athletes – NRES 08/H0405/20 ,

LUEAC R07-P129

Assessment of any bone changes following stress fracture – NRES 08/H0405/21 and

LUEAC R08 –P8

3.1.2   Recruitment

A sample of female endurance athletes at elite level for their age, or who trained at a high volume in their particular sport were recruited from sports federations, Loughborough university athletic student body, and local running and triathlon clubs within the United Kingdom (England, Scotland, Wales and Ireland). Recruitment of participants occurred in a variety of ways including recruitment letters (appendix one), posters (appendix two), press release (appendix 3) meetings with sports federations and foundations, word-of-mouth and emails to recreational coaches and athletes. The inclusion and exclusion criteria for this research were as follows:

Inclusion Criteria

- Female endurance athletes. These were defined as those women who represent the country at an elite level in either endurance running (800m to Marathon) or triathlon or who train to a high volume per week: 8-10 hours per week for an endurance runner or 15-20 hours per week for a triathlete.
- Aged between 18-45 years
- All athletes must be premenopausal.

Exclusion Criteria

- Any injury which has prevented normal training for the past four months or a stress fracture in the last 6 months.
- Pregnancy or lactation within the previous 12 months
- Any contact with medical treatment or diagnosis involving a high level of radiation over the past 12 months
- Any athlete who has a disease or taking medication which may affect bone (Hormonal Contraception was permitted) (Appendix 2).

3.2   Outcome Measurements

3.2.1   Measurement of bone properties

One of the major aims of this research was to determine the bone health of female endurance athletes. The bone properties examined as part of the athlete's profile of bone health included BMD, BMC, and bone geometry.

3.2.2   Dual Energy X-ray Absorptiometry (DXA)

DXA is currently the gold standard and most popular non-invasive measurement of bone mass (Kanis et al. 1994a; Khan et al. 2001; Mazess 1997; Who 1994). By using two distinct energy levels of X-ray the relative composition of the body may be determined (Khan et al. 2001). The attenuation of the X-ray beam will depend largely on the properties of the tissue being studied. BMD (grams of mineral per unit of bone area), and BMC (total grams of bone mineral as hydroxapatite within a measured bone region) are determined at the location of the region of interest (ROI) which is comprised of both bone and soft tissue. BMD and BMC are determined by the attention of the X-ray beam at the ROI. Properties such as BMD and BMC will vary at different regional sites, therefore DXA measurements are normally taken at clinically relevant sites at the lumbar spine (L1-L4), hip, and radius. Some devices such as the Lunar Prodigy allow measurements of the total body density and total body composition.

The advantages of using DXA include: its accuracy and precision, the low level of radiation compared to some other devices, and the ability to determine the body's bone density and composition. The short term precision of DXA is 0.5-1.5% for the lumbar spine and 1-2% for the femur (Baim et al. 2005; Khan et al. 2001; Kohrt et al. 2004).

The limitations of DXA include: lack of distinction between cortical and trabecular bone. DXA is a two dimensional measurement, assumptions determine the three dimensional

architecture of bone, therefore DXA could be measuring areal density rather than volumetric density. To overcome these assumptions equations have been devised (Carter et al. 1992) to correct the differences in size. However these equations are not widely used as they do not have the precision of BMD and make assumptions about bone depth that may have limited accuracy (Faulkner et al. 2006). In athletes bone size or structure may change without changes in BMD, therefore if the correction equations were used the effects of bone strength through exercise would be ignored (Khan et al. 2001).

Despite the limitations, DXA is the gold standard used to diagnose osteoporosis, and continues to be widely used. The device requires no special preparation and is inexpensive to maintain and run compared to other x-ray imaging devices. For research DXA is favorable as the devices are widely available, there is no subjective evaluation, and data can be compared to the reference data developed by the World Health Organization (WHO) (Alexeeva et al. 1994; Kanis et al. 1994a; Khan et al. 2001; Who 1994).

3.2.2.1.1 Bone mineral density (BMD) and bone mineral content (BMC) using DXA

For the purpose of this research DXA (Lunar Prodigy, GE Healthcare, Madison, WI, U.S.A version encore 12.2) was used to determine the participants BMD and BMC of the total body, lumbar spine, hip, and radius. The total body scan determined the total BMD, BMC and body composition. Regional BMD measurements of the lumbar spine, hip, and radius give more precise measures at potential fracture sites.

Prior to scanning a quality control (QC) assessment was carried out to correct any instrumental drift, followed by a manufacturer's lumbar spine phantom to quantify the variation of the measurement. Each scan over the 12-month period was performed and analyzed by the same qualified DXA operator, who followed standardized positioning protocols to maximize reproducibility. Notes were made for each repeated regional scans,

and regions of interests (ROI) were used, with the baseline scan as the reference, to check positioning of follow-up scans. All DXA scans were performed on the dominant side of the body, with dominance being determined by the hand used for writing, and the foot used to kick a ball. Participants were asked to wear similar shorts and t-shirts/vests for each scan, and to remove all jewelry/metal prior to scans commencing.
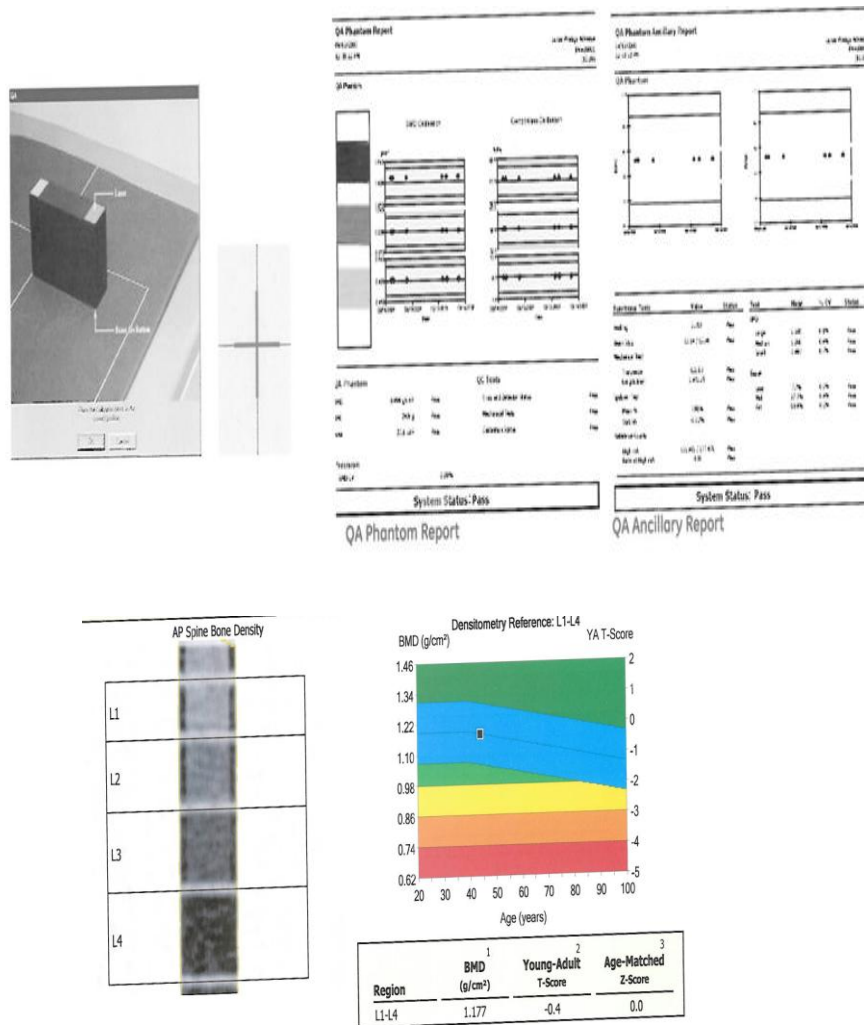
Figure 3.2: Illustrates the quality control (QC) assessment to correct for instrumental drift (above) and the manufacturer phantom lumbar spine to quantify variation in measurement (below) (Taken from Lunar user manual)

Total body scan

The total body scan required the participants to lie in a supine position with the midline of the body centered on the middle of the scanning bed (figure 3.3). The operator ensured the participant was aligned with the body in the allocated scanning area, the head was aligned with the spine and pelvis, and legs were positioned straight with feet together. Straps were placed around the participant's legs and ankles to prevent movement during the scan, which lasted approximately 6 minutes in the standard scanning mode. Participants were asked to breathe lightly and lie as still as possible throughout the scan.
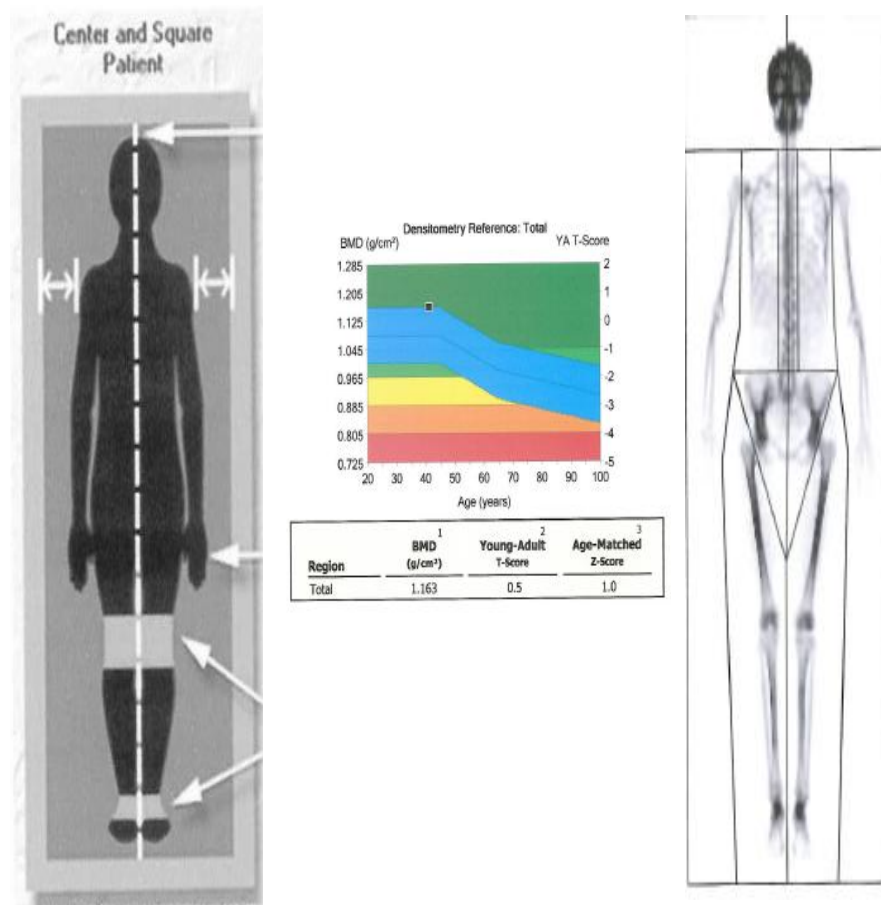
Figure 3.3: Positioning and regions of interest for the total body DXA scan. Left picture shows the positioning of the participant, middle the densitometry graphic, and the right picture, the scanning image. (Taken from Lunar user manual)

Lumbar Spine (L1-L4)

The measurement of the lumbar spine (L1-L4) required the participants to place their legs onto a block adjusted such that there was a 90 degree angle at the hips and knees, minimizing lumbar curvature and positioning the lumbar spine in the scan plane parallel to the DXA bed. To allow accurate identification of the lumbar vertebrae the iliac crest was used as an anatomical land mark. This positioning ensured the entire region from L1-L4 was accurately defined (figure 3.4).
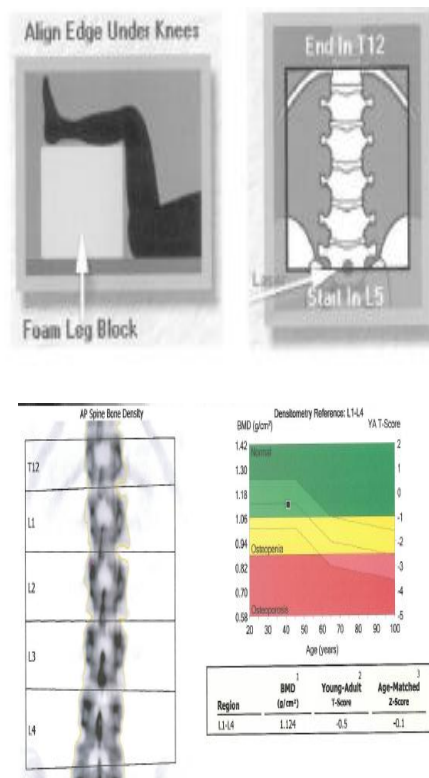
Figure 3.4: Positioning and scanning image for the lumbar spine measurement using DXA, identifying the anatomical landmark of the iliac crest and the regions of interest lumbar vertebrae L1-L4 (Taken from Lunar user manual)

Hip

The participants were positioned supine with the feet apart. A positioning block was placed between the feet. To ensure precise positioning of repeated measurements the hip was placed into a neutral position before the participant was instructed to medially rotate the hip through the knee and into the ankle, such that the foot and knee were medially rotated by 30 degrees and held into position with a velcro strap. The ischial tuberosity was used as an anatomical land mark to ensure the entire region of the hip was accurately identified, with a straight femoral shaft, minimization of the lesser trochanter through the medial rotation of the hip, and with the visual image of the entire femoral head and greater trochanter. The main ROI for this research were the femoral neck and trochanter (figure 3.5).
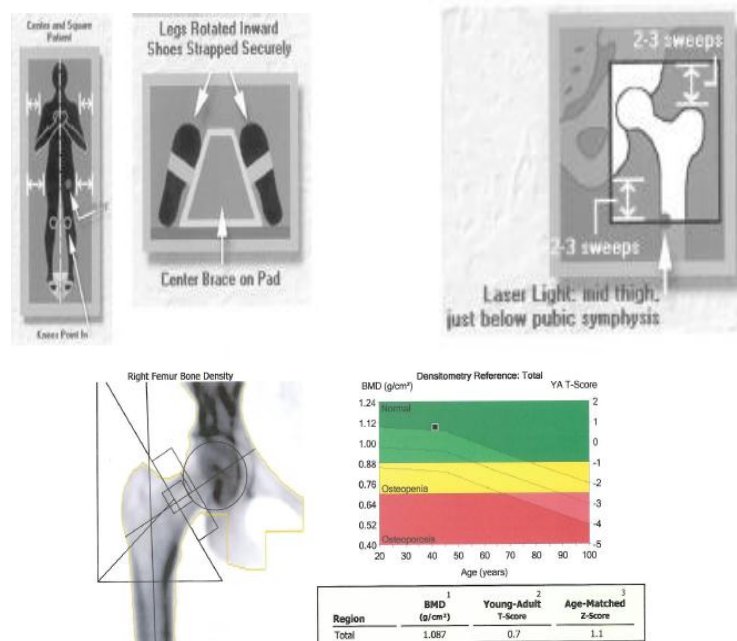
Figure 3.5: Shows the positioning of the participant for the hip measurement. Top left illustrates the medial rotation of the hip, knee and ankle, top right, shows the regions of interest (ROI) the greater trochanter, femoral head, femur shaft, the bottom picture shows the ROI and BMD densitometry graph (Lunar user manual)

Radius

The participant sat in a chair with the dominant forearm resting on a positioning board on the DXA bed. The forearm was positioned flat, with the palm down. The condyles of the radial and ulna were aligned with a cross on the positioning board. The condyle of the ulna was used as the anatomical landmark to ensure the entire ROI was identified. The main ROI on the radius for this research was the radius 33% (figure 3.5).
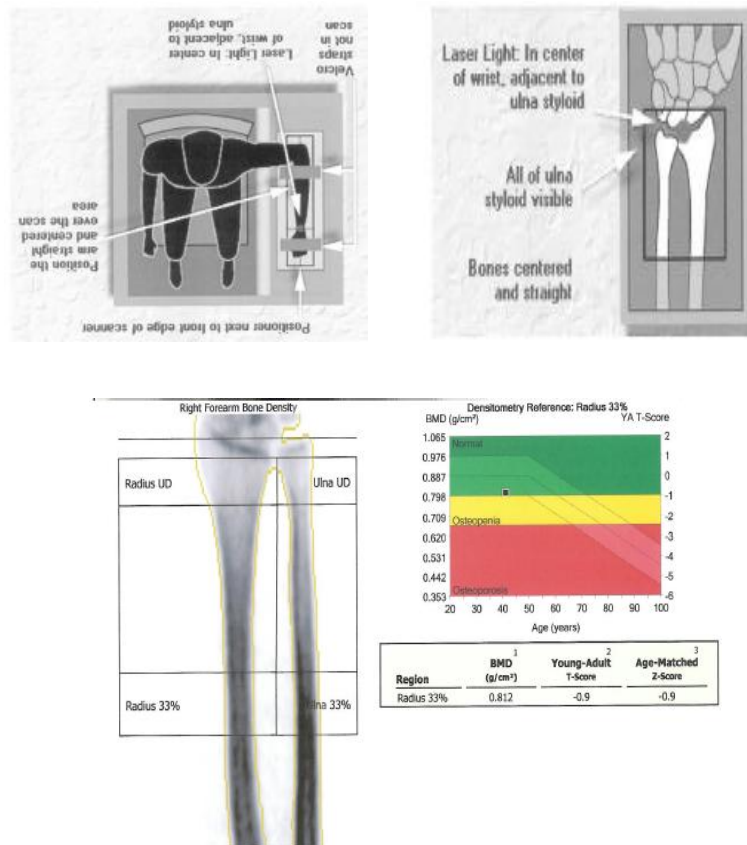
Figure 3.6: Illustrates the positioning and regions of interest (ROI) for the radius. The top left picture shows the position of the participant with the forearm palm down on the positioning board, the top right shows the anatomical landmark of the radial and ulna condyles, the bottom picture illustrate the ROI 33% distal radius (Lunar user manual)

3.2.2.1.2 Bone Geometry measures using DXA

The structure of bone may be as important as BMD and BMC (Beck 2007; Bonnick 2007; Faulkner et al. 2006). Bone strength is determined by bone mass, bone architecture, bone properties as well as bone geometry. In recent years DXA has been used to indirectly assess geometric properties, via a software estimation of structural parameters. The advanced hip structural analysis (AHA) algorithms require precise positioning of the hip (refer above, Hip), to measure femoral neck ROI, CSA, CSMI and minimal neck width. Section modulus (Z) is calculated from CSMI and the minimal neck width (y). Precise positioning of the hip during repeated scans is important to avoid errors in the estimation of geometric parameters (Bonnick 2007). As the femur does not have a perfectly circular cross-section the estimated CSMI can be altered when rotation is varied (Beck 2003). As DXA is a two dimensional image in one plane the three dimensional geometric properties of the hip can only be estimated using DXA (Bonnick 2007; Khan et al. 2001). A technique allowing three dimensional measurements (such as QCT) was not available for this research.

Procedure

The Lunar Prodigy AHA will traverse the narrow neck region of the femur to derive estimates of CSA, CSMI, Z and other geometric properties (Faulkner et al. 2006) (refer to figure 3.7). The values computed by the AHA software for Lunar Prodigy, GE Healthcare, Madison, WI, U.S.A, are used to estimate the structural properties of the hip. The values are not for clinical diagnosis of a disease.

The same positioning used to measure the BMD ROI of the hip (refer to hip) were used to estimate the geometric properties CSA, CSMI, Z and minimal neck width using the AHA software (encore 2006, version 12.2). To ensure positioning of repeated measurements were similar the DXA operator remained the same for each measurement.
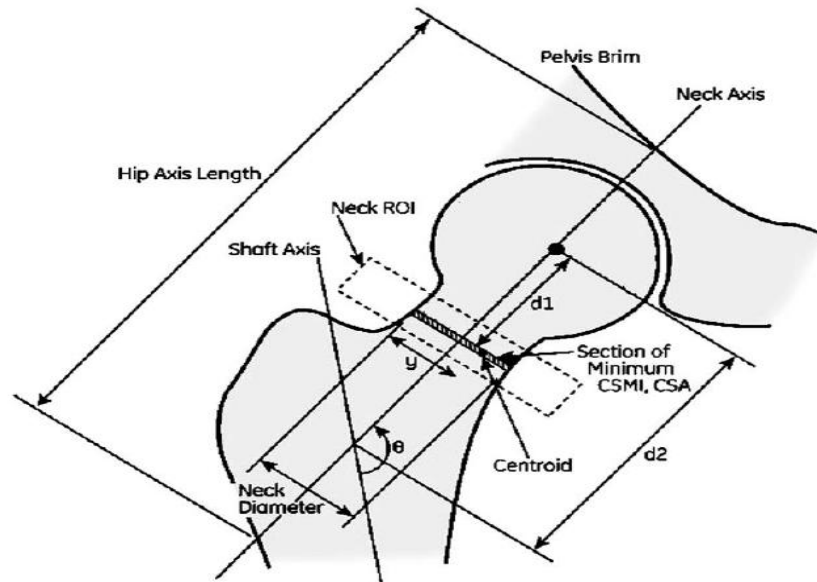
Figure 3.7: Illustrates the Lunar advanced hip analysis neck region of interest used to derive bone geometric properties CSA, CSMI, Z and other geometric properties (taken from (Faulkner et al. 2006).

3.2.2.1.3 Analysis of the DXA scans

Scan analysis to determine regional BMD, BMC of the total body, lumbar spine and radius and geometric properties of the femoral neck (CSA, CSMI, Z, minimal neck width) were performed by the same scanning operator for each scan. All scans were analyzed on completion of the 12-month monitoring phase to minimize analysis error. ROI of each scan were used to check for correct positioning thus reducing coefficients of variance.

3.2.2.1.4 Coefficients of variance (CV) for DXA measurements

DXA precision requires accurate positioning of the participant at each scan, consistent analysis, and routine instrument QA by the operator. The poorer the operators' precision the larger the change in BMD required to determine a meaningful change (Baim et al. 2005). Since the rate in change in BMD is very small it is essential that measurement precision is good, to detect clinically meaningful change (Baim et al. 2005). An

assessment of reproducibility was carried out on all regional measurements during the 12 month follow-up assessment in 13 out of the 70 female endurance athletes to determine the investigators precision error or coefficients of variance (CV) for scan positioning and scan analysis. All regional measurements were scanned and analyzed twice. DXA precision error (CV) was calculated using the root mean squared (RMS-SD) technique highlighted by Gluer et al (Gluer et al. 1995) to give unbiased estimate of precision error. The calculated precision errors (CV) measured for this research (table 3.1) are comparable with precision reports which have been published for the lumbar spine (1.0-1.2%), femoral neck (1.11 -2.2%) and trochanter (1.16 -1.5%) (Henzell et al. 2000; Khan et al. 2001; Lewiecki and Miller 2003; Lilley et al. 1991; Morgan et al. 2003).

Table 3.1: An assessment of reproducibility of the bone parameters measured by DXA: n=13, mean RMS-SD, CV %.

| | | *Precision errors* | |
| | | | *Coefficients of* |
Scanned Area	*Mean*	*RMS-SD*	*Variance %*
Bone mineral density (g/cm2)			
Total body	1.136	0.008	0.73
Lumbar spine (L1-L4)	1.128	0.004	0.42
Femoral neck	1.061	0.009	0.88
Trochanter	0.825	0.011	1.27
Distal radius (33%)	0.828	0.009	1.14
Bone mineral content (g)			
Total body	2364	17	0.75
Lumbar spine (L1-L4)	61.04	0.69	1.14
Femoral neck	4.95	0.06	1.23
Trochanter	9.73	0.17	1.78
Distal radius (33%)	2.01	0.06	2.77
Femoral neck bone geometry			
CSA (mm2)	154	5	3.55
CSMI (mm4)	9995	317	3.17
Z (mm3)	659	30	4.61
Minimal neck width (mm)	28.8	0.3	1.03
Body Composition			
Fat (%)	18	0.3	1.91
Tissue (kg)	52.1	0.1	0.12
Fat (kg)	10.1	0.2	2.22
Lean mass (kg)	42	0.3	0.59
Fat Free mass (kg)	44.3	0.2	0.52

3.2.3 Isometric knee extensor strength

Skeletal muscle strength and endurance has previously been identified as a physiological risk factor of stress fracture (Bennell et al. 1999). As discussed in chapter two, muscle strength may play a dual role as a protector and possible contributor in stress fracture development (Bennell and Grimston 2001; Bennell et al. 1999; Milgrom et al. 2007). There is evidence in military studies that recruits with lower muscle strength have a five times greater risk of stress fracture compared to recruits with greater muscle strength (Hoffman et al. 1999). In endurance athletes the distribution of stress fracture is predominantly in the lower weight bearing extremities of the tibia, metatarsals and femur. A custom made muscle rig was used to indirectly assess the isometric knee extensor strength in one action. The groups of knee extensor muscles are potentially easier to isolate compared to muscles involved in plantar/dorsi flexion, therefore giving more reproducible measures of muscle strength. Function of other muscle groups was not assessed due to time, cost and equipment availability constraints.

Procedure

Each participant was asked to perform a series of three maximal isometric knee extensions, giving an indirect assessment of maximal knee extensor force. The participants were asked to sit in the muscle rig with 90 degree flexion of the hip and knees. To prevent activation of the gluteal muscles a restraint was positioned around the participants' pelvis to isolate the knee extensor muscles. A restraint attached to a force gauge was placed around the participant's ankle. Prior to the three maximal isometric knee extensions participants were asked to take three familiarization sub-maximal warm up trials on each leg to prevent a learning effect. Instruction was given to all participants to produce a maximal effort muscle contraction which increased smoothly to maximal tension

within 4-5 seconds. To ensure each participant was given the same level of motivation during maximal trials the investigator gave the following verbal instruction:

"Three, two, one, GO push, push, push, push and stop"

The coefficient of variance (CV) was assessed in a pilot study of 17 female recreation runners and triathletes prior to the research carried out in this thesis. The CV for the right and left leg were 4.6% and 5.5% respectively.

3.2.4 Nutritional assessment

As discussed in chapter two, athletes' nutritional habits are strongly linked to the interrelated spectrum of the female athlete triad (dietary restraint, low bone density and menstrual dysfunction) (Nattiv et al. 2007), and potential intrinsic risk factors for stress fracture (Bennell et al. 1999; Bennell et al. 1996a; Cobb et al. 2003; Nattiv et al. 2007), it was therefore considered important to assess dietary intake in this research. The selection of the most appropriate method for the assessment of dietary intake in athletes is difficult, because of the erratic eating patterns, inaccurate labeling of sports supplements and the ability to quantify serving sizes (Barnard et al. 2002; Heaney et al. 2010). The acceptable gold standard method in the general population and possibly in athletes is the 7-day weighed food inventory (Burke et al. 2003; Magkos and Yannakoulia 2003). However, in athletes the validity of estimates is influenced by the variation in training periodization (Magkos and Yannakoulia 2003).

A 3-4 day diet analysis is typically used in athletes (Bennell et al. 1995; Bennell et al. 1996a; Burke et al. 2003) as it reduces the burden and potentially improves the compliance. The 3-4 day analysis however, is only adequate to assess macronutrient intake rather than micronutrients (calcium) which would require approximately 7 to 10 days for adequate assessment (Heaney et al. 2010).

Food frequency questionnaires (FFQ) have been used in previous studies in athletes to monitor nutritional intake, including the composition of the diet derived from macro-nutrients (carbohydrates, fats, proteins) (Frederick and Hawkins 1992; Heaney et al. 2010; Nogueira and Da Costa 2004; Soric et al. 2008). Even though FFQ's are less accurate in assessing the energy intake at an individual level there are a number of potential benefits for using FFQ's in the athletic population which include: the ability to assess nutritional intake over an extended period of time to reduce the variation caused by periodization of training, they are easy to administer, quicker and cheaper than other dietary methods, require minimal coding (Willett 1998). If used in combination with a diet diary will give a complete analysis of the athlete's eating behaviour (Burke et al. 2003; Welsh 2005). FFQs offer a choice of foods, so international differences in diet could make them population specific. The use of FFQ often yields higher values for the total energy and macronutrients compared to a weighed inventory however, when analyzed as the percentage of total energy the values are often comparable between the two methods (Bingham et al. 1994; Lietz et al. 2002). FFQ's have been used previously to determine potential risk factors for stress fracture in athletes based in the United States and Australia (Bennell et al. 1995; Bennell et al. 1996a; Cobb et al. 2003; Kelsey et al. 2007), but differences in food types and composition between these countries and the United Kingdom may make these FFQ's less valid in United Kingdom based athletes.

Despite the wide spread use of the dietary tools highlighted above to measure nutritional intake of athletes, all tools are limited in their ability to accurately assess true dietary intake due to the participant burden, reliance on recall, day to day variation in habitual intake and daily training (Magkos and Yannakoulia 2003; Willett 1998).

3.2.5 Food frequency questionnaire (FFQ)

The European Prospective Investigation into Cancer (EPIC) FFQ was developed for use in one of the largest nutritional studies within the United Kingdom to improve the definition of the association of diet and cancer (appendix 6) (Bingham et al. 2001). Within the Norfolk arm of the investigation the scope for the questionnaire has widened from cancer to other diseases such as osteoporosis (Bingham et al. 2001). The FFQ comprises of a list of 130 foods with additional short answer questions related to the consumption of milk, breakfast cereal and fat. The questionnaire is designed for participants to estimate over a 12-month period how often specific foods were eaten. The EPIC FFQ has previously been validated for the use in adults in the United Kingdom, providing a reasonable assessment of habitual diet (Bingham et al. 1994; Kroke et al. 1999; Lietz et al. 2002; McKeown et al. 2001). Therefore the EPIC FFQ was used in this research based on previous validations to assess the frequency of consumption of total calorie intake, carbohydrates, protein, fat, and micronutrients.

Procedure

Participants were asked to fill in the EPIC FFQ, during each 6 month visit, estimating over the previous 12-months how often specific foods were eaten. Privacy was given to each participant to ensure truthful responses were given. Data from the EPIC FFQ was coded into grams per serving for each food listed (Crawley 1993) ,and were analyzed using computerized food analysis software (CompEat pro, version 5.8, Nutritional systems, Colsterworth) to estimate total energy, carbohydrate, fat, protein and calcium intake at each phase of training. The CompEat Pro software, comprises of 3555 foods, from the Royal Society of Chemistry food data base (McCance and Widdowson 2002) allowing for the report of up to 158 nutrients.

3.2.6 Four-day diet diary

The 4-day diet analysis was used in conjunction with the EPIC FFQ to give a complete analysis of the participants eating behaviours (Burke et al. 2003; Welsh 2005). A four-day diet analysis was completed following each laboratory visit (appendix 7). The athletes were asked to record on a supplied diary everything they ate and drank for a total of four days (three week days and one weekend day). To avoid changes in diet during competition the four days of analysis were during a normal training week avoiding race day preparation. Serving sizes were recorded using household measures such as cups, bowls and spoonfuls. Data from the four-day diet diary were analyzed using computerized food analysis software (CompEat, pro version 5.8, Nutritional systems, Colsterworth) as mentioned above to estimate energy, carbohydrate, fat, protein and calcium intake at each phase of training.

3.2.7 Reproducibility and validity of nutritional methods

Prior to this research reproducibility of the four-day diet against a 7-day weighed inventory was determined in a group of recreational runners. Fourteen recreational runners were recruited and asked to firstly complete the EPIC FFQ questionnaire. They were then asked to record on a diary, everything they ate and drank for 4-days using household measurements. After a three day rest they were provided with a set of scales and asked to weigh and record everything they ate and drank for seven days. The RMS CVs were for total energy intake of 14%, carbohydrate 7%, protein 16%, fat 13% and calcium 24%. This variation may have been due to a number of factors but most likely is due to weekly variation in nutritional intake (Lietz et al. 2002).

The EPIC FFQ reported significantly higher energy intake compared to the 4 day diet and 7-day weighed inventory (FFQ: 4067 (278) kcal, 4-day diary, 1902 (165) kcal, 7-day weighed inventory: 2060 (128) kcal, $p<0.001$) which is consistent with previous studies

(Bingham et al. 1994; Lietz et al. 2002; McKeown et al. 2001). However, estimates of the percentage of energy intake from carbohydrate (CHO), fat, and protein from the FFQ and 4-day diary were comparable to the 7-day weighed inventory, (FFQ: CHO 55 (3) % fat 28(3 1)% protein 17 (1) %, 4-day diary: CHO 56 (3) % fat 27 (3)% protein 17 (1) %, and 7-day weighed inventory: CHO 56 (3)% fat 29 (3)% protein 15 (1) %), with no significant differences between methods. Although, the 7-day diet is accepted as the gold standard method for the analysis of dietary intake the validity of estimates is influenced by the variation in training periodization, and reduced compliance due to increased burden for the athletes. The use therefore of the 4-day diet and the EPIC FFQ within this research would seem adequate to assess the nutritional behaviours of the endurance athletes.

3.2.8 Eating psychopathology and exercise cognitions

Athletes were requested to complete two self reported measures; the Eating Disorder Examination Questionnaire (EDE-Q) and the Compulsive Exercise Test (CET). The EDE-Q is a self reported version of the eating disorder examination (EDE) (Fairburn and Beglin 1994). The EDE-Q is a 36 item questionnaire which provided 4 subscale scores, a global score and a specific section of diagnostic questions related to binge/purging. Each item on the questionnaire is scored from 0-6. The CET is a 24 item self reported questionnaire designed to assess four main domains of compulsive exercise (compulsivity, affect regulation, weight and shape driven exercise, and behavioral rigidity (Taranis et al. 2009).

Participants were asked to fill out both the EDE-Q and the CET (Appendix 8) during each laboratory visit, truthfully answering the questionnaires of their cognitions and behaviors over the last 28 days. To aid truthful responses on the questionnaires privacy was given to each participant and reassurance was given that all answers on the questionnaires would remain confidential.

3.2.9 Menstrual function

Menstrual function data were collected retrospectively by asking participants to complete a menstrual function questionnaire which included questions regarding age at menarche, classification of the number of menses per year, and oral/hormonal contraception use (Appendix 7). To allow prospective monitoring of menstrual function, athletes were asked to record each month for a period of 12-month, the date of their menstrual flow, and the number of days the flow lasted. Current menstrual function was assessed during each assessment visit to determine any annual change in menstrual status.

3.2.10 History questionnaires

Retrospective information of the participant's weight, musculoskeletal health, nutritional, menstrual, injury and training history was collected by asking each participant to complete a questionnaire at baseline and 12-month assessment periods (appendix 9). Each of the sub-sections within the questionnaire was modified from previous questionnaires used to assess bone health and stress fracture risk. History of weight was asked to determine if participants were concerned with thinness during competition. Participants were asked if there was a family history of osteoporosis, or if they had ever been diagnosed with either low BMD or eating disorders, components of the female athlete triad. History of stress fractures/stress reactions was assessed by questionnaire. These were defined as a fracture/reaction clinically diagnosed by a sports physician and confirmed with a positive diagnosis on X-ray, CT, or MRI. For each stress fracture/reaction, athletes recorded the age when the stress fracture occurred, location, time of year, and method of diagnosis. The participants were asked to complete the questionnaire as truthfully as possible. To ensure truthful responses on the questionnaire privacy was given to each participant and reassurance was given that all answers on the questionnaires would remain confidential. The questionnaire was piloted in a sample of 17 recreational runners prior to this research

to evaluate the effectiveness of the questionnaire. Prior to the analysis the question was coded.

3.2.11 Prospective measure of training

Current training and training history were assessed using questionnaire at baseline and during the 12-month follow-up session. Participants were asked to record the number of training phases per year, the event they were training for, frequency of sessions per week, weekly training duration (hours per week), subjective measure of training intensity per training phase (%) and the predominant training surface. Participants were also asked to keep a month training diary for the 12-month period which they were asked to fill in daily. Participants were asked to record the type, duration, intensity and total mileage of training, and any injuries experienced over the month. The diary was analyzed and spilt into intensity, frequency and duration of training.

3.2.12 Anthropometric

During each of the three laboratory visits anthropometric data was collected to monitor changes over the 12-month training phase. For each visit participants were instructed to wear the same or similar clothing, consisting of shorts and T-shirt/vest containing no metal. Stature, body mass, girth and segment length were measured using standard procedures (Lohman 1988). Brief descriptions of the anthropometric procedures are reported below.

3.2.13 Stature

Stature was measured using a portable suitcase-mounted stadiometer (Holtain, Pembrokeshire, Ltd) to the nearest 0.01 metre (m). Participants were asked to stand barefoot on the stadiometer platform positioning their heels, buttocks, and scapulae against the backboard, in a relaxed position. The participant's head was placed in the Frankfort horizontal plane, the headboard was lowered to rest on the vertex of the skull

ensuring the hair was compressed. As the participant inhaled deeply, traction was applied and a the maximal measurement was recorded (Lohman et al. 1988). The stadiometer was calibrated prior to every measurement with a metre rule.

3.2.14 Body mass

The mass of the human body was measured to the nearest tenth of a kilogram (kg) (Kent 1998) (Heyward and Wagner 2004), using beam balance scales (Herbert and sons, Ltd, London). Participants were asked to step onto the base ensuring both feet were evenly spread, looking straight ahead. The individual's body mass was recorded once the balance bar was balanced in the centre of the scales. To standardize the measure participants were weighed in shorts and T-shirt/vest first thing in the morning prior to eating breakfast and after voiding.

3.2.15 Body mass index (BMI)

Body mass index is an estimated indicator of overweight or underweight (Kent 1998). BMI was calculated by body mass in kilogram (kg) divided by stature in metres squared. BMI does not take into account a person's body composition. For instance an individual who has a high lean mass such as a weightlifter may be classified as obese on the basis of a high BMI. Thus the assumption that BMI is a good reflection of body fat is not always true (Heyward and Wagner 2004; Kent 1998).

3.2.16 Body composition

Body composition is the relative amount of different components in the body and is often spilt into fat-free mass and fat mass (Kent 1998). There have been a number of methods developed (hydrodensitometry, air displacement plethysmography, hydrometry, dual energy x-ray absorptiometry, and skin-fold measurements), to assess body composition. For the purpose of this research body composition was assessed using dual energy x-ray

absorptiometry (DXA). DXA measurement can provide estimates of 3-compartments: fat mass (FM), lean tissue mass (LTM) and total body bone content (TBBC) (Heyward and Wagner 2004)(Heywood). The procedure used to determine body composition is described in section 3.2.1.1.1 total body. The CV for the measurements of body composition is given in table 3.1.

3.2.17 Circumference and segment measurements

Circumference measurements were recorded to detect changes that may reflect changes in CSA of muscle and/or fat (Lohman et al. 1988). Past research has indicated girth measures of the lower extremities may predict the risk for stress fracture in athletes (Bennell et al. 1996a). For this study the girth measures of the dominant mid thigh and mid calf were recorded following standard techniques (Lohman et al. 1988).

Femur length and mid-thigh circumference

The recommended technique for measuring the thigh circumference required the athletes to sit with their knees flexed at 90 degrees. A tape was placed at the centre of the inguinal crease and the distance to the proximal border of the patella measured. A mark was made at mid-point using a water soluble pen (Lohman et al. 1988). The participant was then asked to stand and face the researcher with their feet approximately 10cm apart, the weight was distributed on the opposite leg, the measured leg was relaxed with the knee slightly flexed (Lohman et al. 1988). A tape measure was placed horizontally around the participant's thigh and the circumference was recorded to the nearest millimetre.

Tibia length and calf circumference

The calf circumference was measured at the mid-point of the tibia to ensure consistent measures throughout the 12-month period. A pen mark was made mid-point between the medial epicondyl of the femur to the medial malleous. The circumference of the calf was

measured, with the participant standing with the feet approximately 10cm apart and weight leaned on the opposite leg, with a tape measure placed horizontally around the calf the circumference was read to the nearest millimeter.

Chapter Four:

Correlates of stress fracture history in female endurance athletes in the United Kingdom

Chapter four presents the retrospective analysis of correlates of stress fracture history. The results from this chapter were presented orally at the British Association of Sport and Exercise Medicine (BASEM) annual conference, Edinburgh, UK in October 2009. The abstract for this chapter is in press (Appendix 10: Abstract).

Correlates of stress fracture history in female endurance athletes

4.1 Introduction

Stress fractures are the most common over use injury in athletes (Bennell et al. 1995). It is thought that stress fractures occur in athletes when microdamage caused by repetitive mechanical load exceeds the biological capacity of the bone. Reports of prevalence vary from 2.7-41.5%. This variation may be due, at least in part, to differences in the population studied and in the classification used (Bennell et al. 1996b; Nattiv et al. 2000).

In studies of military recruits risk factors for stress fracture have included lower lean mass, irregular menses, lower BMD, decreased muscle size and strength, poor skeletal alignment (leg length discrepancy), narrow tibia cross sectional area, and low physical fitness (Beck 2003; Beck et al. 2000; Giladi et al. 1991; Milgrom et al. 1988). Even though the earlier military studies have improved our understanding of the aetiology of stress fracture, these risk factors may not translate well to the athletic population where stress injury is the most common over use injury and where fitness and training may differ markedly (Bennell et al. 1995). In female athletes, previously identified risk factors of stress fracture include lower calf circumference, late age at menarche, menstrual dysfunction, low bone density, disordered eating, and abnormal gait (Bennell et al. 1999; Bennell et al. 1995; Bennell et al. 1996a; Brukner et al. 1998; Carbon et al. 1990; Myburgh et al. 1990; Nattiv 2000; Snyder et al. 2006). However, further studies are needed to resolve conflicting findings in the research. Endurance athletes are thought to be at a particularly high risk for stress fracture injury, due to the negative association of high training intensity and volume and low BMD. In female endurance athletes the high prevalence of menstrual dysfunction has been reported to increase stress fracture risk (Bennell et al. 1999; Bennell et al. 1996a; Nattiv 2000). There are limited studies however,

to determine the correlates of stress fracture in endurance female athletes, and the findings to date are conflicting and based on small sample sizes (Snyder et al. 2006). The high prevalence (1-62%) (Sundgot-Borgen and Torstveit 2007) of eating disorders or disordered eating in female athletes could increase the risk of stress fracture (Bennell and Grimston 2001), however few studies have reported the association between stress fracture risk and disordered eating patterns (Bennell et al. 1995; Kelsey et al. 2007; Myburgh et al. 1990; Nattiv and Armsey 1997). Some have reported an increased risk of stress fracture (Bennell et al. 1995; Nattiv and Armsey 1997) and others no increased risk (Kelsey et al. 2007) when associated with restrictive eating patterns. Given the increasingly accepted role of eating behaviours in the female athlete triad further investigations are required using more sensitive tools to measure both eating and stress fracture occurrence to highlight the role eating attitudes may play in stress fracture risk (Nattiv et al. 1994; Nattiv et al. 2007; Snyder et al. 2006). To our knowledge there is no indication of whether compulsive exercise, which commonly occurs alongside restrictive eating patterns in athletes, has an independent effect on stress fracture risk or whether these behaviours may be mediating the relationship between restrictive eating patterns and risk of stress fracture. A recent epidemiologic review of stress fracture risk in athletes revealed that the literature is contradictory with no compelling consensus for risk factors of stress fracture in athletes (Snyder et al. 2006). The majority of previous studies have not used multivariate models to determine whether risk factors are independent of one another (Snyder et al. 2006).

The main aim of this study was, therefore to investigate the correlates of stress fracture in female endurance athletes. Further objectives were to 1) determine the prevalence of past stress fracture, 2) determine the distribution of stress fractures on the body, and 3) to

determine if eating and exercise cognitions as well as the previously established risk factors are independent predictors of stress fracture in female endurance athletes.

4.2 Methods

4.2.1 Study design

A retrospective cohort design was used with history of stress fractures and current risk factors assessed at one visit. Questionnaires were completed to assess stress fracture history, eating psychopathology, dietary intake, menstrual dysfunction and training history. BMD, BMC, geometric properties and body composition were measured using dual energy x-ray absorptiometry (DXA), and anthropometric measures were taken. The study received ethical approval from the National Research Ethics Service (NRES) and Loughborough University ethical committee. All athletes gave written informed consent.

4.2.2 Subjects

70 female endurance athletes (58 runners, 12 triathletes) aged 18-45 years were recruited from the English Institute of Sport, UK athletics, British triathlon and UK registered running and triathlon clubs. Athletes were required to either be competing at international, national, or county level or training between 8-10 hours per week (runners) or 15-20 hours per week (triathletes) in events from 800m to the marathon or triathlon. Athletes were classified into one of five groups: junior elite (18-23 yrs, competed for England and higher): senior elite (23+ yrs, completed for England and higher), veteran elite (35+ yrs, completed for England or higher), age group elite (consisted of athletes who had made the elite start time for the marathon) and county level (athletes who in the last 12-month competed and were ranked in the top ten of their county). Athletes were excluded from the study if they were not currently training, if they were currently pregnant and lactating and had been in the last 12-months.

4.2.3 Injury history

History of stress fractures/stress reactions was assessed by questionnaire. These were defined as a fracture/reaction clinically diagnosed by a sports physician and confirmed with a positive diagnosis on X-ray, CT, or MRI. For each stress fracture/reaction, athletes recorded the age when the stress fracture occurred, location, time of year, and method of diagnosis.

4.2.4 Menstrual function

Participants were classified into two groups based on their menstrual function in the last 12-months: a/oligomenorrhoeic phenotype ($\leq$ 9 periods per year, this may be two hormonally distinct conditions) and eumenorrhoeic ($\geq$ 10 periods per year). Athletes were asked their age at menarche, number of periods per year and duration of a/oligomenorrhoea since menarche, and current use of hormonal contraception (HC).

4.2.5 Eating psychopathology and exercise cognitions

Athletes completed two self report measures: the Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn and Beglin 1994) and the Compulsive Exercise Test (CET)(Taranis et al. 2009). The EDE-Q is a self reported version of the eating disorder examination (EDE)(Fairburn and Beglin 1994). The EDE-Q is a 36 item questionnaire which provides four subscale scores, a global score and a specific section of diagnostic questions related to binge/purging. Each item on the questionnaire is scored from 0-6 (appendix 5). The CET is a 24-item self reported questionnaire designed to assess four domains of compulsive exercise (compulsivity, affect regulation, weight and shape driven exercise and behavioural rigidity) (appendix 8)(Taranis et al. 2009).

4.2.6   Dietary behaviours

Participants were asked to complete two self reported nutritional measures: the European Prospective Investigation into Cancer (EPIC) food frequency questionnaire (FFQ) (Bingham et al. 1994; Welsh 2005) and a four day diet diary. The EPIC FFQ (Bingham et al. 1994; Welsh 2005) is a self reported questionnaire which gives general information of the type, frequency, and servings of foods the participants have eaten over the past 12-months. The four day food diary was completed by the participants over three week days and one weekend. The FFQ and the 4-day diet diary were coded and entered into a computerized software package (CompEat pro, version 5.8) to determine the total energy intake, and macro and micro-nutritional intake. The 4-day diet was averaged over the four days. The 12-month FFQ was averaged to the number of servings per day prior to analysis.

4.2.7   BMD and geometry

Dual-energy x-ray absorptiometry (DXA) was used to measure BMD and BMC of the total body, lumbar spine, femoral neck, and radius (Lunar Prodigy, GE Healthcare, Madison, WI, U.S.A version 12.2). Bone geometry at the femoral neck was estimated using Lunar advanced hip structural analysis (AHA) algorithms to determine the CSMI, CSA, minimal femoral neck width and Z. All scans were conducted on the dominant side as this is thought to be the most prevalent side for injury (Bennell et al. 1996a).

4.2.8   Anthropometric measures

Height and body mass were measured using standardized protocols using a stadiometer (Holtain, ltd, Pembrokeshire) and beam balance scale (Herbert and sons ltd, London) respectively. Body composition was assessed by DXA to determine percent body fat, total lean and fat tissue. Circumferences of the dominant femur and tibia were estimated with a tape measure held horizontally at the midpoint between the inguinal crease and the patella

for the femur, and midpoint between the proximal tibia border and the medial malleolus for the tibia

4.2.9 Statistical analysis

All statistical analyses were performed using SPSS16.0 (SPSS Chicago, Illinois, USA). Potential correlates of stress fracture were compared between athletes with and without stress fracture history using a one way analysis of variance (ANOVA). Subjective ratings of eating psychopathology and compulsive exercise were compared between athletes with and without a history of stress fracture using a Mann-Whitney U test. Categorical associations of stress fracture history were evaluated using the Chi-squared test. Statistically significant correlates of stress fracture identified in univariate analyses at $p<0.05$ were entered into a logistic regression analyses along with theory driven variables BMI (as a measure for body size) and HC (to determine an independent contribution of menstrual function) to determine their independent contribution to stress fracture. For this analysis, eating psychopathology and compulsive exercise variables were re-coded into two subgroups using a median split. Statistical significance was considered at the 5% probability level ($p< 0.05$).

4.3 Results

Nineteen (27%) athletes experienced a total of 24 stress fractures. Distribution of the athletes' events, competitive level and number of stress fractures are summarised in table 4.1. Common sites of stress fracture history were the metatarsals (46%), tibia/fibula (38%), calcaneus (13%), and the femur (4%) (figure 4.1).

The mean (SE) age of stress fracture was 21.4 (1.4) years, with stress fractures diagnosed on average 3.2 years from the start of training. Age, height, body mass and body composition did not differ according to stress fracture history (table 4.2)

A high prevalence of menstrual dysfunction was evident, with 42.9% of athletes categorized as a/oligomenorrhoeic ($\leq$ 9 periods/year), 57.1% eumenorrhoeic ($\geq$ 10 periods a year). Of the 70 athletes, 21 (30%) (4 a/oligomenorrhoeic and 17 eumenorrhoeic) were currently using oral contraceptives (HC).

Table 4.1: Summary of athletes' events and competitive levels

Events	Athletes (N =70)	Level					Number (%) with stress fracture History
		Junior Elite (18-23 yr)	Senior Elite (23+ yrs)	Vet Elite (35+ yrs)	Age Group elite (marathon)	Club (County level)	
Middle Distance (800m, 1500m)	16	5	0	1	5	5	5 (31%)
Long Distance (3k, 5k,10k)	42	6	1	3	13	19	10 (24%)
Triathlon	12	3	2	1	4	2	4 (33%)
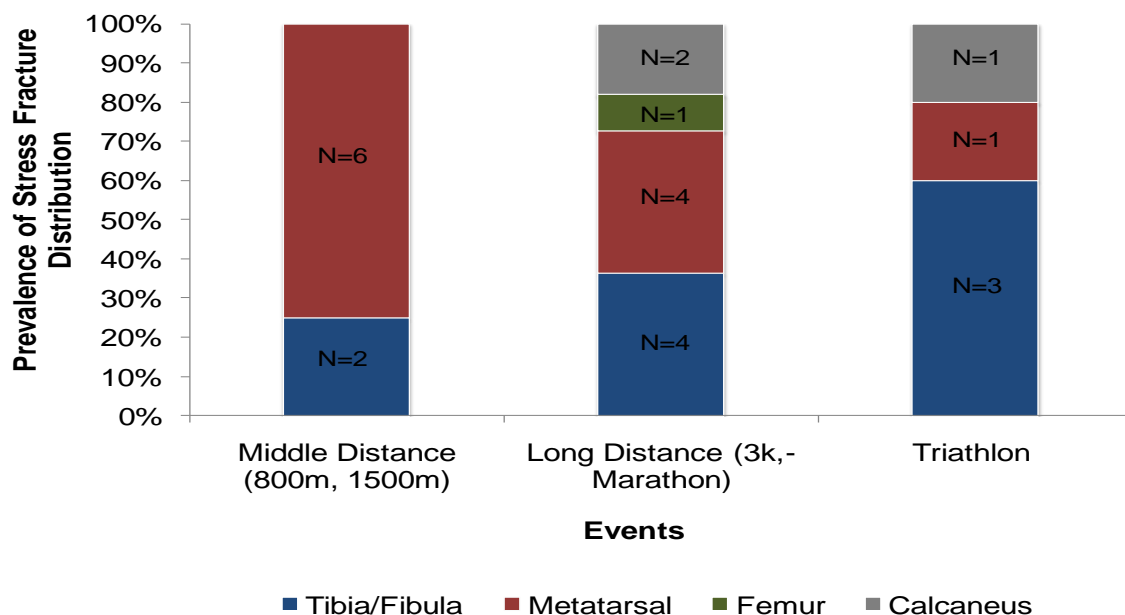
Figure 4.1: The prevalence of stress fracture/reaction distribution according to endurance event. N refers to the number of stress fractures/reactions at each bone location in each event.

Table 4.2: Characteristics of athletes according to stress fracture (FX) history: (mean (SE)

Characteristics	FX (n = 19)	Non-FX (n = 51)
Age (years)	25.6 (1.4)	26.1 (1.8)
Height (m)	1.67(0.01)	1.67 (0.01)
Weight (kg)	56.2 (1.1)	54. 9 (0.8)
Body fat (%)	16.6 (1.1)	17.4 (0.9)
Calf girth circumference (cm)	32.0 (0.53)	31.2 (0.25)
Training		
Years of training (yrs)	7.4 (1.0)	7.8 (0.9)
Age started competing	18.3 (1.6)	18.4 (1.2)
Hours spent training per week	14.2 (1.0)	12.1 (0.6)
Bone Mineral Density (g/cm²)		
Total body	1.160 (0.016)	1.151 (0.009)
Femoral neck	1.068 (0.028)	1.089 (0.015)
Lumbar spine	1.144 (0.0.36)	1.119 (0.014)
Radius	0.832 (0.014)	0.818 (0.009)
Bone Mineral content (g)		
Total body	2482 (70)	2425 (42)
Femoral neck	4.9 (0.1)	5.1 (0.1)
Lumbar spine	60.5 (2.8)	60.2 (1.4)
Radius	2.1 (0.1)	2.0 (0.4)
Bone Geometry		
CSMI (mm⁴)	9941 (592)	9672 (347)
CSA (mm²)	154 (4)	157 (3)
Minimal femoral neck width (mm)	28.9 (0.6)	28.4 (0.3)
Section Modulus (mm³)	677 (27)	674 (17)
Menstrual History		
**Age at Menarche	13.9 (0.4)	14.1(0.3)
**History of amenorrhoea	15 (78.9%) *	26 (51.0%)
**Current a/oligomenorrhoeic	13 (68.4%) *	17 (33.3%)
**Current eumenorrhoeic	6 (31.6%)	34 (66.7%)
Hormonal Contraception		
**Current hormonal contraception users (HC)	4 (21.1%)	17 (33.3%)
** Current hormonal contraception use in a/oligomenorrhoeic (HCA)	4(21.1%)	3(64.7%)

*significant difference between stress fracture and non stress fracture groups: *P < 0.05*
** Reported as the number of people and percentage
The prevalence of a/oligomenorrhoea was significantly higher *(p=0.008),* in the stress

fracture than non-stress fracture group, as was a history of amenorrhoea *(p=0.035)* (table

4.2) although age of menarche did not differ significantly between groups (13.9 (0.4) years, 14.1 (0.3) years, *p=0.691*). Similarly no significant differences were found in calf girth measurements, BMD, BMC (total body, lumbar spine, femoral neck radius) and femoral neck geometric properties (CSA, CSMI, minimal femoral neck width and Z) between the stress fracture and non-stress fracture group (table 4.2).

Athletes with a history of stress fracture scored higher on the global scores for both the EDE-Q and the CET (p=0.049, p=0.006 respectively) and had a higher behaviour rigidity score on the CET (p=0.038) than those without stress fracture (table 4.3).

Table 4.3: Eating disorder examination and compulsive exercise test questionnaire responses according to stress fracture history median (inter-quartile range).

Characteristics	FX (N =19)	Non-FX (N=51)	
EDE-Q			
Restraint	1.6 (0.6-3.2)	1.0 (0.4-1,8)	
Eating Concerns	0.6 (0.2-1.4)	0.2 (0.2-0.6)	
Shape Concerns	1.9 (0.9-3.6)	1.3 (0.5 -2.0)	
Weight Concerns	1.2 (0.6-3.0)	1.0 (0.4-1.8)	
Global Scores	1.7 (0.6-2.7)	0.9 (0.5-1.6)	*
CET			
Compulsive Exercise	3.4 (2.6-3.9)	2.8 (1.9-3.5)	
Shape and weight	2.4 (1.8-3.4)	2.0 (1.6-2.6)	
Mood Regulation	4.0 (3.4-5.0)	3.8 (3.0-4.4)	
Lack of exercise enjoyment	0.7 (0.0-1.3)	0.3 (0.0-1.0)	
Behaviour Rigidity	3.7 (3.0-4.0)	3.0 (2.7-3.7)	*
Global Scores	2.9 (2.4-3.1)	2.4(2.1-2.8)	*

*Significant difference between stress fracture and non-stress fracture groups: Mann Whitney-U test *P < 0.05.*

Comparison of means, using an one way analysis of variance (ANOVA), revealed no significant differences between groups in total energy intake, percentage of energy intake from carbohydrates, protein and fat, and calcium (mg) when assessed using the four day diet analysis and the FFQ (table 4.4).

The variables that differed according to stress fracture history in the univariate analysis, current a/oligomenorrhoea, eating psychopathology scores, and compulsive exercise scores, along with BMI and current HC use were entered into a multiple logistic regression. A/oligomenorrhoea *(p=0.010, B=1.492),* was found to be a significant predictor of stress fracture history, increasing the relative risk by 4.4 times compared to athletes who were eumenorrhoeic (table 4.5, model B). This risk persisted when current HC was entered into the regression model (table 4.5, model C) with a/oligomenorrhoea significantly predicting *(p=0.013, B1.457)* stress fracture risk independent of HC use (p=0.423, B-0.542). Eating psychopathology was not a significant predictor of stress fracture *(p=0.405, B=0.490)* when entered into the regression model with BMI and a/oligomenorrhoea (table 4.5, model E). However, when a measure of compulsive exercise was added to the regression model whose who were a/oligomenorroea were 6.1 times *(p=0.005, B 1.813)* more likely to have a stress fracture than whose who were not (table 4.5, model D). Compulsive exercise was identified as a risk of stress fracture history independently of a/oligomenorrhoea with compulsive exercisers having a relative risk of stress fracture 5.8 times *(p=0.009, B=1.754)* greater than those with non-compulsive attitudes. These findings persisted when a measure of eating psychopathology was added to the regression model (table 4.5, model F).

Table 4.4: Dietary intake of athletes according to a history of stress fracture measured using a 4-day diary and an FFQ: mean (SE). Macro nutrients are reported as the percentage of the energy intake.

Four-day diet diary	FX (n=17)	Non-FX (n=44)
Energy Intake (kcal)	1862 (167)	1875 (55)
CHO (%)	55.2 (1.3)	56.6 (0.8)
Protein (%)	14.8 (0.7)	16.2 (0.5)
Fat (%)	30.0 (1.2)	27.2 (0.8)
Calcium (mg)	887 (92)	944 (42)

FFQ	FX (n=19)	Non-FX (n=51)
Energy Intake (kcal)	2959 (301)	2560 (88)
CHO (%)	52.5 (1.6)	54.6 (0.9)
Protein (%)	17.6 (1.0)	18.3 (0.5)
Fat (%)	29.9 (1.5)	27.1 (0.9)
Calcium (mg)	1546 (128)	1391 (65)

Table 4.5: Independent predictors of stress fracture history, *odds ratio (95% CI)*

	Model A	Model B	Model C	Model D	Model E	Model F
Constant (B)	-3.27	4.6	-4.7	-8.4	-4.8	-8.8
Cox & Snell **R²**	0.006	0.103	0.111	0.200	0.112	0.205
Variables						
BMI	1.1 (0.8-1.6)	1.2(0.8-1.7)	1.2 (0.8-1.2)	1.2 (0.8-1.8)	1.1 (0.8-1.6)	1.2 (0.8-1.9)
Current a/oligomenorrhoea		4.4 (1.4-13.9) *	4.3 (1.4-13.5) *	6.1 (1.7-21.8) **	4.1 (1.3-13.1) *	6.9 (1.6-26.3) **
Current HC			0.6 (0.2-2.2)			
Global CET				5.8 (1.6-21.4) **		7.3 (1.6-33.5) *
Global EDE-Q					1.6 (0.5-5.2)	0.6 (0.2-2.7)

Logistic Regression with stress fracture history as the dependent variable, significant difference reported as: * $P< 0.05$, **$P < 0.01$, Cox & Snell R^2 = variation in models
BMI = Body mass index, HC= Hormonal contraception, CET =Compulsive Exercise Test, EDE-Q = Eating Disorder Examination-Questionnaire.

4.4 Discussion

This study is novel in that it has identified independent predictors of stress fracture history using a multivariate approach, identifying that eating psychopathology, and exercise cognitions as well as amenorrhoea may play a role in stress fracture aetiology.

This study found that 27% of female athletes had a history of stress fracture, which is consistent with past retrospective studies in females (2.7-41.5%) (Snyder et al. 2006). Variations between studies may be caused by the type of sporting event studied, (athletes who participate in repetitive loading sports which emphasize leanness often report the highest prevalence of stress fracture), the classification of stress fracture, (studies which rely on active stress fracture diagnosis the prevalence of stress fracture will be higher than if reported passively), and the age of the athletes recruited (stress fractures are often greatest in the early twenties) (Snyder et al. 2006). Our findings are consistent with Kelsey et al (Kelsey et al. 2007), who reported from a prospective study of 127 female cross country runners a prevalence of stress fracture history of 31%. Similarly, Dixon et al (Dixon and Fricker 1993), reported a stress fracture history of 27% in female gymnasts. Conversely, Bennell et al, (Bennell et al. 1995) reported a prevalence of stress fracture history in 54 female track and field athletes of 41.5%. When characterized as endurance athletes this prevalence increased to 50%. The variance between our findings compared with the Bennell et al (Bennell et al. 1995) study could be caused by a number of factors such the definition used to determine stress fractures, the age and potential sample bias. Retrospective studies recruiting for a study on stress fractures may unknowingly recruit a sample of athletes with a greatest interest in stress fracture because of a past history, thus biasing the sample and possibly resulting in increased relative risk of stress fracture history.

The most common site of stress fractures for female endurance athletes in this study was the metatarsals (46%), tibia/fibula (38%), followed by the calcaneus (13%), which is in support with an earlier study of female distance runners (Arendt et al. 2003), but conflicts with a number of other studies which have reported the greatest proportion of stress fracture to occur at the tibia (44-63%) (Barrow and Saha 1988; Bennell and Brukner 1997; Bennell et al. 1995; Bennell et al. 1996b; Matheson et al. 1987). Bennell et al (Bennell et al. 1995) reported the highest proportion of stress fractures in track and field (sprinters, jumpers, middle/distance) athletes to occur at the tibia (33%) with only 20% of stress fractures occurring at the metatarsals. Similarly, previous studies (Benazzo et al. 1992; Bennell et al. 1995; Bennell et al. 1996b) have reported significant associations with specific events and fracture risk, with distance runners more likely to suffer long bone (tibia and femur) stress fractures (Bennell et al. 1995), and sprinters and jumpers foot (metatarsal and tarsal) stress fractures (Benazzo et al. 1992). However, because these studies reported distribution of stress fracture over multiple events and sports the numbers of athletes with stress fracture in each group were small and therefore may not have given a representative comparison of stress fracture distribution in a specific event such as endurance running. The increased distribution of metatarsal stress fractures in our study of female endurance athletes may have been associated with a history of a change in training surface, training volume, or load bearing when running, which all have previously been associated with a greater proportion of metatarsal stress fracture (Crossley et al. 1999), however further studies would be needed to clarify this explanation.

Current menstrual dysfunction was prevalent in this sample of athletes with 43% of athletes reporting to be currently a/oligomenorrhoeic. Of the athletes who had a history of stress fracture 47% were currently a/oligomenorrhoeic and 31% were eumenorrhoeic, whilst the remaining 22% were currently a/oligomenorrhoeic and taking hormonal

contraceptives. Our study showed compelling evidence that a history of amenorrhoea (78%) is prevalent in athletes with a history of stress fracture, supporting earlier findings (Bennell et al. 1995; Carbon et al. 1990; Grimston and Zernicke 1993; Myburgh et al. 1990; Warren 1992).

The present study found no significant differences in age at menarche between groups, however in accordance with previous literature, this study did show evidence of delayed menarche in the athletic population (Malina and Bouchard 1991). The average age of menarche in the general European population is 12.3 years (Morris et al. 2011) where as in this population of athletes the average age of menarche was 13.9 years in the stress fracture group and 14.1 years in the non stress fracture group. This delayed age at menarche was not associated with an increased risk for stress fracture. Previous research is contradictory with some studies identifying age at menarche to be associated with stress fracture (Bennell et al. 1995; Bennell et al. 1996a; Carbon 1992), and others finding no association (Kelsey et al. 2007; Myburgh et al. 1990). Prospectively (Bennell et al. 1996a), it has been shown that for every 1 year increase in age at menarche, the risk of stress fracture increases by a factor of 4.1. The possible association between age at menarche and stress fracture could be due to a lower rate of BMD accrual during adolescence in individuals with later age at menarche, thus possibly decreasing peak bone mass (Bennell and Grimston 2001; Bennell et al. 1996a). As the present study did not find significant differences in either age at menarche or current BMD, it may be possible to suggest that stress fracture history in this sample of athletes is associated with secondary rather than primary amenorrhoea or delayed menarche. Current menstrual dysfunction was identified as an independent predictor of stress fracture history, with a/oligomenorrhoeic athletes 4.3 times more likely to have a history of stress fracture compared with those who were currently eumenorrhoeic. This finding is in support of Bennell et al, (Bennell et al. 1995)

who reported athlete with a history of menstrual dysfunction to be 6 times more likely to stress fracture than athletes with no history of menstrual dysfunction.

Athletes with a history of stress fracture scored higher on the EDE-Q than athletes with no stress fracture history, thus showing a greater concern for eating psychopathology in athletes with stress fracture history. This finding supports earlier work which has used a less robust measure of eating psychopathology (Bennell et al. 1995). However, unlike previous studies (Bennell et al. 1995) our findings did not show eating psychopathology to be an independent predictor of stress fracture history therefore the relationship between restrictive eating patterns and stress fracture may be masked by the a/oligomenorrhoea, which is independently increasing the risk of stress fracture. The restrictive eating patterns of athletes may contribute to the a/oligomenorrhoea by reducing energy availability thus affecting the endocrine system resulting in menstrual dysfunction.

A novel addition to the literature from the present study is the finding that a history of stress fracture is associated with compulsive exercise behaviours. Using the compulsive exercise test (CET) (Taranis et al. 2009) athletes with a history of stress fracture scored higher on questions related to behavior rigidity and global scores for the CET than the non stress fracture group. This may indicate that athletes who stress fracture are more likely to train through illness or injury to prevent deviating from training habits than the non-stress fracture group. It was also shown that compulsive exercise, unlike eating psychopathology, is an independent predictor of stress fracture, with compulsive exercisers 7.3 times more likely to have a history of stress fracture than non-compulsive exercisers. This finding was independent of a/oligomenorrhoea and eating psychopathology. It is therefore important to examine training behaviours as well as menstrual status when identifying at risk athletes for stress fracture.

This study did not identify an association between dietary intake and stress fracture history, no significant differences in nutritional habit between groups were found when determining the percentage of intake from carbohydrates, proteins and fats and calcium intake (mg) from an FFQ and 4-day diet analysis. However, this finding cannot rule out the role changes in nutritional intake may have on stress fracture risk if monitored prospectively. Previous prospective studies have shown conflicting findings of the risk of stress fracture due to nutritional habits, some have shown low dietary calcium intake to be a significant predictor of stress fracture (Kelsey et al. 2007), whereas others have identified that neither calcium nor energy intake was associated with stress fracture (Bennell et al. 1996a). These differences in findings may be due to the variation in measurement tools (food diaries verses FFQ) used to quantify nutritional intake. The only possible cause for the variation in findings could be the geographical location in which the studies were carried out (Australia (Bennell et al. 1996a), United States (Kelsey et al. 2007) and the present study United Kingdom). Different countries may fortify foods with calcium, therefore increasing daily intake. However, if this was the case we would expect the athletes in the United States where food is fortified to have increased calcium and vitamin D status, however it is Kelsey et al (Kelsey et al. 2007), which have shown low calcium intake to be associated with stress fracture, whereas both the studies in Australia and the United Kingdom reported calcium intake to be no different between stress fracture and non stress fracture groups. Similarly calcium intake in these studies was within the recommended value for daily calcium intake. Further studies may be required to determine it nutritional habits and calcium intake are potential risk factors of stress fracture in athletes.

Several of the risk factors that have been associated with stress fracture in previous studies were not significantly associated with stress fracture history in this study. BMD and

calf circumference were not significantly different between the groups in this sample of athletes. Previous research (Bennell et al. 1996a) has suggested that a lower calf girth circumference increases the risk of stress fracture in track and field athletes independently of all other factors. Our findings that BMD was not a significant correlate of stress fracture history support earlier work (Bennell et al. 1995; Carbon et al. 1990). However, the evidence for low BMD is conflicting with some showing BMD to be lower in stress fracture cases (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv 2000) and others showing no effect (Bennell et al. 1995; Carbon et al. 1990). Furthermore, it is unclear whether low bone density increases stress fracture risk, or whether amenorrhoea increases risk of both low bone density and stress fracture.

No differences in age, weight, height, and body composition were detected, supporting earlier work which failed to find differences in anthropometric measures between stress fracture and non-stress groups (Bennell et al. 1995). Training history was similar between groups with no differences found between years competing; hours spent training per week and sessions per week. However the sample size is modest in this study, and as the study is retrospective with stress fracture occurring on average 4.2 +/- 6.8 years ago, some of these factors such as BMD, bone geometric properties and nutritional intake, may have changed in the interim, obscuring possible associations.

The retrospective nature of this study incurs some limitations, such as potential recruitment bias, self reporting of history of stress fracture and the ability to recall data. Athletes were measured at variable intervals following stress fracture during which time behaviours, and other risk factors may have changed. Thus given the potential limitations of retrospective studies, further prospective studies are necessary to assess the risk factors of stress fracture in female endurance athletes.

Strengths of this research include the concentration on endurance female athletes, which reduces the potential confounding effects of discipline specific differences in stress fracture aetiology. This study used more robust measures of eating behaviours (EDE-Q) (Fairburn and Beglin 1994), showing that athletes with stress fracture history are more likely to have concerns with societal expectation for thin female athletes and restrictive eating behaviours than non stress fracture. The use of the compulsive exercise test is novel and has highlighted the importance for medical physicians to monitor the compulsive behaviour towards exercise in athletes independent of menstrual dysfunction.

In conclusion this study found that athletes with a history of stress fracture were more likely to have current menstrual dysfunction, as well as a history of amenorrhoea, than the non-stress fracture group. Disordered eating was associated with increased risk of stress fracture, but this was not independent of menstrual dysfunction. There were no indications that current BMD, body composition, and training regimens had any association with stress fracture history in these endurance athletes. Exercise cognitions, as well as amenorrhoea, were identified as independent predictors and may have a role in stress fracture development.

Chapter Five
Comparison of bone geometry and bone density according to menstrual function in female endurance athletes

Chapter five presents the results of the second retrospective analysis comparing bone geometry and bone density in female endurance athletes and sedentary controls according to menstrual function. The results from this chapter were presented orally at the American College of Sports Medicine (ACSM) conference, Baltimore, USA, in May-June 2010. The accepted abstract has been published in the MSSE supplement May 2010 (Appendix 11: abstract).

Comparison of bone geometry and bone density according to menstrual function in female endurance athletes and sedentary controls

5.1 Introduction

The prevalence of amenorrhoea ($\leq$ 3 periods a year) in athletes (44%) is substantially greater than in the general population (2-5%) (Bennell et al. 1997b), increasing the risk of future osteoporotic fractures. In eumenorrhoeic women exercise is often seen as a potential preventive measure for osteoporotic fractures, as the increased high impact loads can lead to increases in BMD relative to sedentary women (Bennell et al. 1996a; Snow-Harter and Marcus 1992). However in amenorrhoeic athletes the increased BMD accompanied with exercise is often counteracted by oestrogen deficiency (Bass 2003; Saxon and Turner 2006).

It is well established that amenorrhoeic athletes have substantially lower BMD at the lumbar spine and femoral neck than their regularly menstruating peers (Drinkwater 1994; Drinkwater et al. 1984; Grimston 1990; Micklesfield et al. 2007; Myburgh et al. 1993). Oestrogen deficiency in the amenorrhoeic athletes may promote bone loss, particularly from the endosteal surface, resulting in lower BMD, which could possibly be accompanied by structural adaptations (Bass 2003; Saxon and Turner 2006).

BMD has been used as a proxy for bone strength, suggesting that decreases in bone density is tantamount with declining bone strength (Cummings et al. 1993; Hui et al. 1988). However bone strength encompasses the bone's architecture, geometry, cortical porosity, and tissue mineralization which cannot be individually identified in BMD measurements (Bonnick 2007). Therefore it is appropriate to consider other estimates of bone strength

such as Z, which can be calculated using bone densitometry in the form of hip structural analysis (HSA) to fully understand the benefits of exercise.

Whilst it is known that exercise and oestrogen are beneficial to bone density (Frost 1999; Saxon and Turner 2006) it is unknown how bone geometry and hence the bone's bending strength, is affected in amenorrhoeic athletes. Exercise may enhance the bone's accrual on the periosteal (outer) bone surface, thus conferring greater resistance to bending, whereas oestrogen may inhibit periosteal apposition (Saxon and Turner 2006). It is possible that in amenorrhoeic athletes, where exercise is accompanied by low oestrogen levels, the low BMD is accompanied by structural differences such as increased bone diameter and Z (Bass 2003). Thus the aim of this study was to compare bone geometry and density of a/oligomenorrhoeic athletes, eumenorrhoeic athletes and eumenorrhoeic controls.

5.2   Methods

5.2.1 Study design

A cross-sectional design was used to compare bone status in female endurance athletes and sedentary controls according to menstrual function. BMD, BMC, and body composition were assessed using Dual-energy X-ray absorptiometry (DXA) (Lunar prodigy, GE Healthcare, Madison, WI, U.S.A, encore version 12.2). DXA was also used to estimate bone geometric parameters using the advanced hip structural analysis (AHA) software. Questionnaires were completed to assess current and past menstrual function.

5.2.2 Subjects

68 female endurance athletes (55 runners and 12 triathletes) and 88 healthy sedentary controls aged between 18-45 years were recruited. Athletes were recruited from sporting federations and registered running and triathlete clubs within the United Kingdom. They

were required to be competing at an elite level for their age or training at a high volume (8-10 hours per week for a runner and 15-20 hours per week for a triathlete) in events from 800m to the marathon or triathlon. Athletes were excluded if they were currently injured or had been in the past 12 months. Athletes and controls were excluded if they had been pregnant or lactating in the past 12 months and if they had any medical conditions or were taking medications which were likely to affect bone metabolism. The 88 controls were recruited within the Loughborough community from a previous intervention study, the aim of which was to determine the optimum weekly frequency of exercise to increase bone mass in premenopausal women who do not regularly participate in physical activity (Bailey and Brooke-Wavell 2010). Controls were screened to exclude individuals who; had a body mass index > 30 kg/m^2, participated in high impact or weight bearing exercise more than 1 h/week, and those who were not regularly menstruating (< 10-13 menstrual cycles per year) (Bailey and Brooke-Wavell 2010).

5.2.3 BMD and geometry

DXA was used to measure BMD and BMC of the lumbar spine (L1-L4), and femoral neck. Further measurements were assessed in the athletic group to determine BMD and BMC of the total body, and 33% distal radius to address further thesis aims. Bone geometric properties of the femoral neck were estimated using the AHA software (Lunar Prodigy, GE Healthcare, Madison, WI, U.S.A version encore 12.2) to determine CSA, Z, minimal neck width and strength Index.

5.2.4 Menstrual function

Questionnaires were used to assess menstrual function and hormonal contraceptive use. Participants were classified into three groups: a/oligomenorrhoeic phenotype athletes (AA) (< 9 periods per year, may be two hormonally distinct conditions), eumenorrhoeic athletes (including those taking hormonal contraception) (EA) (> 10 periods per year), and

eumenorrhoeic controls (EC). Participants who reported hormonal contraception use for less than 12 months were excluded from the study.

5.2.5 Calculation of menstrual index in athletes

Menstrual index was calculated using a modified version of the equation derived by Grimston et al, (Grimston et al. 1990) to determine an objective measure of menstrual history in female athletes, thus avoiding the ambiguity associated with the changes in menstrual function (amenorrhoea, oligomenorrhoea and eumenorrhoea) over a life span. Athletes were asked to record their age at menarche (M), the number of years they had been amenorrhoeic (0 – 3 periods per year) and oligomenorrhoeic (4 – 10 periods per year) since menarche. Using the equation below menstrual index (MI) was calculated:

$$MI = \frac{11.5 \ (no. \ yrs) + 7 \ (no. \ yrs) + 1.5 \ (no. \ yrs)}{C - M}$$

Where MI is a number representing the average menses per year over the entire length of menstruation, 11.5, 7 and 1.5 are the midpoints of the menstrual categories eumenorrhoea, oligomenorrhoea and amenorrhoea respectively, M represents age at menarche, and C is the current age.

Based on the MI categorizes derived by Grimston et al (Grimston et al. 1990), athletes were categorized into two groups, athletes with a history of menstrual regularity MI $\geq$ 10 (MI-Reg A) and athletes with a history of menstrual irregularity MI < 10 (MI-IRReg A). Sedentary control (EC) subjects reported, had no history of amenorrhoea.

5.2.6 Anthropometric measures

Height and body mass were assessed using standardised protocols using a stadiometer, a beam and balance scale respectively in all participants. Body composition in athletes was assessed using DXA.

5.2.7 Statistical analysis

Descriptive statistics (mean and standard errors) were used to characterize the sample. Analysis of variance (ANOVA) was used to compare means between menstrual function groups, with a Tukey's HSD Post Hoc test determining which groups differed. An analysis of covariance (ANCOVA) was used to adjust for age, height, and body mass. All statistical analysis was carried out using SPSS 16.0 (SPSS Chicago Illinois, USA). Level of significance was set at p value <0.05.

5.3   Results

The physical characteristics of the three groups can be found in table 5.1. Of the sixty-eight endurance athletes 24 (35%) were currently a/oligomenorrhoeic ($\leq$ 9 periods per year) and forty-four (65%) were eumenorrhoeic. Nineteen (43%) of the eumenorrhoeic athletes were currently taking hormonal contraception. According to the menstrual index (MI), 26 (40%) athletes had a history of menstrual irregularity. Three athletes did not provide adequate information to determine menstrual history using the MI.

Eumenorrhoeic controls had significantly lower height, and greater age and weight than both athlete groups, whether athletes were classified according to either current menstrual function or menstrual history (Table 5.1). Physical characteristics of athletes did not differ according to either menstrual function or menstrual history (Table 5.1). Percent body fat of the eumenorrhoeic and amenorrhoeic athletes was (mean (SE)) 17.7 (0.9) and 16.1 (1.1)% respectively (Table 5.1).

Table 5.1: Physical Characteristics of athletes and sedentary controls according to current menstrual function (mean (SE)

	Current Menstrual Function			Menstrual History (MI)	
	EC (N=88)	EA (N=44)	AA (N=24)	MI-Reg A (N=39)	MI-IRReg A (N=26)
Age (years)	32.8 (0.9)	26.9 (1.2) [a]*	23.9 (1.3) [a]*	27.6 + 1.1 [a]*	23.0 (1.4) [a]*
Body Mass (kg)	61.8 (1.1)	55.4 (1.0) [a]*	55.4 (1.0) [a]*	55.7 (1.1) [a]*	54.4 (0.9) [a]*
Height (m)	1.63 (0.01)	1.66 (0.01) [a]*	1.68 (0.01) [a]*	1.67 (0.01) [a]*	1.67 (0.01) [a]*
BMI (Kg/m^2)	23.2 (0.4)	20.0 (0.2) [a]*	19.6 (0.3) [a]*	20.0 (1.6) [a]*	19.6 (1.5) [a]*
Body Fat (%)	-	17.7 (0.9)	16.1 (1.1)		
Age at Menarche (yrs)	-	13.9 (.03)	14.5(.03)	-	-

*$p<0.05$ in ANOVA , [a] sig diff from EC
EC: Eumenorrhoeic control, EA: Eumenorrhoeic Athletes, AA: A/oligomenorrhoeic Athletes, MI-Reg A: Menstrual Index regularly menstruating Athletes, MI-IRReg A: Menstrual Index Irregularly Menstruating Athletes.

5.3.1 Bone mineral density (BMD) and bone mineral content (BMC)

Comparisons of BMD and BMC according to current menstrual function are shown in table 5.2. BMD of the femoral neck was significantly higher in eumenorrhoeic athletes than a/oligomenorrhoeic athletes and eumenorrhoeic controls by 8% and 11% respectively *(p<0.001)*. Lumbar spine BMD was significantly higher *(p=0.005)* in the eumenorrhoeic controls than the a/oligomenorrhoeic athletes (8% difference). There were no significant differences at the lumbar spine between the two athlete groups.

Similarly BMC of the femoral neck was significantly (*p<0.001*) higher (12%) in the eumenorrhoeic athletes than the eumenorrhoeic controls. However, there were no significant differences in BMC between a/oligomenorrhoeic athletes and other groups.

5.3.2 Bone geometry

Bone geometric properties estimated at the hip using DXA are summarised in table 5.2. The CSA of the hip was significantly higher in eumenorrhoeic athletes compared to a/oligomenorrhoeic athletes and controls (9% and 11% difference respectively, *p<0.001*). Z was significantly higher in eumenorrhoeic athletes than eumenorrhoeic controls *(11% difference, p=0.001)*. The strength index of the bone was significantly higher in eumenorrhoeic and a/oligomenorrhoeic athletes than eumenorrhoeic controls *(22% and 13% differences respectively, p<0.001)*.

Table 5.2: Comparisons of BMD, BMC and geometric measures in female athletes and sedentary controls according to current menstrual function and menstrual index: mean (SE)

	Current menstrual Function			Menstrual History (MI)	
	EC (N= 88)	EA (N= 44)	AA (N=24)	MI-Reg A (N=39)	MI-IRReg A (N=26)
Bone mineral density (BMD)					
BMD Femoral Neck (g/cm^2)	0.999 (0.014)	1.118 (0.015) [a]	1.023 (0.020) [b]	1.113 (0.014) [a]	1.059 (0.022)
BMD Spine (L1-L4) (g/cm^2)	1.188 (0.014)	1.148 (0.020)	1.092 (0.026) [a]	1.168 (0.020)	1.069 (0.024) [a c]
Bone mineral content (BMC)					
BMC Femoral Neck (g)	4.6 (0.1)	5.2 (0.1) [a]	4.8 (0.1)	5.1 (0.1)	5.0 (0.1)
BMC Spine (L1-L4) (g)	61.4 (1.1)	62.0 (1.7)	57.7 (1.9)	63.1 (1.8)	56.3 (1.8) [c]
Femoral neck geometric measures					
CSA (mm^2)	144 (2)	161 (3) [a]	148 (4)[b]	159 (3) [a]	154 (3) [a]
Minimum neck width (mm)	28.5 (0.2)	28.4 (0.3)	28.9 (0.5)	28.4 (0.4)	28.4 (0.4)
Section modulus (Z) (mm^3)	592 (10)	667 (19) [a]	625 (21)	665 (22) [a]	624 (18)
Strength Index	1.61 (0.03)	2.06 (0.0)6 [a]	1.87 (0.09) [a]	1.94 (0.06) [a]	2.04 (0.10) [a]

[a] sig diff from EC, [b] sig diff from EA, c sig diff from MI-Reg A p<0.05 ANOVA according to Tukey's Post Hoc test
EC: Eumenorrhoeic control, EA: Eumenorrhoeic Athletes, AA: A/oligomenorrhoeic Athletes, MI-Reg A: Menstrual Index regularly menstruating Athletes, MI-IRReg A: Menstrual Index Irregularly Menstruating Athletes.

In the athlete groups, despite the significantly lower CSA in the amenorrhoeic athletes compared to eumenorrhoeic athletes, there were no other significant differences in geometric properties (Z, strength index and minimal neck width) and hence the strength in bending was relatively maintained in the amenorrhoeic athletes.

5.3.3 BMD and bone geometry according to menstrual index (MI)

Sixty-five of the athletes recruited were re-grouped according to menstrual index; three of the athletes were excluded from the analysis as they did not report enough history of entire menstrual status. Those who reported a history of menstrual irregularity (MI-IRReg A) (calculated < 10 on MI) had significantly lower lumbar spine (L1-L4) BMD than MI-Reg athletes *(p = 0.007)* and EC groups *(p<0.001).* Lumbar spine (L1-L4) BMC in MI-IRReg athletes was significantly lower than MI-Reg athletes *(p=0.026).* MI-IRReg athletes had significantly higher CSA (p=*0.045*) and strength index (*p< 0.001*) than EC group. Similarly MI-Reg athletes had significantly higher femoral neck BMD, CSA (p <0.001), Z (*p = 0.002*) and strength index (*p < 0.001*) than EC (table 5.2), however there were no significant differences at the femoral neck in the athlete groups.

5.3.4 Adjustments for differences in physical characteristics

After adjustment for age, body mass and height, significant differences in BMD, BMC and bone geometric properties at the hip according to current menstrual function persisted (Table 5.3). However, at the lumbar spine differences were no longer significant between groups.

When athletes were categorized for MI after adjustments for physical characteristics significant differences in BMD, BMC and geometric properties (CSA, Z and SI) persisted between MI-Reg compared to eumenorrhoeic controls, however MI-IRReg athletes showed significantly higher BMD, BMC and CSA at the femoral neck than EC. MI-Reg

athletes had a significantly higher BMD at the lumbar spine compared to MI-IRReg athletes. There were no other significant differences after adjustment for physical characteristic between athlete groups according to MI.

5.3.5 Comparisons of total body and radius bone measurements according to current menstrual function in athletes

Comparisons of BMD and BMC at the total body and 33% distal radius between the athlete menstrual function groups showed no further significant differences between the athlete groups at the total body (BMD, AA 1.139 (0.013), EA 1.163 (0.010) g/cm^2, BMC, AA 2415 (56) , EA 2469 (48) g), and 33% distal radius (BMD AA 0.821 (0.013), EA 0.825 (0.009) g/cm^2, BMC 2.11 (0.07), EA 201 (0.05) g.

Table 5.3: BMD, BMC and geometric parameters (adjusted for age, height and body mass) between athletes and controls according to current menstrual function and menstrual index: Mean (SE).

	Current Menstrual Function			Menstrual History (MI)	
	EC (N= 88)	EA (N= 44)	AA (N=24)	MI-Reg A (N=39)	MI-IRReg A (N=26)
Bone mineral density (BMD)					
BMD Femoral Neck (g/cm^2)	0.985 (0.013)	1.138 (0.018) [a]	1.037 (0.025) [a b]	1.134 (0.019) [a]	1.077 (0.24) [a]
BMD Spine (L1-L4) (g/cm^2)	1.168 (0.014)	1.173 (0.020)	1.121 (0.027)	1.190 (0.021)	1.103 (0.025) [c]
Bone mineral content (BMC)					
BMC Femoral Neck (g)	4.8 (0.1)	5.3 (0.1) [a]	4.5 (0.1) [a b]	5.2 (0.1) [a]	5.0 (0.1) [a]
BMC Spine (l1-L4) (g)	58.7 (1.9)	63.4 (1.4)	60.5 (1.0)	64.0 (1.5) [a]	58.6 (1.8) [a]
Femoral neck geometric measures					
CSA (mm^2)	150 (4)	164 (3) [a]	142 (2) [b]	162 (3) [a]	157 (4)[a]
Section modulus (Z) (mm^3)	623 (20)	676 (14) [a]	588 (10)	670 (15) [a]	635 (19)
Strength Index	1.84 (0.08)	2.02 (0.06) [a]	1.64 (0.04)	1.9 (0.61) [a]	2.0 (0.08) [a]

Covariates appearing in the model are evaluated at the following values: Age = 29.8yrs, Height = 1.65m and body mass = 59.0kg.

* $p<0.05$ in ANCOVA Bonferroni adjustments, [a] sig diff from EC, [b] sig diff from EA. C sig diff from MI-Reg A

5.4 Discussion

This study is novel in that it compares bone geometry according to menstrual function in female endurance athletes and sedentary controls. A/oligomenorrhoeic athletes did have lower BMD compared to eumenorrhoeic athletes but this was still higher than the eumenorrhoeic controls and section modulus did not differ according to menstrual status in athletes. The lower BMD in a/oligomenorrhoeic compared to eumenorrhoeic athletes may be partly compensated by the structural adaptation such that estimated strength in bending did not differ significantly between the athlete groups despite the lower BMD in the amenorrhoeic athletes.

Menstrual dysfunction was prevalent in this sample of athletes with twenty four (35%) of the athletes reporting current menstrual dysfunction and 40% reporting a history of menstrual dysfunction when calculated as MI. These findings are consistent with previous reports which have indicated menstrual dysfunction is present in 1-44% (Bennell et al. 1997b) of athletes depending on the sport population surveyed and the definition of menstrual dysfunction (Bennell et al. 1997b; Ducher et al. 2009). However, contrary to previous findings (Warren 2002), there were no significant differences in height, body mass and age between the a/oligomenorrhoeic, and eumenorrhoeic athletes, although the sedentary control group were significantly older, heavier and shorter than the athlete groups.

Athletes had a significantly higher femoral neck BMD than the eumenorrhoeic sedentary controls in accordance with earlier studies (Bass 2003; Bennell et al. 1996a; Bennell et al. 1997b; Snow-Harter and Marcus 1992; Torstveit and Sundgot-Borgen 2005c). Torstveit et al (Torstveit and Sundgot-Borgen 2005c) found low BMD was three times more likely in non-athletes than in athletes, with athletes having 3-20% higher BMD compared to non-athlete controls. In the present study eumenorrhoeic athletes had approximately 14%

higher BMD than controls after adjustments for age, height and weight. A/oligomenorrhoeic athletes had values intermediate between eumenorrhoeic athletes and controls. Between the athlete groups an 8% higher BMD at the femoral neck was found in eumenorrhoeic athletes compared to currently a/oligomenorrhoeic athletes, which indicates that exercise may partly counteract oestrogen deficiency (Bass 2003; Ducher et al. 2009; Saxon and Turner 2006). Similarly gymnasts who engage in a higher impact activity have greater BMD than normally active individuals despite the later menarche and periods of amenorrhoea (Ducher et al. 2009; Robinson et al. 1995).

A novel addition to the current literature by the present study was the use of the advanced hip structural analysis (AHA) software to estimate the effects exercise may have on bone strength in athletes and sedentary controls according to menstrual function. It was found that eumenorrhoeic athletes had significantly greater resistance to bending at the hip, with higher BMD and section modulus at the femoral neck, after adjustment for weight, height and age compared to eumenorrhoeic controls. Despite the lower BMD in amenorrhoeic athletes the bone's resistance to bending (Z) was relativity maintained when compared to eumenorrhoeic athletes. A previous study in anorexic women who are potentially amenorrhoeic and physically inactive has shown that low BMD is accompanied with lower structural properties (Z, cortical thickness), hence the bones resistance to bending was compromised at the hip compared to healthy controls (DiVasta et al. 2007). This suggests that exercise may lead to structural adaptations to bone despite oestrogen deficiency, and therefore potentially lowering the risk of osteoporotic fractures in amenorrhoeic athletes with lower BMD (Eser et al. 2009) compared to eumenorrhoeic controls. The measure of bone structural properties in athletes with oestrogen deficiency may therefore be a better indicator of poor bone health than BMD alone.

Interestingly there were no significant differences in bone width between any of the groups. Previously, it has been shown that a wide femoral neck is associated with increased risk of hip fracture in the elderly (Frost 2003). Similarly, exercise is believed to enhance periosteal expansion, thus resulting in a wider bone (Bass 2003). By showing that femoral neck width remained the same between athletes and controls it may be possible to suggest, at least in young adults, that exercise may not result in enlarged periosteal dimensions of the femoral neck, but rather it results in thicker cortical walls, thus increased strength in bending, possibly resulting from increased endocorticol contraction. The similar bone width between groups found in the present study, does support earlier work in athletes and controls (DiVasta et al. 2007; Heinonen et al. 2001; Nikander et al. 2005). Nikander et al (Nikander et al. 2005), compared different types of sports loading (high impact, odd impact and low repetitive loading) with a control group and found that even though the femoral neck section modulus was significantly higher (16-26%) in all sports compared to the controls the width of the bone remained similar. Our observation of no differences in bone width only applies to the femoral neck, increases in outer diameter of bone have been reported in other long bones such as the tibia and radius (Haapasalo et al. 2000).

It has previously been suggested that exercise and oestrogen will have a beneficial effect on bone, however in athletes who are amenorrhoeic oestrogen deficiency counteracts the beneficial effects to BMD by inhibiting the endocorticol contraction resulting in lower BMD (Bagi et al. 1997; Seeman 2002). In the present study a/oligomenorrhoeic athletes seemed to maintain Z even through their BMD was significantly lower than eumenorrhoeic athletes. For section modulus to be maintained with lower BMD in amenorrhoeic athletes implies that the reduced amount of bone present is distributed further from the axis of the bone. As femoral neck width was no different, this seems unlikely to be explained by periosteal

expansion, so these findings suggest that the amenorrhoeic women have relatively more cortical and less trabecular bone (Bagi et al. 1997; Seeman 2002). Exercise in the presence of oestrogen deficiency may thus be maintaining cortical bone at the expense of trabecular bone at the femoral neck. Further studies are needed to clarify this suggestion as it is not possible to distinguish between cortical and trabecular bone using DXA.

In the present study it was found that there were no significant differences in lumbar spine BMD after adjustments of age, height and weight between groups, possibly suggesting that the lumbar spine BMD is influenced by body size. Our findings conflict with earlier research (Bennell et al. 1996a; Christo et al. 2008; Cobb et al. 2003; Hind 2008) which have reported amenorrhoeic athletes to have substantially lower BMD at the lumbar spine compared to eumenorrhoeic athletes, but higher BMD compared to sedentary controls. The lower BMD reported in previous studies in amenorrhoeic athletes is believed to be caused by the increased responsiveness to hormonal stimuli at the lumbar spine due to the higher ratio of trabecular (62-70%) to cortical bone (30-38%), (Bennell et al. 1997b). A possible explanation for the lack of differences in lumbar spine BMD between a/oligomenorrhoeic, eumenorrhoeic athletes and controls could be that many of the a/oligomenorrhoeic athletes in this study took part in resistance training, which may compensate for potential oestrogen deficiency related losses in BMD at the lumbar spine by increased loading. Torstveit et al (2005)(Torstveit and Sundgot-Borgen 2005c), showed that high impact loading exercise at the spine through resistance training could have a protective effect in athletes with menstrual dysfunction (Torstveit and Sundgot-Borgen 2005c).

When the athletes were re-categorized to express history of entire menstrual function it was found that athletes with a history of menstrual irregularity over the entire menstrual profile had significantly lower lumbar spine BMD than athletes with a history of regular

menstruation, there were no significant differences in femoral neck BMD and geometric parameters. Therefore it could be argued that long term menstrual irregularity has a greater effect on hormonal status, which results in a lower lumbar spine BMD over a life span. Whereas, it would seem the femoral neck BMD is affected by the current shorter bouts of menstrual irregularity.

The limitations of this study include that; bone geometric properties were only estimated at the hip using AHA, as DXA only gives a 2-dimensional image in one plane. Any slight change in positioning of the femoral neck could result in changes in the measure of the geometric properties of the hip, such as a higher estimate of cortical thickness. Throughout the course of the study, all DXA scans were performed by the same operator using standard protocols while paying extreme attention to positioning for this measurement therefore as much as possible was done to ensure consistency of measurements between participants. In order to achieve a more accurate measure of bone geometry a 3-dimensional image would be preferable such as given by CT or pQCT. However in a sample of reproductive aged women there may be ethical concerns about exposing them to increased radiation of this magnitude. Menstrual status was assessed retrospectively, which is not an accurate assessment of oestrogen depletion. The gold standard for measurements of oestrogen depletion requires measurement of serum or urinary metabolites of oestrogen throughout the menstrual cycle (Ducher et al. 2009) which was beyond the scope of this investigation. The control group was not matched for age, height, and body mass to the athlete groups which would have been the optimal study design, as matching for body mass may offset the effects of exercise. However physical characteristics were added as covariates and findings did persist.

The findings from this study may have implications for the clinical and applied sports medicine field illustrating that athletes who have low BMD due to oestrogen depletion may

potentially be protected against future osteoporotic fractures as the strength of the bone is relatively maintained due to the participation in loading sports. A/oligomenorrhoeic athletes also have better or no worse BMD than eumenorrhoeic controls despite the size differences.

It can therefore be concluded from this study that eumenorrhoeic athletes have substantially higher hip BMD and Z than sedentary controls. Femoral neck Z and hence strength in bending was relatively maintained in athletes with menstrual dysfunction despite their lower BMD at this site, indicating possible structural adaptation.

Chapter Six
A twelve month prospective analysis of stress fracture incidence in female endurance athletes

Chapter six presents the first of three prospective results chapters, determining the incidence rate of stress fracture in female endurance athletes over a 12-month analysis. The results from this chapter were presented as a poster at the United Kingdom Sports and Exercise Medicine (UKSEM) annual conference, London, Nov 2010. The accepted abstract is in press (Appendix 12: abstract)

A twelve-month prospective analysis of stress fracture incidence in female endurance athletes

6.1   Introduction

It is well established that stress fractures are the most common overuse injury in military recruits and athletes, causing disruption to training, and in severe cases early career termination (Beck et al. 2000; Bennell and Grimston 2001; Bennell and Brukner 1997; Bennell et al. 1995; Jones et al. 2002; Rauh et al. 2006; Shaffer 2001; Snyder et al. 2006). Retrospective data collection is the most common method used to determine the incidence of stress fracture. This method surveys a group of individuals and determines incidence from a history of stress fracture (Snyder et al. 2006). Even though this method can determine incidence, it often results in sampling bias, and recall error. A more accurate method, and often considered the gold standard for determining incidence of stress fracture, is to prospectively monitor a cohort of uninjured individuals over a set period of time, and document the new cases of stress fracture, allowing therefore, the comparison of injured and non-injured groups (Bennell and Brukner 1997; Shaffer 2001; Snyder et al. 2006).

Incidence rates of stress fracture reported in prospective studies have varied widely. Several large studies have been conducted in military recruits commencing training (Almeida et al. 1999; Beck et al. 1996; Shaffer et al. 1999). Incidence rates ranged from 0.2 to 4.0% in males and 1 to 7% in women monitored over a basic training period (Kelly et al. 2000; Shaffer et al. 1999). In athletes, only three studies have prospectively monitored incidence and risk of stress fracture, reporting incidence to range between 8.7 and 20.7%

in track and field athletes (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000), with female endurance athletes having the greatest incidence (30%) (Bennell et al. 1996a).

Previously identified risk factors of stress fracture in female athletes have included: menstrual irregularity, low BMD, disordered eating, energy deficiency, decreased strength, and decreased calf girth (Bennell et al. 1999; Kelsey et al. 2007; Nattiv 2000; Snyder et al. 2006). More recently it has been shown that compulsive exercise and amenorrhoea, are independent risk factors of stress fracture history (chapter 4). Although these factors have been comprehensively assessed in athletes the study designs have largely been cross sectional with measurements obtained following the stress fracture injury. Few studies prospectively monitored risk factors and findings are often contradictory, especially regarding the role of age at menarche (Bennell et al. 1996a; Kelsey et al. 2007), with some reporting a later age at menarche (Bennell et al. 1996a) to be associated with stress fracture and others reporting no association (Kelsey et al. 2007).

The awareness of the female athlete triad which includes low bone density, disordered eating and menstrual irregularity (all known risk factors for stress fracture)has increased in athletes and coaches over the last decade (Nattiv et al. 2007). This increased awareness could have lead to measures being implemented to prevent stress fracture development, thus lowering the incidence of stress fracture in female endurance athletes. Therefore, the purpose of this study was to prospectively determine the annual incidence of stress fracture in female endurance athletes. A secondary objective was to determine whether previous identified risk factors were present in stress fracture cases.

6.2   Methods

6.2.1   Study design

This was a 12 month prospective study recruiting participants at three time points each six-months apart between May 2008 and April 2009. Athletes were prospectively monitored for one year with regular email contact and face-to-face visits at 6-month intervals to determine the incidence rate of stress fracture injury. Questionnaires were used to determine history of stress fracture, menstrual function, and training. BMD, BMC, geometric properties and body composition were measured using dual energy x-ray absorptiometry (DXA), and anthropometric measures were taken.

6.2.2   Subjects

The cohort included 70 female endurance athletes (58 runners, 12 triathletes) aged between 18 to 45 years. At baseline, athletes were required to be healthy and un-injured and competing at international, national level or training at least 8 to 10 hours per week for endurance athletes and 15 to 20 hours per week for a triathlete. Athletes were excluded from the study if they were currently injured preventing normal training, or were pregnant or lactating.

6.2.3   Diagnosis of stress fracture

During the 12-month study, athletes were monitored for the signs and symptoms of a stress fracture injury. The main signs and symptoms of stress fracture are localized bone tenderness, swelling of surrounding soft tissue and increased pain during loading. Athletes were asked to report all stress fracture symptoms in a monthly training diary based on methods used in previous studies (Bennell et al. 1996b; Matheson et al. 1987). At each 6 month assessment athletes were asked if they had been diagnosed with a stress fracture.

In order for a stress fracture to be classified athletes were asked to give evidence of a positive diagnosis from a medical physician supported with a MRI, CT or X-Ray.

6.2.4 Stress fracture history

At baseline and during a 12 month follow up session history of stress fractures/reactions were assessed by questionnaire. Athletes were asked to record if they had ever had a stress fracture/reaction in their life time, the age the stress fracture occurred, the location, time of year and form of diagnosis. A history of stress fracture/reaction was included only if the injury had been diagnosed by a medical physician with a positive finding on a MRI, CT or X-Ray.

6.2.5 Menstrual function

Current and past menstrual history was assessed at baseline to determine if athletes were currently amenorrhoeic (0-3 periods per year), oliogomenorrhoeic (4-9 periods per year, or eumenorrhoeic (10-13 periods a year). History of menstrual dysfunction (amenorrhoea or oligomenorrhoea) and the duration of dysfunction, as well as age at menarche were recorded.

6.2.6 Training

Current training and training history was assessed using questionnaire at baseline and during the 12-month follow-up session. Athletes were asked to record the number of training phases per year, the event they were training for, frequency of sessions per week, weekly training duration (hours per week), subjective measure of training intensity per training phase (%) and the predominant training surface. Athletes were also asked to keep a month training diary for the 12-month period which they were asked to fill in daily.

6.2.7   BMD and geometry

DXA (Lunar Prodigy, GE Healthcare, Madison, WI, U.S.A version encore 12.2) was used to measure BMD and content of the total body, lumbar spine (L1-L4), femoral neck, tibia, and radius. Femoral neck, CSMI, CSA, and Z (Z) were estimated. All scans were carried out using standardized protocols previously mentioned.

6.2.8   Eating psychopathology and exercise cognitions

Athletes completed two self report measures: the Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn and Beglin 1994) and the Compulsive Exercise Test (CET)(Taranis et al. 2009). The EDE-Q is a self reported version of the eating disorder examination (EDE)(Fairburn and Beglin 1994). The EDE-Q is a 36 item questionnaire which provides four subscale scores, a global score and a specific section of diagnostic questions related to binge/purging. Each item on the questionnaire is scored from 0-6 (appendix 5). The CET is a 24-item self reported questionnaire designed to assess four domains of compulsive exercise (compulsivity, affect regulation, weight and shape driven exercise and behavioural rigidity) (appendix 8)(Taranis et al. 2009).

6.2.9   Dietary behaviours

Participants were asked to complete a self reported nutritional measure: the European Prospective Investigation into Cancer (EPIC) food frequency questionnaire (FFQ) (Welsh 2005). The EPIC FFQ (Bingham et al. 1994; Welsh 2005) is a self reported questionnaire which gives general information of the type, frequency, and servings of foods the participants have eaten over the past 12-months. The FFQ was coded and entered into a computerized software package (CompEat version 2.4) to determine the total energy intake, and macro and micro-nutritional intake.

6.2.10 Isometric knee extensor force (kg)

Maximal Isometric knee extensor force was determined in the participants using a custom built muscle rig, which gave an indirect assessment of maximal knee extensor force. The participants seated with 90 degree flexion at the hip and knees and strapped to prevent movement of the pelvis and contribution of the gluteal muscles. A restraint attached to a force gauge was placed around the participant's ankle. Participants performed three sub maximal warm up trials on each leg to prevent a familiarization effect prior to the completion of three maximal efforts. Instruction was given to all participants to produce a maximal effort muscle contraction which increased smoothly to maximal tension within 4-5 seconds.

6.2.11 Anthropometric measurements

Height, body mass, body composition and calf girth were assessed using standardized protocols previously mentioned. Height, body mass and calf girth were measured using a stadiometer (Holtain, Ltd, Pembrokeshire), a beam balance scale (Herbert and son Ltd, London) and a tape measure respectively. Body composition was assessed using DXA.

6.2.12 Power analysis

Sample size estimates were calculated prior to the initiation of this study. To yield a power of 80%, a sample size of 146 participants was required to detect a within study group difference in stress fracture incidence of 35% vs. 15% with a bone density split at the median. Due to the strict inclusion criteria of this study, recruiting only athletes who were of elite standard or training at high volumes, it was only possible to recruit a final sample of 70 female endurance athletes.

6.2.13 Statistical analysis

All statistical analyses were carried out using SPSS 16 (SPSS Chicago Illinois, USA). One-way analysis of variance (ANOVA) was used to compare means between the athletes who withdrew and those who completed the 12-month assessment to determine if a dropout bias was present. Categorical comparisons were evaluated using chi squared tests. A comparison of means between groups of athletes who stress fractured and the non stress fracture group are reported as the mean and standard error. Confidence intervals at 95% are reported in the stress fracture group.

6.3   Results

Nine of the 70 female endurance athletes who were followed for 12-months withdrew from the study, representing an overall attrition rate of 12.8%. Figure 6.1 illustrates the recruitment and overall withdrawal rate from the study. Comparison of means were carried out to determine if bias was present in those who completed the 12-month prospective study compared to the nine athletes who withdrew (Table 6.1). No significant differences were found in physical characteristics, training history, and BMD. A greater proportion of the 61 analyzed athletes had a history of stress fracture, a history of menstrual dysfunction and was currently a/oligomenorrhoeic compared to the athletes who withdrew from the study. Only current a/oligomenorrhoea however, was significantly different ($p=0.041$). .
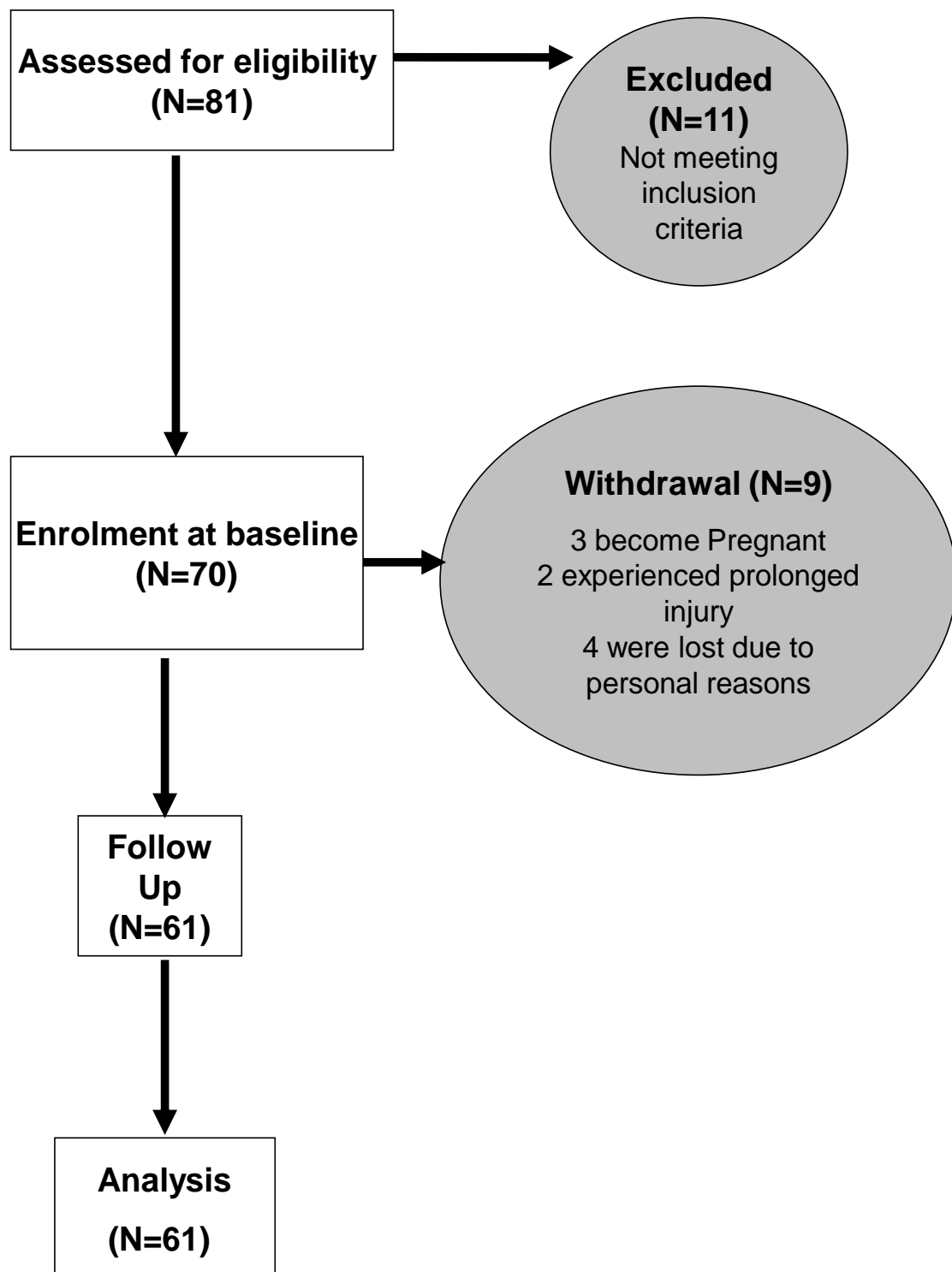
Figure 6.1: Illustrates the baseline recruitment and overall withdrawal rate of the study.

Table 6.1: Characteristics of analyzed athletes compared to the nine athletes who withdrew from the 12-month prospective study: mean (SE).

	Analyzed athlete group (N=61)	Athletes who withdrew (N=9)
Age (years)	25.3 (0.9)	30.2 (2.3)
Body Mass (kg)	54.8 (0.7)	58.6 (3.1)
Height (m)	1.67 (0.01)	1.69 (0.02)
BMI (Kg/m^2)	19.8 (0.19)	20.4 (0.76)
Body Fat (%)	17.0 (0.6)	18.5 (2.8)
Calf Girth (cm)	31.4 (0.3)	32.7 (0.6)
Training		
Years of competitive training	7.6 (0.7)	7.8 (2.7)
Weekly training duration (hrs)	12.6 (0.6)	13.2 (1.8)
History of Stress Fracture (%)	18	0
Menstrual Function		
Age at Menarche (yrs)	14.0 (0.2)	14.2 (0.4)
Current A/oligomenorrhoea (%)	47.5*	11.1
History of Amenorrhoea (%)	60.7	44.4
Current Hormonal contraception Use (%)	31.1	22.2
Bone mineral density (BMD) (**g/cm2**)		
Femoral neck	1.080 (0.014)	1.116 (0.039)
Lumbar spine (L1-L4)	1.117 (0.169)	1.183 (0.039)
Total body	1.154 (0.008)	1.147 (0.268)
Distal Radius (33%)	0.824 (0.007)	0.810 (0.025)

* Significant difference between the analysis and withdrawal group: $p < 0.05$

6.3.1   Stress fracture incidence

Two (3.3%) out of the 61 athletes sustained a stress fracture over the 12-month period. There were no other reports of symptoms consistent with stress fracture from athletes, which could have been excluded by a medical diagnosis. Table 6.2 provides baseline descriptive characteristics of the individual stress fracture cases compared to the 59 controls who did not sustain a stress fracture.

The two cases of stress fracture were diagnosed by a sports medical physician and confirmed with a positive MRI scan during the spring of 2009. The female athletes who presented with the stress fractures had been competing on average for a period of 7.6 years and were middle distance runners. At the time of the stress fracture the athletes were predominantly competing at a national level for their age in the 800m and were training on average 14.5 hours per week.

Stress fracture cases: Case one and case two stress fracture athletes were of similar age: 18 and 21 years respectively. Both athletes reported to be eumenorrhoeic with no previous history of amenorrhoea, and were not currently taking hormonal contraception. Healthy values for BMD were measured in both cases. Case one had no indications of differences in total energy intake or percentage of carbohydrates (CHO), proteins and fats from total calories compared to the mean of the non-stress fracture group (Case 1: mean total energy: 3350 kcal, CHO: 51%, proteins: 18%, and fats: 31%, Non-stress fracture: total energy: mean (SE) 2668(121) kcal, CHO: 54%, proteins: 19%, and fats: 27%). Eating psychopathology (Case 1: global score: 0.36, non-stress fracture 1.37(1.4)), and compulsive exercise (Case 1: CET global score 1.90, non-stress fracture 2.6(0.7), scores were both lower in case one when compared with the non-stress fracture group.

Table 6.2: Baseline descriptive characteristics of stress fracture cases (mean) and non-stress fracture group (mean (SE) and 95% CI)

	Stress Fractures		Non-Stress Fracture Group (n=59)		
	Case One	Case Two	Mean(SE)	95% Confidence Interval for mean	
				Lower	Upper
Physical Characterlistics					
Age (years)	18.	21	25.6 (0.6)	23.6	27.5
Body mass (kg)	46.7	62.5	54.8 (0.7)	53.5	56.1
Height (m)	1.60	1.70	1.67 (0.01)	1.65	1.68
BMI (kg/m^2)	18.3	21.6	19.8 (0.2)	19.4	20.1
Body fat (%)	15.7	16.2	17.0 (0.7)	15.7	18.4
Calf girth (cm)	31.1	33.1	31.4 0.3)	30.7	31.9
Training History					
Years of competitive training	7.3	10.0	7.6 (0.7)	6.1	9.1
Weekly training duration (hrs)	9.5	19.5	12.5 (0.6)	11.4	13.8
Menstrual Function					
Age at menarche	13.0	16.0	14.0 (0.2)	13.5	14.5
Bone Mineral Density (g/cm^2)					
Total body	1.118	1.178	1.154 (0.008)	1.137	1.171
Lumbar spine (L1-L4)	1.139	1.196	1.115 (0.017)	1.080	1.149
Femoral neck	1.209	1.164	1.077 (0.014)	1.049	1.106
Distal radius 33%	0.774	0.863	0.824 (0.001)	0.809	0.840
Bone Geometry					
CSA (mm^2)	154	177	155 (2)	150	160
CSMI (mm^4)	7238	12341	9594 (294)	9004	10185
Min neck width (mm)	25.8	30.6	28.5 (0.2)	28	29.1
Section modulus (mm^3)	529	801	641 (15)	610	671
Isometric knee extensor force (kg)					
Peak right	38.6	42.7	38.8 (0.9)	37.0	40.5
Peak left	35.5	28.3	36.9 (0.9)	35.0	38.8

However in case two the total calorie intake, and percentage of CHO was lower and percentage of protein and fat intake were higher compared to the mean of the non-stress fracture group (Case 2: mean total energy: 2008 kcal, CHO: 40%, proteins: 21%, and fats: 39%). Similarly, eating psychopathology (global score: 3.26) and compulsive exercise (global score: 2.98) scores were higher in case two than the mean for the non-stress fracture group.

Case one was diagnosed with a stress fracture in the dominant side of the sacrum this was her first reported stress fracture. Case two reported to have had one previous stress fracture. The current stress fracture, in case two, was located at the 2nd metatarsal of the right foot (dominant side) which was the exact location of the first stress fracture, approximately three years previously. Both case athletes had a stronger dominant leg observed via a maximal leg extensor test than the non-stress fracture group.

6.4   Discussion

The annual incidence of stress fracture shown here was 3.3% in female endurance athletes when calculated by the number of female athletes diagnosed with a stress fracture over a 12-month period. This is a substantially lower incidence rate compared to previous prospective studies (Bennell et al. 1996b; Kelsey et al. 2007; Nattiv et al. 2000), which could be indicative of increased awareness by coaches and female athletic groups over the past decade of the risk factors of stress fracture and the female athlete triad (low BMD, low energy availability and menstrual dysfunction). To our knowledge this is one of the largest prospective studies to monitor stress fractures in female endurance athletes, aged between 18 and 45 years, located in the United Kingdom.

The incidence of stress fracture (3.3%) in this study is comparable to previous studies in female military recruits (1-7%) (Kelly et al. 2000; Shaffer 2001; Shaffer et al. 1999) and

athletic studies (0.5 -7.8%) which, have determined incidence rates from medical records (Arendt et al. 2003; Goldberg and Pecora 1994; Matheson et al. 1987). Our findings support Goldberg et al (Goldberg and Pecora 1994), who reported an incidence rate of 3.7% over three years (1.9% yearly incidence) when retrospectively reviewing stress fracture incidence from medical records in track athletes.

The incidence of stress fracture reported here in athletes (3.3%) is substantially lower than that of previous studies which have reported incidence rates to range between 8.7 – 20.7% in male and female track and field athletes (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000), and 7.6% in female cross country runners over 12 months (Bennell et al. 1996a; Kelsey et al. 2007).

The potential difference between the present study and the previous prospective studies in athletes, which have reported higher incidence of stress fracture, is the age range of the athletes. The female athletes in this study were aged between 18-45 years. Thus substantially lower incidence in the present study could be due to a higher average age of the athletes (25.3 years). Previous prospective studies have been largely based on 18-26 year old athletes who are likely at the peak of their training intensity and volume. For this reason, this age group may be more vulnerable to stress fracture as an over use injury than the wider age range recruited in this study. Endurance athletes are known to continue training beyond the age of 26 years, making our sample and therefore our estimate of stress fracture incidence more representative of the endurance athlete population as a whole.

Interestingly, the two cases of stress fracture in the present study did not present with previously identified risk factors of stress fracture, menstrual irregularities, or low BMD (Bennell et al. 1996b; Kelsey et al. 2007). Neither reported a history of menstrual irregularities and both were eumenorrhoeic during the year prior to the stress fracture

diagnosis. Low BMD was not present in either case with an average Z score of 0.2 at lumbar spine and Z=1.5 femoral neck. One of the two cases reported a past history of stress fracture, but with such a small incidence rate it cannot be concluded if a past history of stress fracture is a relevant risk factor in this population of female endurance athletes.

Female athletes tend to be delayed in biological maturation compared to the general population of girls (Malina and Bouchard 1991). The present study supports this literature with an average age of menarche in the female athletes reported as 14.0 years compared to the general European population of 12.3 years (Morris et al. 2011). Delayed age at menarche (+ 2 years of the average population) a potential risk factor for stress fracture was evident in case two with an age at menarche of 16.0 years, but not in case one (13.0 years).

Bennell et al (Bennell et al. 1996b) indicated that age at menarche and decreased calf girth increases the probability of stress fracture occurring in female athletes. They state that for every additional year of menarche, and for every 1cm decrease in calf girth the risk of stress fracture increased fourfold. When applying this algorithm to the findings in this study we found case one and case two would have been at no greater risk of developing a stress fracture than the average of the non-stress fracture group. Interestingly case one, who had an age at menarche of 13 years and a calf girth of 31.1cm, could have been considered to be at a decreased risk of stress fracture than the non-stress fracture group. Therefore in this cohort of athletes this approach did not seem to discriminate stress fracture cases. In fact it would seem to overestimate the risk of stress fracture in this sample of endurance athletes. This may reflect a change in stress fracture risk factors over the last decade or differing sample characteristics between studies. However, in the sample as a whole, age at menarche and BMD were similar to those reported previously whilst there was, if anything a greater prevalence of menstrual dysfunction. The present

study reported a similar age at menarche (14.2 years), a higher percentage of current menstrual dysfunction (47.5%) and a similar femoral neck BMD (1.080 g/cm^2), compared to the findings of the Kelsey et al (Kelsey et al. 2007) (menarche 13.1 years, 33% current menstrual dysfunction, femoral neck BMD 0.986 g/cm^2) and Bennell et al (Bennell et al. 1996b) (menarche 15.1 years, 30% current menstrual dysfunction, femoral neck BMD 1.181 g/cm^2).

It seems evident that the causes of stress fracture in the present study were not related to the potential risk factors of stress fracture such as menstrual dysfunction but could have been a result of changes in training. Cases of stress fracture in the current study were both diagnosed at the start of the track season. This could have corresponded with a sudden change from endurance winter training to the increased intensity of summer track sessions in these two athletes. Previous studies (Goldberg and Pecora 1994; Shaffer et al. 1999) have reported stress fracture diagnosis to increase following a sudden change in training regimen, such as after a competitive track season (Goldberg and Pecora 1994). Findings in military recruits with low prior physical activity and poor physical fitness were reported to develop three times as many stress fractures compared to those who were physically fit (Shaffer 1999).

The risk for stress fracture in case one may have been increased due to age. At age 18 the Sacrum may not have been fully fused therefore increasing the risk for micro damage to occur resulting in a stress fracture. The fusion of the sacral vertebra will often begin at puberty, with complete fusion reported to occur between 25 and 33 years of age and is often related to the load bearing aspects of the region (Esses and DJ 1997). Differences in stress fracture location in the present study and previous work (chapter 4) (Bennell et al. 1996a; Bennell et al. 1996b; Kelsey et al. 2007; Matheson et al. 1987; Nattiv 2000), may explain the conflicting findings in the literature of risk factors for stress fracture. It may

therefore be pertinent to determine incidence and risk factor of stress fracture at different anatomical fracture sites to fully understand stress fracture aetiology.

Limitations of the current study include the modest sample size, which was inadequate to evaluate risk factors of stress fracture. However, this was one of the largest studies prospectively monitoring the incidence of stress fracture in female endurance athletes. Bennell et al (Bennell et al. 1996a), reported an annual incidence of 21.7% in a sample of 46 female athletes. Thus, in comparison it is clear that the current study has a larger cohort of participants, a lower incidence rate and overall a higher power to detect any possible statistically significant differences. However as the current study had such a low incidence rate (3.3%), and did not meet the sample size estimated to detect differences in incidence, it was not possible to construct meaningful data driven logistic regression models to identify the statistically significant predictors of stress fracture as previously planned. Instead, the two cases were considered descriptively. Stress fracture diagnosis was passively monitored, which required athletes to report symptoms to either the researchers or medical doctors this may have lowered the incidence of stress fracture as some athletes may not have reported potential symptoms.

Menstrual function was assessed though questionnaire which may have lead to recall bias. To accurately measure menstrual function an assessment of oestrogen depletion could have been used, however this would have required measuring serum or urinary metabolites throughout the menstrual cycle which may have deterred many athletes from participating. The nature of recruitment may have lead to a biased sample of endurance athletes who had a high percentage of stress fracture history and menstrual irregularities. However, the high percentage of stress fracture history and menstrual irregularity did not seem to increase the likelihood of stress fracture incidence in this sample of athletes.

The findings from this study may have implications to the clinical and applied sports medicine field suggesting an even lower boundary to the potential range of incidence figures presented in the United Kingdom. This could be a result of increased awareness of the female athlete triad (low BMD, menstrual dysfunction, and low energy availability), and better preventive management strategies for athletes who present with possible risk fractures of stress fracture in the United Kingdom.

The present study is one of the largest prospective studies to date, and the first to identify incidence of stress fracture in endurance female athletes in the United Kingdom. It can therefore be concluded from this study that the annual incidence of stress fracture in female endurance athletes based in the United Kingdom is 3.3%. Furthermore, the incidence of stress fracture does not seem particularly high in amenorrhoeic athletes possibly due to the increased awareness and management of stress fracture risk factors over the past decade.

Chapter Seven
Seasonal bone changes in female endurance athletes

Chapter Seven presents prospective analysis, quantifying the seasonal bone changes in female endurance athletes during a 12-month training phase. The results from this chapter have been accepted to be presented at the fore coming American College of Sports Medicine (ACSM) conference, Denver, USA, in May-June 2011, and awarded an SSHB travel grant. (Appendix 13: abstract).

Seasonal bone changes in female endurance athletes

7.1 Introduction

An athletes' competitive season will often determine the circadian variations in the training programs (Peiser et al. 2006). In endurance athletes there is often a summer and winter competitive season which will result in variations in training. Summer competitive training often consists of shorter interval sessions of higher cardiovascular intensity, whereas, the winter training/competitive phase may consist of higher volumes of training at moderate cardiovascular intensity. It is well established that sports participation will induce changes in BMD (Gass and Dawson-Hughes 2006), but it is unclear whether seasonal variations in training (volume and intensity) may alter the mechanical bone loading effects in endurance athletes.

A handful of prospective studies in athletes have investigated the effects of training and detraining on BMD and body composition over a competitive season (Barry and Kohrt 2008; Bonis et al. 2009; Carbuhn et al. 2010; Constantino et al. 1996; Klesges et al. 1996; McClanahan et al. 2002; Snow et al. 2001; Vuori 2000), indicating that the benefit of athletic training on bone may not persist if training is markedly reduced. Snow et al (2001) have reported distinct patterns with seasonal gains and off-seasonal losses in BMD of gymnasts, who have an eight month competitive training season and a four month period of detraining from high impact mechanical loading.

Similarly, changes in volume and intensity of cardiovascular training may have a detrimental effect on bone mineral density, with evidence in endurance athletes suggesting an inverse relationship between BMD and volume of training. High volumes of low impact running have been shown to result in lower BMD of the lumbar spine (Hetland et al. 1993; Macdougall et al. 1992). Equally, in the response to exercise which is very intense there

has been an observation of decreased BMD, trabecular thinning and structural adaptations (Forwood and Burr 1993). Quantifying the intensity of bone loading during athletic training is not an easy process, however, it has been indicated that the magnitude of bone loading intensity will increase parallel to the increase in exercise intensity (Kohrt et al. 2004), with short bursts of dynamic loading activities such as jumping resulting in increased BMD more than activities which are determined as low impact repetitive sports such as endurance running (Nikander et al. 2009; Nikander et al. 2005).

The prevalence of amenorrhoea in female athletes can range from 1 to 44% depending on the sporting event (Bennell et al. 1997b) and in athletes that have prolonged amenorrhoea the beneficial effects of exercise are often counteracted by oestrogen deficiency (Bass 2003; Saxon and Turner 2006). In men high volumes of running may contribute to low testosterone and high cortisol levels leading to bone loss (Drinkwater et al. 1984; Marcus et al. 1985), thus high intensity and high volumes of training in women who are amenorrhoeic may lead to a hormonal mechanism which reduces BMD over a competitive season when training volume is increased. Whilst it is known that gymnasts have bone gains over a competitive training season despite menstrual irregularities (Taaffe et al. 1997) it is unknown if there are seasonal variations in female endurance runners who have similar prevalence of menstrual irregularities to gymnasts. In amenorrhoeic athletes, if there are any seasonal losses it is possible that these may not be recovered thus contributing to the lower BMD in amenorrheoic athletes compared to eumenorrhoeic counterparts.

Previously, studies of seasonal variations in athletes have rarely considered other factors which could possibly contribute to seasonal changes in bone such as age related accrual and sunshine/vitamin D status. Age related accrual of BMD will often peak in the third decade (Heaney et al. 2000), where it levels out until the fifth decade, diminishing into old age. Any positive seasonal increases therefore in athletes before the third decade may be

due to annual age related accrual in bone and not seasonal changes in training parameters.

Higher vitamin D (25-OH-D concentration) status has been associated with increased BMD (Bischoff-Ferrari et al. 2006), with optimal levels of 25-OH-D (75.0 nmol/L) often met entirely through endogenous synthesis, when the skin is exposed to ultraviolet-B radiation (sun exposure) (Holick 2008; Larson-Meyer and Willis 2010). Seasonal fluctuations in 25-OH-D will mean people may be at or above the optimal level during the summer season resulting in increased BMD, but not in the winter (Dawson-Hughes et al. 1997; Hypponen and Power 2007; Patel et al. 2001; Vieth et al. 2004). Any seasonal changes in BMD therefore in athletes during the summer season (April –October) may be a result of increased concentration of 25-OH-D, and possibly not due to mechanical adaptation to training.

Whilst it seems evident that there are bone gain and losses in sports which have a definite competitive season and offseason it remains unclear what seasonal bone variations occur in endurance athletes who have a continuous summer competitive season (April – September), followed by a winter competitive/training season (October – March) resulting in changes in training intensity and volume, thus possible changes in bone loading forces (Bennell et al. 1997a; McClanahan et al. 2002). Furthermore, it is unknown whether seasonal variation differs between amenorrhoeic and eumenorrhoeic athletes and whether other factors such as vitamin D status could be contributing to any seasonal bone changes in endurance athletes. Therefore the aim of this study was to quantify seasonal variations of bone parameters in endurance athletes, comparing eumenorrhoeic (EA) and amenorrhoeic (AA) endurance runners. A secondary objective was to determine seasonal changes in Vitamin D in female endurance runners.

7.2 Methods

7.2.1 Study design

A prospective monitoring design was employed, to observe the seasonal bone changes in female endurance runners and triathletes during 12-month training phase consisting of a summer (April-October) and winter (October to March) competitive season. Athletes were recruited between April 2008 and April 2009 and prospectively monitored with regular email contact and face-to-face visits at three times points over 12-months at the beginning of the summer and winter competitive/training seasons, which are often linked to changes in training parameters (figure 7.1). BMD, BMC, bone geometric parameters and body composition were measured using dual energy x-ray absorptiometry (DXA). Menstrual function was assessed using questionnaires and training was prospectively monitored via questionnaire and training diaries. Anthropometric measures were taken and maximal knee extensor force measured to examine changes in muscle strength associated with seasonal changes in training.

Figure 7.1: A flow diagram to illustrate the recruitment and prospective monitoring of female endurance athletes over the 12-month period

Intake	Spring 2008	Autumn 2008	Spring 2009	Autumn 2009	Spring 2010
(#of athletes recruited at each)					
1 (13)	B >>>>>>>>	6-F >>>>>>>	12-F		
2 (39)		B >>>>>>>>>	6-F >>>>>>>	12-F	
3 (18)			B >>>>>>>>	6-F >>>>>>>	12- F

Visits

Baseline (B): DXA, anthropometry, questionnaires , muscle strength

6 month Follow-up (6-F): DXA, anthropometry, menstrual status, muscle strength

12 month Follow-up (12-F): DXA, anthropometry, questionnaires, muscle strength

>>>>>>> = Prospective monitoring of training

7.2.2 Subjects

Participants were recruited at the beginning of the 2008 and 2009 summer season and, 2008 winter season. At baseline the cohort included 70 female endurance athletes (58 runners and 12 triathletes) aged 18 to 45 years. Recruitment criteria required athletes to be uninjured; competing at an international, national level or training at least 8 to 10 hours per week for an endurance runner and 15 to 20 hours per week for a triathlete. Athletes were excluded from the study if they were pregnant or lactating or had been in the last 12 months.

7.2.3 Bone parameters

DXA was used to measure BMD and content of the lumbar spine (L1-L4), and hip (femoral neck and trochanter). Femoral neck CSMI, CSA, minimal neck width, and Z were estimated. All scans were carried out using standardized protocols as previously described in chapter 3.

7.2.4 Menstrual function

Current menstrual function was assessed via questionnaire at baseline, 6 month and 12-months to determine if athletes were currently a/oligomenorrhoeic phenotype (0-9 periods per year, this may be two hormonally distinct conditions) or eumenorrhoeic (10-13 periods a year), and to determine any seasonal variation in the athletes' menstrual function. The questionnaire at baseline also assessed the athletes' menstrual history (years of dysfunction), and age at menarche.

7.2.5 Training

Training was prospectively monitored via monthly training diaries. Athletes were asked to record the type, duration, and intensity of training each day for the 12-month period. A questionnaire on training in the previous 12 months was also assessed in all athletes at

baseline and 12–months. Athletes were asked to report the events they were training for, frequency, duration per week, intensity and surface of training for the summer and winter seasons.

7.2.6   Isometric knee extensor force (kg)

Maximal isometric knee extensor force was determined in the participants using a custom built muscle rig, which gave an indirect assessment of maximal knee extensor force. The participants were strapped into the muscle rig with 90 degree flexion at the hip and knees to prevent activation of the gluteal muscles. A restraint attached to a force gauge was placed around the participant's ankle. Participants performed three sub maximal warm up trials on each leg to prevent a familiarization effect prior to the completion of three maximal efforts. Instruction was given to all participants to produce a maximal effort muscle contraction which increased smoothly to maximal tension within 4-5 seconds.

7.2.7   Anthropometric measures

Height, body mass, body composition and calf girth were assessed using standardized protocols previously described. Height, body mass and calf girth were measured using a stadiometer (Holtain, Pembrokeshire), a beam balance scale (Herbert and sons Ltd, London) and a tape measure respectively. Body composition was assessed using DXA.

7.2.8   25-Hydroxy vitamin D EIA

Blood samples for the determination of the serum 25(OH)D concentration were drawn and analyzed in a sample of endurance runners in the spring and autumn. After coagulation at room temperature for one hour the samples were centrifuged at 3000 rpm for 2 minutes at 18 degrees Celsius to allow serum separation. The serum was then frozen and stored at -20°C for later analysis. Total serum 25 (OH) D concentration was measured with an enzyme immunoassay EIA kit (immunodiagnostiosystems Ltd (IDS Ltd) UK). The serum

25 (OH) D concentrations of the endurance runners collected during the spring were compared with spring levels of a control group who were aged between 18 and 30 years, who took part in less than 30 minutes of physical activity a week.

7.2.9 Statistical analysis

All statistical analysis was performed using SPSS for windows (version 18), statistical software package. Comparisons of means at baseline were carried out to determine whether athletes who completed the 12-month study differed from those who withdrew. Repeated measures analysis of variance (ANOVA) with age as a covariate, was used to determine any significant age-related annual changes from baseline to the 12-month follow-up period. Repeated measures analyses of variance (ANOVA) were used to determine if seasonal variations were present in the group of endurance athletes who adhered to the 12-month follow-up. The comparisons of means for the summer (spring to autumn) and winter (autumn to spring) were carried out independently to determine the changes in physical characteristics, bone parameters, knee extensor force, and training over the two seasons. The endurance athletes were classified into two groups (endurance runners and Tri-athletes) and further repeated measures analysis of variance (ANOVA) was carried out to determine the seasonal changes of physical characteristics, bone and muscle strength in the two different sporting events.

Repeated measures analysis of variance (ANOVA) with a between subject factor of menstrual function (a/oligomenorrhoea and eumenorrhoea) was used to detect any season times group interactions of endurance runners according to menstrual function. Multiple paired t-tests with a bonferroni correction were used where there was a significant mean or interaction effect to determine the significant seasonal changes within the menstrual function groups. Effect size was calculated to determine the magnitude of

observed effect between variables and reported as r. Level of statistical significance was considered at *P< 0.05.*

7.3 Results

Of the 70 female endurance athletes who were recruited at baseline, nine withdrew from the study for the following reasons: three became pregnant, two developed a long term injury, and four were lost to follow-up due to personal reasons (refer to figure 6.1). Comparisons of means were carried out and reported in chapter 6.3 (table 6.1) to determine if sample bias was present in those who completed the 12-month prospective study compared to the nine athletes who withdrew. As reported in chapter 6, there were no significant differences in physical characteristics, BMD and training history. Athletes who adhered to the study had a significantly higher prevalence of current amenorrhoea presented than those who withdrew, possibly indicating a bias towards menstrual dysfunction in this sample of athletes.

7.3.1 Baseline characteristics

The baseline descriptive characteristics of the 61 endurance athletes who adhered to the 12-month monitoring period are shown in table 6.1. A high prevalence of menstrual dysfunction was reported with 60.7% of athletes experiencing a history of amenorrhoea and 47.5% reporting current a/oligomenorrhoea, the average age at menarche was 14.0 (0.2) years. Of the 61 endurance athletes 20 reported to currently take hormonal contraception (13 a/oligomenorrhoeic, 7 eumenorrhoeic). The endurance athletes reported training 12.6 (0.6) hours per week for the previous year and had been competing competitively for 7.6 (0.7) years.

7.3.2 Annual changes

Repeated measures ANOVA with age as a covariate revealed no significant annual changes in physical characteristics (height, body mass, body fat % and BMI), bone parameters BMD and BMC (total body, spine, femoral neck, trochanter and distal radius) and bone geometric parameters (Z and minimal neck width) in the group of 59 endurance athletes (table 7.1). The BMD and bone geometric measurements at 12-months were within +/-1% of baseline, where as the BMC measurements at 12 months were within 5% of baseline.

7.3.3 Seasonal changes

Results of the repeated measures ANOVA on the 61 endurance athletes showed no seasonal variation in physical characteristics (height, weight, body fat %) over the summer or winter season (Table 7.2).

Seasonal changes in endurance athletes were shown in bone parameters with trochanter BMD increasing significantly over the summer (5.4%, f=15.338, *p<0.001*) with no significant change over the winter (1.0%, f=4.211, *p=0.207*). In contrast trochanter BMC increased significantly over the winter (3.0% f=8.778, *p=0.004*) with no significant changes over the summer (1.0%, f=0.584, *p=0.435*). Femoral neck BMD decreased significantly over the winter (1.0% f=4.211, *p=0.045*) with no significant change over the summer (0%, f=0.19, *p=0.890*). Minimal femoral neck width increased in the group as a whole over the winter (1.1%, f=7.078 *p=0.01*) with no significant change over the summer (0%, f=0.100, *p=0.753*). Calf girth significantly increased over the winter (2.8%, f=37.633, p<0.001) with no significant changes over the summer (1.0%, f=0.417, p=0.521). Interestingly as calf girth increased, left peak isometric knee extensor force increased significantly over the winter (4.0% f=9.855, p=0.003), with no changes over the summer (1.0%, f=0.167, p=0.684).

Table 7.1: Annual change from baseline to 12-month of physical characteristics, and bone parameters (N=61), mean (SE).

	All Endurance Athletes (n=61)		
	Baseline	12-month	*p-values*
Physical Characteristics			
Body Mass (kg)	54.8 (0.6)	55.1 (0.6)	*0.592*
Height (m)	1.67 (0.01)	1.67 (0.01)	*0.237*
BMI (kg/m^2)	19.7 (0.2)	19.8 (0.2)	*0.639*
Body Fat (%)	16.7 (0.6)	16.8 (0.7)	*0.872*
Bone mineral density (BMD) (g/cm^2)			
Femoral neck	1.080 (0.014)	1.072 (0.014)	*0.523*
Trochanter	0.863 (0.014)	0.867 (0.113)	*0.432*
Lumbar spine (L1-L4)	1.118 (0.019)	1.118 (0.019)	*0.235*
Total body	1.154 (0.009)	1.157 (0.008)	*0.861*
Distal radius (33%)	0.823 (0.008)	0.827 (0.008)	*0.136*
Bone Mineral Content (BMC) (g)			
Femoral neck	5.0 (0.1)	5.0 (0.1)	*0.797*
Trochanter	10.0 (0.3)	10.3 (0.3)	*0.520*
Lumbar spine (L1-L4)	59.5 (1.3)	58.9 (1.4)	*0.432*
Total body	2424 (36)	2389 (55)	*0.357*
Distal radius (33%)	2.0 (0.0)	2.1 (0.0)	*0.458*
Bone Geometry			
CSA (mm^2)	155 (2)	155 (2)	*0.258*
min Neck Width (mm)	28.5 (0.3)	28.7 (0.3)	*0.916*
Section Modulus (Z) (mm^3)	637 (15)	639 (13)	*0.488*

Covariate appearing in the model is evaluated at the following values: Age=25.4years
No significant changes in repeated measures ANOVA with P >0.05

The results of an ANCOVA comparing the effect of order in which the athletes entered the study (spring starters or winter starters, figure 7.1) revealed that the significant increase in left peak isometric knee extensor force was not due to an effect of order; however the increase in the right peak isometric knee extensor force was due to an order effect, which may have represented greater familiarization and thus an artificial increase in strength. There was no significant change in the right peak isometric knee extensor force; however there was a trend to suggest that an increase in mean force over the winter season was approaching significance, with no change in mean force over the summer. There were no significant seasonal variations in other bone parameters or physical characteristics (table 7.2).

7.3.4 Seasonal change according to endurance sport

Of the 61 endurance athletes fifty-two were endurance runners and 9 were tri-athletes. When analyzing the two sports separately the results of the repeated measures ANOVA showed there were no significant seasonal variations in weight, height, body fat % in either the tri-athletes or the endurance runners

7.3.5 Seasonal changes in triathletes

In the small group of tri-athletes (n=9), Spine BMC, CSA, and calf girth significantly increased over the winter with no significant summer changes. Femoral neck significantly decreased over the summer with no significant changes in the winter. There were no other observed seasonal changes in physical characteristics, bone parameters or muscle strength (table 7.3).

Table 7.2: Seasonal variations in training typically defined as summer (April to October) and winter (October to April) in female endurance athletes (n=61) (mean (SE), effect size and mean significant change)

	Summer (n=60)				Winter (n=61)			
	Spring	Autumn	r	p-value	Autumn	Spring	r	p-value
Physical Characteristics								
Height (m)	1.67 (0.01)	1.67 (0.01)	0.2	0.099	1.67 (0.01)	1.67 (0.01)	0.2	0.243
Body Mass (kg)	55.7 (0.7)	55.5 (0.7)	0.1	0.409	54.9 (0.6)	55.3 (0.6)	0.2	0.167
Body Fat (%)	16.9 (0.7)	16.9 (0.7)	0.0	0.914	16.9 (0.7)	16.9 (0.7)	0.0	0.936
Calf Girth (cm)	32.2 (0.3)	32.3 (0.3)	0.1	0.521	31.6 (0.2)	32.5 (0.2)	**0.6**	**0.000**
Bone mineral density (BMD) (g/cm²)								
Femoral neck	1.076 (0.014	1.075 (0.014)	0.0	0.890	1.083 (0.014)	1.075 (0.013)	**0.3**	**0.045**
Trochanter	0.863 (0.133)	0.910 (0.015)	**0.5**	**0.000**	0.860 (0.014)	0.869 (0.013)	0.1	0.271
Lumbar spine (I1-L4)	1.120 (0.017)	1.117 (0.017)	0.1	0.209	1.116 (0.017)	1.120 (0.017)	0.0	0.178
Bone Mineral Content (BMC)(g)								
Femoral neck	5.0 (0.1)	5.0 (0.1)	0.1	0.706	5.0 (0.1)	5.0 (0.1)	0.1	0.298
Trochanter	10.3 (0.2)	10.4 (0.2)	0.1	0.448	9.9 (0.3)	10.2 (0.2)	**0.4**	**0.004**
Lumbar spine (I1-L4)	60.2 (1.4)	59.8 (1.3)	0.2	0.058	59.3 (1.3)	59.1 (1.4)	0.0	0.735
Bone Geometry								
CSA (mm²)	157 (2)	156 (2)	0.1	0.461	155 (2)	155 (2)	0.0	0.840
Min neck width (mm)	28.8 (0.3)	28.8 (0.3)	0.0	0.753	28.4 (0.3)	28.7 (0.3)	**0.3**	**0.010**
Section modulus (Z) (mm³)	650 (15)	649 (14)	0.0	0.916	638 (14)	639 (13)	0.0	0.824
Isometric Knee Extensor Force (kg)								
Peak left	37.7 (1.0)	37.4 (1.1)	0.1	0.684	37.6 (0.9)	39.1 (1.0)	**0.4**	**0.003**
Peak right	40.0 (0.9)	39.4 (1.0)	0.1	0.435	39.3 (0.9)	40.8 (1.0)	**0.3**	**0.029**

Significant changes in repeated measures ANOVA with p<0.05

Table 7.3: Seasonal variations in triathletes training typically defined as summer (April to October) and winter (October to April), mean (SE), effect size and significant mean differences.

| | Tri-Athletes | | | | | | | |
| | Summer (n=8) | | | | Winter (n=9) | | | |
	Spring	Autumn	r	P-value	Autumn	Spring	r	P-value
Physical Characteristics								
Height (m)	1.70 (0.02)	170 (0.02)	0.0	0.921	1.68 (0.02)	1.68 (0.02)	0.2	0.700
Body mass (kg)	59.7 (2.4)	58.9 (2.4)	0.3	0.323	59.3 (2.0)	58.6 (1.9)	0.2	0.500
Body fat (%)	18.3 (2.5)	17.3 (1.9)	0.3	0.406	20.5 (2.1)	18.3 (2.5)	0.5	0.180
Calf girth (cm)	33.0 (0.6)	33.2 (0.8)	0.2	0.610	31.9 (0.7)	33.0 (0.6)	**0.7**	**0.013**
Bone mineral density (BMD) (g/cm²)								
Femoral neck	1.054 (0.038)	1.039 (0.036)	**0.7**	**0.039**	1.048 (0.040)	1.061 (0.040)	0.4	0.261
Trochanter	0.852 (0.288)	0.880 (0.036)	0.4	0.216	0.840 (0.032)	0.857 (0.030)	0.5	0.116
Spine (L1-L4)	1.080 (0.045)	1.079 (0.043)	0.2	0.604	1.080 (0.046)	1.094 (0.043)	0.6	0.086
Bone Mineral Content (BMC)(g)								
Femoral neck	5.0 (0.2)	4.9 (0.2)	0.5	0.184	4.9 (0.2)	5.0 (0.2)	0.5	0.103
Trochanter	9.6 (0.5)	10.1 (0.6)	0.6	0.092	9.5 (0.6)	9.8 (0.5)	0.4	0.260
Lumbar spine (l1-L4)	58.5 (3.9)	58.1 (4.1)	0.2	0.601	57.5 (4.1)	59.2 (3.9)	**0.7**	**0.029**
Bone Geometry								
CSA (mm²)	155 (6)	154 (6)	0.2	0.519	152 (6.5)	156 (6.3)	**0.7**	**0.018**
Min neck width (mm)	29.0 (0.6)	29.4 (0.8)	0.5	0.128	28.3 (0.7)	28.9 (0.5)	0.5	0.143
Section modulus (Z) (mm³)	648 (37)	645 (34)	0.1	0.878	617 (34)	638 (31)	0.4	0.230
Isometric Knee Extensor force (kg)								
Peak left	40.8 (3)	39.5 (3)	0.3	0.418	40.6 (3.8)	41.2 (2.6)	0.3	0.368
Peak right	41.6 (3)	41.8 (3)	0.0	0.949	41.5 (2.5)	41.9 (2.9)	0.1	0.853

Significant changes in repeated measures ANOVA with p<0.05

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7.3.6 Endurance runners

In endurance runners, paired tests with a bonferroni correction applied revealed no differences in frequency of training (winter: 9.0 (0.4), summer: 8.0 (0.4) sessions/week, t=1.691, p= 0.097), and hours spent training over the seasons (winter: 11.8 (0.7), summer: 11.5 (0.8) hrs/week, t=-0.842, p=0.404). However there were significant differences in the percentage of training intensity with the higher intensity training being greater in the summer (34.4 (2.3) % than the winter (24.0 (1.6) %, t=5.229, p<0.001). Serum samples of 38 endurance runners revealed no significant spring (112.4 (9.5) nmol/l) and autumn (104.5 (6.2) nmol/l) variation in serum 25 (OH) D levels (*p=0.489*). When comparing the spring serum 25 (OH) D levels of the controls (C) with that of the spring levels for the endurance runners (ER) it was revealed that the endurance runners had significantly higher serum 25 (OH) D levels compared to the controls (ER:112.4 (9.5), C: 77.2 (6.6) nmol/l, *p=0.009*).

Similar results were shown in the endurance runners to that observed in the entire group with femoral neck BMD decreasing significantly over the winter (2.0%, f=8.795, *p=0.005*), with no significant changes over the summer (0.0%, f=0.360, *p=0.551*). Minimal neck width increased significantly over the winter 1.1%, f=4.566, *p=0.037*) with no significant change over the summer 0.7%, f=1.019, *p=0.630*) Trochanter BMD increased significantly over the summer (6.0%, f=13.447, *p=0.001*), with no change over the winter (0%, f=0.454, p= 0.503), whereas trochanter BMC increased significantly over the winter (3%, f=7.200, p=0.010). Calf girth (5%, f=28.563, *p<0.001*) and left peak isometric knee extensor force (4%, f=9.137, p=0.01) increased over the winter with no changes over the summer (0%, f=0.275, *p=0.603* and 1%, f=0.027 p=0.933) respectively (table 7.4).

Table 7.4: Seasonal variations in training of endurance runners typically defined as summer (April to October) and winter (October to April), mean (SE), effect size and significant mean differences.

	Runners							
	Summer (n=51)				Winter (n=52)			
	Spring	Autumn	r	P-value	Autumn	Spring	r	P-value
Physical Characteristics								
Height (m)	1.67 (0.01)	1.67 (0.01)	0.3	0.066	1.66 (0.01)	1.66 (0.01)	0.2	0.275
Body Mass (kg)	55.0 (0.7)	54.9 (0.7)	0.1	0.679	54.1 (0.6)	54.7 (0.6)	0.3	0.054
Body Fat (%)	16.7 (0.7)	16.8 (0.8)	0.0	0.760	16.3 (0.7)	16.7 (0.7)	0.1	0.315
Calf Girth (cm)	32.0 (0.3)	32.1 (0.3)	0.1	0.603	31.6 (0.2)	32.4 (0.2)	**0.6**	**<0.001**
Bone mineral density (BMD) (g/cm2)								
Femoral neck	1.080 (0.015)	1.082 (0.015)	0.1	0.551	1.089 (0.015)	1.077 (0.014)	**0.4**	**0.005**
Trochanter	0.865 (0.015)	0.915 (0.017)	**0.5**	**<0.001**	0.870 (0.015)	0.871 (0.009)	0.1	0.503
Lumbar spine (L1-L4)	1.130 (0.019)	1.123 (0.019)	0.2	0.261	1.123 (0.018)	1.125 (0.018)	0.1	0.460
Bone Mineral Content (BMC)(g)								
Femoral neck	5.0 (0.1)	5.1 (0.1)	0.0	0.836	5.0 (0.1)	5.0 (0.1)	0.3	0.068
Trochanter	10.4 (0.3)	10.4 (0.3)	0.0	0.933	10.0 (0.3)	10.3 (0.3)	**0.4**	**0.010**
Lumbar spine (L1-L4)	60.5 (1.5)	60.1 (1.4)	0.3	0.068	59.6 (1.4)	59.1 (1.5)	0.1	0.413
Bone Geometry								
CSA (mm^2)	157 (3)	157 (3)	0.1	0.63	156 (2)	155 (2)	0.1	0.594
Min neck width (mm)	28.9 (0.3)	28.7 (0.3)	0.1	0.318	28.4 (0.3)	28.7 (0.3)	**0.3**	**0.037**
Section modulus (Z) (mm^3)	650 (17)	650 (16)	0.0	0.976	642 (16)	639 (15)	0.1	0.668
Isometric Knee Extensor force (kg)								
Peak left	37.2 (1.1)	37.0 (1.2)	0.0	0.971	37.1 (1.0)	38.8 (1.1)	**0.4**	**0.004**
Peak right	39.8 (1.0)	39.0 (1.1)	0.1	0.379	38.9 (1.0)	40.6 (1.0)	0.3	0.020

Significant changes in repeated measures ANOVA with p<0.05

7.3.7 Seasonal changes according to menstrual function in endurance runners

Of the 52 endurance runners who were monitored to determine seasonal changes of bone parameters, 28 of the athletes were categorized as being currently eumenorrhoeic and 24 were a/oligomenorrhoeic. Nineteen endurance runners reported to currently take hormonal contraceptives (13 eumenorrhoeic and 6 a/oligomenorrhoeic). No changes in menstrual function were reported on 6-month questionnaires. Comparisons of means (ANOVA) showed no significant differences in physical characteristics at baseline between the eumenorrhoeic and a/oligomenorrhoeic runners (table 7.5). Similar to the results presented in section 7.3.4.2 there were no significant seasonal (summer and winter) changes in height, weight and body fat percentage according to menstrual function in the endurance runners.

Table 7.5: Baseline descriptive characteristics of endurance runners according to menstrual function (mean (SE).

	A/oligomenorrhoeic (n=24)	Eumenorrhoeic (n=28)
Age (years)	24.6 (1.5)	26.1 (1.3)
Body mass (kg)	54.4 (0.8)	53.8 (1.1)
Height (m)	1.665 (1.132)	1.662 (0.879)
BMI (kg/m^2)	19.6 (0.3)	19.4 (0.2)
Body fat (%)	16.1 (1.0)	16.5 (0.9)

In eumenorrhoeic athletes, trochanter BMD increased over the summer (mean (SE) 0.885(0.019) to 0.947(0.017) g/cm^2, *p=0.002*) with no significant change over the winter (0.880 (0.018) to 0.885 (0.018), g/cm^{2,} *p=0.153*); figure 7.1 a).

In a/oligomenorrhoeic athletes, femoral neck BMD decreased over the winter (1.065 (0.021) to 1.052 (0.020) g/cm^2, p=0.030) with no significant change over the summer (1.050 (0.020) to 1.052 (0.020), p=0.770). Left peak quadriceps muscle strength significantly increased over the winter (38.2 (1.2) to 40.8 (1.4) p=0.005) with no significant changes over the summer (38.9 (1.5) to 37.9 (1.4) p=0.525; figure 7.2 C).

Calf girth significantly increased over the winter in both the eumenorrhoeic (31.5 (0.3) to 32.4 (0.3) p<0.001) and a/oligomenorrhoeic (31.6 (0.3) to 32.4(0.3) p=0.004) athletes with no significant change over the summer (EA: 32.1 (0.5) to 32.0 (0.4) p=0.852 and AA: 31.9 (0.4) to 32.2 (0.3) p=0.160) respectively figure 7.2 d. There were no significant seasonal variations in other bone parameters, season time's group interactions or annual changes according to menstrual function.
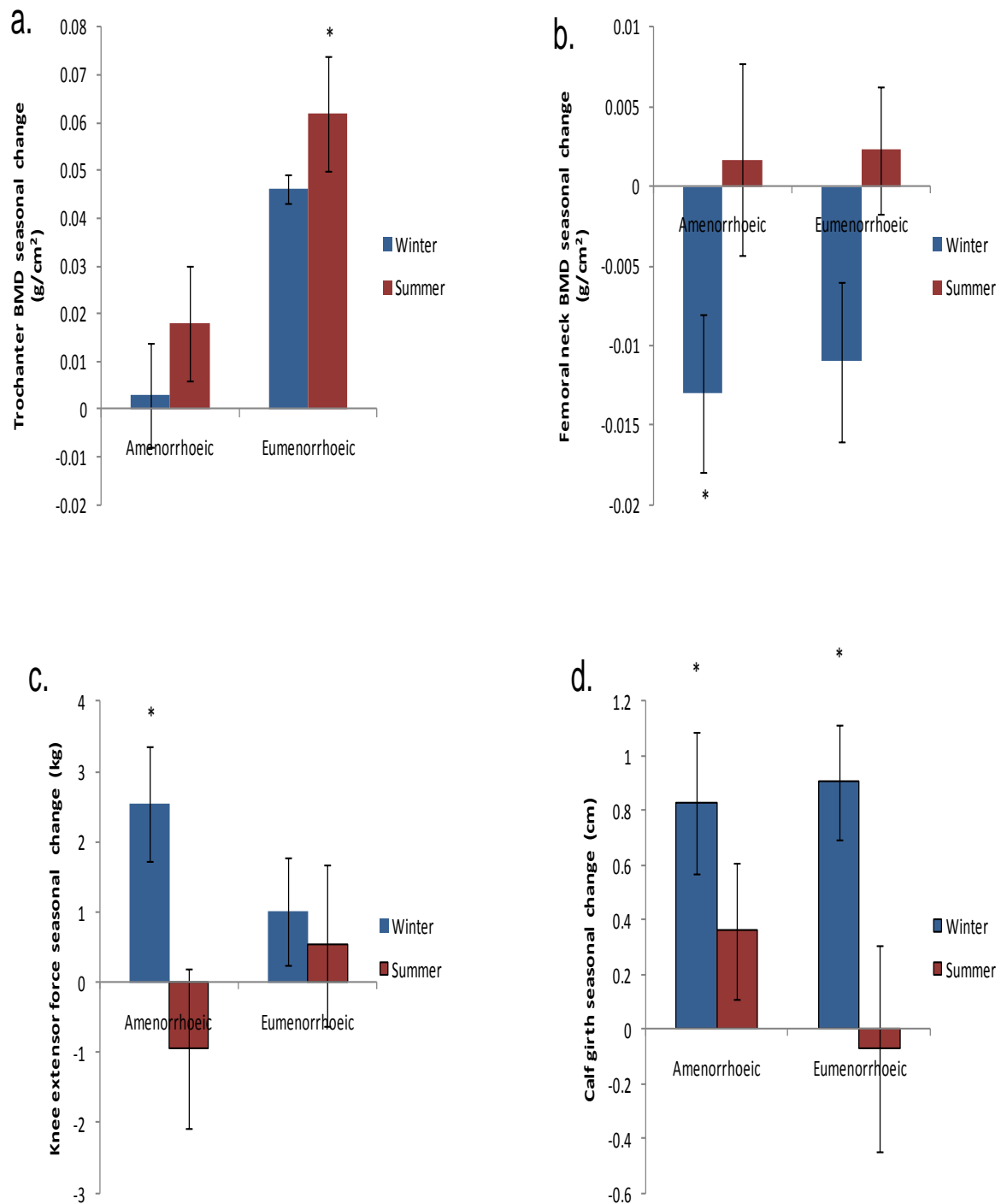
Figure 7.2: Mean (SE) seasonal changes (winter and summer) in eumenorrhoeic and amenorrhoeic runners of a. Trochanter BMD, b. femoral neck BMD, c. left peak isometric extensor force and d. calf circumference (EA: n=28, AA: n=24, P< 0.05)

7.1   Discussion

This study is novel in that it is, to our knowledge, the largest prospective study to report seasonal bone changes in female endurance athletes, and the differences in bone changes in eumenorrhoeic and amenorrhoeic endurance runners over both the summer and winter season. We report that there are significant decreases in BMD at the femoral neck in amenorrhoeic athletes during the winter which are not recovered over the summer season, although the increase in the width of the femoral neck may partly compensate the BMD loss to maintain strength in bending.

There were no annual or seasonal changes in physical characteristics of the endurance athletes indicating that the changes observed in bone parameters due to seasonal variations in training from the summer and winter competitive seasons and not due to annual increases seen with age-related bone change (Heaney et al. 2000; Melton 1996). Due to the mean age (25.3 years) of the athletes in the present study it is possible to suggest that peak bone accrual had already occurred (Heaney et al. 2000). These findings were in contrast to previous prospective studies (Bennell et al. 1997a) in which significant annual differences were found over a 12 month period. A possible explanation for these differences could have been the difference in mean age and possibly the training regimes of the athletes in each study. In the bennell et al (Bennell et al. 1997a) study the mean age of the athletes was 20.1 years and at this age bone would still be being accrued whilst in this study the mean age was 25. 3 years.

The levels of 25 (OH) D in the endurance runners were at the optimal recommended value for adults (Bischoff), and were significantly higher in the runners compared to sedentary controls during the spring. There were no significant seasonal differences in the runners, with change negligible compared to differences between individuals. 25-OH-D is typically lower in the spring in the United Kingdom (Patel et al. 2001), with values reported to be

sub-optimal in previous studies in British adults in the spring (Hypponen and Power 2007). The relatively good values in the athletes may be related to sunshine exposure with 44% reporting warm weather training during the weeks prior to spring testing. Optimal levels of vitamin D can be met entirely through endogenous synthesis, when the skin is exposed to ultraviolet-B radiation (sun exposure) (Holick 2008; Larson-Meyer and Willis 2010).

Over the winter season, when athletes reported participating in a high percentage of moderate intensity training, there were significant decreases in femoral neck BMD (by 0.7%) with no significant recovery over the summer season. This finding supports earlier detraining studies (Snow et al. 2001; Winters and Snow 2000) which have revealed decreases in BMD at the hip during periods of detraining. The reduction in BMD in the present study however, was not contributed to by detraining and BMD losses were markedly smaller than previously reported (Snow et al. 2001). Endurance athletes do not have an identified season and offseason, so decreases in femoral neck BMD were more likely caused by changes in training intensity from short bursts of high intensity interval training in the summer to continuous moderate intensity training in the winter (low impact repetitive loading) (Kohrt et al. 2004; Nikander et al. 2009; Nikander et al. 2005). It has previously been reported that high volumes of cardiovascular training, which results in repetitive low impact bone loading, is negatively associated with BMD values (Barry and Kohrt 2008; Hetland et al. 1993; Hind et al. 2006; Macdougall et al. 1992; Stewart and Hannan 2000). A study in male runners (MacDougall 1992) reported an inverse association between BMD and training volume when the weekly training exceeded 20 miles. The skeleton will adapt to the current activity needed to maintain strength, increasing the volume/loading cycles of exercise above the established level confers no additional benefits (Rubin and Lanyon 1984; Umemura et al. 1997). This theory could explain the decrease in femoral neck BMD over the winter season in the present study.

Endurance athletes may potentially have increased the volume (miles per week), while decreasing the intensity of training during the winter without increasing the hours spent training, furthermore changes in volume of training (hours per week) in the present study may not have been identified possibly due to the crude measure of training. In contrast, during the summer when the percentage of high intensity training was greatest, trochanter BMD increased (by 5.4%), this magnitude of change over the summer competitive season is consistent with previous findings (Klesges et al. 1996; Snow et al. 2001; Winters and Snow 2000).

Even though femoral neck BMD decreased in endurance athletes over the winter bone size and muscle strength did increase. Trochanter BMC and femoral neck width increased in magnitude, 3.0% and 1.1% respectively. Increases in bone size were accompanied by increases in muscle strength and calf girth by 4.0% and 2.8% respectively. The increased muscle strength and bone size could be related to increases in resistance training over the winter months. It is well documented that resistance training has a direct and positive site specific effect on bone properties (Kelley et al. 2002; Kohrt et al. 2004).

The competitive season of endurance athletes will often determine the circadian variations in the specific training programs. The present study recruited both endurance runners and triathletes. In general the training and competitive season of an endurance runner will involve high intensity short burst interval training in the summer for track and road racing and potentially high volume moderate intensity training in the winter for cross-country or longer road events. In contrast triathletes who participate in a combination of running, cycling and swim training will often compete (high intensities) during the summer months, while training during the winter. These differences in seasonal training patterns could be related to variations seen in seasonal bone changes in the present study. When splitting the endurance athletes into runners and triathletes it was found that both runners and

triathletes had significant increases in calf girth, 2.5% and 3.4% respectively, over the winter with no changes over the summer. Runners however, had additional increases in femoral neck width (1.0%), and knee extensor force (4.4%) and decreases in femoral neck BMD (1.2%), over the winter, which were not observed in the triathletes. In contrast, triathletes showed increases in lumbar spine BMC of 2.9%, and CSA of 2.6% over the winter months. During the competitive summer season runners showed significant increases in trochanter BMD (5.4%), however triathletes showed no significant changes in bone. These findings in triathletes do support an earlier study which reported no significant changes in bone during a competitive season (McClanahan et al. 2002). It is important although, to note that the previous prospective study (McClanahan et al. 2002) only followed the triathletes for a 24 week period, which may not have allowed adequate time to obtain seasonal changes, as bone formation generally takes more than 12 weeks and bone mineralization requires 3-4 months (Wolman and Reeve 1995). The present study monitored the triathletes over a 12 month period with 6 month assessments and found with a longer monitoring period that there were no adverse seasonal changes in bone in triathletes over a competitive season. However further studies with larger samples sizes are required with triathletes to support these findings.

Menstrual dysfunction was prevalent in this sample of athletes with twenty-four (46%) of the athletes reporting current menstrual dysfunction, with no reports of change in menstrual status over the 12 month period. This prevalence of menstrual dysfunction is consistent with previous reports (Bennell et al. 1997b), however contrary to previous cross sectional studies there were no significant differences in physical characteristics between the a/oligomenorrhoeic and eumenorrhoeic athletes (Drinkwater et al. 1984; Marcus et al. 1985; Nelson et al. 1986).

In accordance with earlier studies (Bonis et al. 2009) trochanter BMD increased significantly over the summer in eumenorrheic athletes, and was relatively maintained over the winter season. In contrast, amenorrhoeic athletes had significant decreases (1.1%) in femoral neck BMD over the winter training season which was not recovered over the summer. This is of concern as it is unclear in the literature whether full bone recovery is obtained once menses resumes with some studies indicating a full recovery (Hind 2008; Hind 2010) and others alluding to irreversible bone losses despite several years of normal menses (Keen and Drinkwater 1995; Keen and Drinkwater 1997).

Even though amenorrhoeic athletes seem to have higher BMD values than previously reported (Hind et al. 2006; Pollock et al. 2010; Zanker et al. 2004), the continued loss of bone at the femoral neck (1.1%) year on year during the winter training season is a cause for concern, and may increase the risk for early onset osteoporosis prior to the menopausal years. Previous studies have indicated that the annual age-related loss of BMD at the femoral neck during the menopausal years is approximately 1%, with a greater loss of trabecular rather than cortical bone (Beck et al. 2006; Khan et al. 2001; Melton 1996; Melton et al. 2000a; Melton et al. 2000b).

In contrast to previous studies (Klesges et al. 1996; Snow et al. 2001; Winters and Snow 2000) there were no significant seasonal changes at the lumbar spine over the summer and winter, possibly due to the seasonal change in training being smaller in the present study than in previous studies which indicated 4 months of disuse. Bone gain at the spine is reported to continue well into the third decade, but at the hip the bone gain is suggested to end early in the third decade (Heaney et al. 2000). In the present study there were no significant seasonal changes at the lumbar spine in either the eumenorrhoeic or amenorrhoic athletes, suggesting that the seasonal variations in loading may be greater at the hip than at the spine (Snow et al. 2001; Taaffe et al. 1997).

This study has several limitations, firstly there was no direct comparison with a non-athlete control group therefore it is not clear whether bone changes are due to seasonal changes in training or other seasonal effects such as age related bone accrual and reabsorption. Sex steroid concentrations were not assessed and there might have been some variation even without evident changes in menstrual function. It is therefore possible that hormonal changes contributed to the observed seasonal changes. The sample size of the triathlete group in the present study was small and may not be adequate to detect a seasonal change in this subgroup. There were no significant changes in BMC at the femoral neck in amenorrhoeic athletes, possibly due to the measure not being sensitive enough to detect small seasonal changes, it is therefore, not possible to determine if increases in mineral neck width are due to seasonal changes or a potential redistribution of bone.

The strengths of the study are that, this is the largest prospective study which has observed bone changes in endurance athletes over both the summer and winter competitive season determining that seasonal changes in bone are due to changes in training rather than other seasonal factors such as seasonal variations in 25-OH-D concentration.

Potential clinical implications can be drawn from this study suggesting that although amenorrhoeic endurance athletes do not have BMD as low as reported previously (Hind 2008; Hind et al. 2006; Pollock et al. 2010), bone loss is a cause for concern, and that this may occur particularly in the winter suggests this may be an opportunity for intervention.

It can be concluded from this study that there are positive and negative seasonal bone changes in female endurance athletes which are smaller than those indicated in previous detraining studies. Eumenorrhoeic athletes increased trochanter BMD over the summer, and this was maintained over the winter. Conversely, amenorrhoeic athletes lost femoral neck BMD over the winter and this was not recovered during the summer, although the

increase in width of the femoral neck may partly compensate BMD loss to maintain strength in bending. Seasonal losses in BMD that are not recovered may contribute cumulatively to bone loss in amenorrhoeic runners.

Chapter Eight
Magnitude and timescale of bone change
following a stress fracture injury

Chapter eight presents the final prospective analysis, determining the time and magnitude of bone loss following a stress fracture injury, and subsequent bone regain following retraining.

Magnitude and timescale of bone parameters attenuation following a stress fracture injury

8.1   Introduction

Stress fractures are common overuse injuries in athletes which are thought to result from repetitive loading to bone without adequate time for repair (Bennell et al. 1996c; Khan et al. 2001; Shaffer 2001). Without adequate repair through bone remodeling, symptoms of bone stress will develop, leading to micro cracks, resulting in stress fracture injury (Bennell et al. 1996c; Brukner and Bennell 1997; Niemeyer et al. 2006).

Reviews of guidelines for stress fracture management (Bennell and Brukner 2005a; Bennell et al. 1996c; Brukner et al. 1998; Carmont et al. 2009; Romani et al. 2003) conclude that stress fractures with brief history and symptoms will often heal within 4 to 6 weeks of diagnosis and return to sport may occur within 6 to 8 weeks following the stress fracture. This is usually gradual thus progressive increases in bone loading through weight bearing activity is needed (Bennell and Brukner 2005a; Carmont et al. 2009). The healing of bone following a stress fracture injury is determined by the absence of localized pain and tenderness during physical activity and does not require a CT scan for confirmation (Bennell and Brukner 2005a; Khan et al. 2001; Romani et al. 2003).

Although few studies have prospectively reported reoccurrence rates of stress fracture, there are indications that it is as high as 23% over a 12-month period (Bennell et al. 1996a; Bennell et al. 1996b; Nattiv 2000; Snyder et al. 2006), but there is no consensus in the literature identifying a cause of stress fracture reoccurrence. A number of risk factors of stress fracture could determine the cause of stress fracture reoccurrence especially in female athletes who are reported to be at the greatest risk for stress fracture due to a high

prevalence of menstrual dysfunction (1-44%) (Bennell et al. 1997b) and disordered eating (1-62%) (Sundgot-Borgen and Torstveit 2007). Low bone density has been identified as a potential risk factor in some (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv 2000) but not all studies (Bennell et al. 1995; Carbon et al. 1990; Grimston et al. 1991; Myburgh et al. 1990)(Chapter 4 and chapter 6) in female athletes. In previous studies, however bone mineral density was not measured at the time of stress fracture diagnosis, therefore it may be feasible that changes in bone mineral density following a stress fracture could contribute to reoccurrence of stress fracture by reducing the bone strength (Bennell et al. 1999), resulting in an accumulation of micro-damage when athletes begin retraining following injury (Caler et al. 1981). Sudden increases or changes in training intensity and volume have previously been associated with increased risk of stress fracture (Brunet et al. 1990; Goldberg and Pecora 1994; Shaffer 2001). It is well established that there is an association between fracture and low bone mineral density, therefore clinically accurate BMD measurements can predict the likelihood of a fracture (Cummings et al. 1993; Melton et al. 1993). However, in athletes BMD tends to be above the level seen in the average population due to the increased mechanical loading (Bennell and Brukner 2005b) (Kannus et al. 1994a; Khan et al. 2001; Nordstrom et al. 2005b). There are no normative BMD databases for athletes with which to determine potential low bone mass. Similarly there is no indication of whether bone changes following stress fracture are associated with reoccurrence rates.

During the recovery of fractures and musculoskeletal injuries, the associated non-weight bearing or immobilization leads to rapid loss of trabecular and cortical bone. Bone loss of between 2.5 to 18% has been reported, with the greatest loss of bone observed 3 to 4 months following an injury (Alfredson et al. 1998; Magnusson et al. 2001; Petersen et al. 1997; Therbo et al. 2003; Veitch et al. 2006). Both cross-sectional (Kannus et al. 1994b;

Karlsson et al. 1993) and prospective studies (Clement et al. 1999; Kannus et al. 1992; Petersen et al. 1997; Therbo et al. 2003) have shown that the loss of bone density following post-traumatic injury is not completely reversible, with bone loss being detected in contralateral limbs and in bones which are more proximal to the injured area (Cattermole et al. 1997; Kannus et al. 1992; Magnusson et al. 2001; Therbo et al. 2003).

It is believed that early weight bearing after an injury can limit the degree of bone loss (Emami et al. 2001). However Petersen et al (Petersen et al. 1997) still reported bone loss in patients treated with partial weight bearing after ACL surgery. Following a stress fracture injury, the management consists of active rest (non weight bearing activities, swimming etc) for up to 6 weeks, followed by a period of progressive weight bearing to return to full training. The 6 weeks of active rest may promote bone loss, resulting in weakened bone more liable to fracture.

To our knowledge it is unknown whether there is a similar magnitude of bone loss following a stress fracture as seen with traumatic injuries. If bone loss is occurring at a similar timescale following stress fracture as that of a traumatic injury then athletes may be returning to competitive training at the point at which the bone is at its weakest. Therefore the purpose of this study was to 1) determine the time and magnitude of bone loss following a stress fracture and 2) determine the subsequent bone regain following retraining.

8.2   Methods

8.2.1   Study design

A case control prospective design was used to assess bone change following a stress fracture injury and subsequent bone regain following re-training. BMD, BMC, bone geometric properties, and body composition was assessed at five time points following

stress fracture diagnosis (baseline, 6-8 weeks, 3-4 months, 6-8 months, 12 months) over a 12-month period using DXA. Questionnaires were used to assess menstrual function, and training was prospectively monitored.

8.2.2 Subjects

Eight female athletes diagnosed with a lower extremity stress fracture (cases) and seven controls matched for age, event and menstrual status were included in the study. Stress fracture cases were recruited through sports medical physicians working for the English Institute of Sport (EIS), English Cricket Board (ECB) and UK Athletics (UKA), after a positive diagnosis was confirmed with a MRI, CT or X-Ray.

Controls were required to be un-injured and normally training and were recruited through the English Institute of Sport (EIS), English Cricket Board (ECB), UK Athletics (UKA) and local sporting clubs, and were normally training. Controls were screened to exclude any athlete who was currently injured, had a previous stress fracture in the past 12-months or who had recently had exposure to high levels of medical radiation. Stress fracture cases and controls were excluded from the study if they were currently taking any medication which could affect bone metabolism, and who were pregnant or lactating or had been in the past 12-months.

Stress fracture cases had measurements of both the injured and contra-lateral limb. In control participants, "injured" and "contra-lateral" limbs were used as comparisons to the cases and were allocated based on the dominance of the lower extremity in the stress fracture cases. If the stress fracture occurred in the dominant leg of the case the dominant leg of the control was allocated as the injured comparison leg and the non-dominant leg the healthy limb and vice versa.

8.2.3 Bone parameters

BMD and BMC were assessed at the total body, lumbar spine (L1-L4), and both hips (femoral neck, trochanter) using DXA. The lumbar spine and dual hips were used as they are common regions of interest for the diagnosis of osteoporosis and risk of hip fracture. Bone geometric properties were estimated using the hip structural analysis at the femoral neck to assess CSA, Z, and buckling ratio.

8.2.4 Menstrual function

Questionnaires were used to assess menstrual function at baseline. From the baseline questionnaires stress fractures cases were matched with a control female athlete for menstrual status. Changes in menstrual function were monitored at each assessment and through questionnaire at the 12 month period.

8.2.5 Training

Athletes were asked to keep a monthly training log following the stress fracture. All therapy and training sessions (cross training, pool sessions, periods of running and sports specific training) were recorded for the 12-month period. From the training logs, training mode, duration, frequency, and estimate of intensity was determined at each assessment point to determine the time point at which weight bearing activity was reintroduced. This time point was used to determine the post injury and retraining phases following stress fracture.

8.2.6 Anthropometric measures

Anthropometric measures were taken at each of the five assessment points. Body mass, height and calf girth were measured using standardized protocols using a stadiometer (Holtain, Pembrokeshire), balance beam scale (Herbert and sons Ltd, London) and tape measure respectively. Body composition was assessed by DXA to determine changes over time in percent fat, total lean mass, and tissue mass.

8.2.7 Statistical analysis

All statistical analyses were performed using SPSS for windows (version 16.0) statistical software package. Comparisons of means using a one way-analysis of variance (ANOVA) were carried out at baseline to compare physical characteristics between the stress fracture cases and matched controls. Paired t-tests with a bonferroni correction applied, were used to determine the mean change in BMD, BMC and geometric properties from baseline to the point of retraining following a stress fracture.

Bone regain following stress fracture was determined in a sub-sample of athletes who adhered to at least 6-8 months follow-up. Comparisons of means at baseline were carried out to determine whether athletes (stress fracture cases and controls) who adhered to the study differed from those who withdrew. Repeated measures analysis of variance (ANOVA) was used to determine any significant periods of bone change in BMD, BMC (total body, lumbar spine, femoral neck, trochanter) and geometric properties (strength index, Z, buckling ratio) following stress fracture injury in the injured and contra-lateral limb of case and control athletes independently. Multiple paired t-tests with a bonferroni correction were used where there was a significant mean difference to determine the significant time point and limb in which bone change occurred in stress fracture cases and controls. Repeated measures analysis of variance (ANOVA) with a between subject factor of case (stress fracture and control subjects) determined if there was a bone change times group interaction according to subjects (stress fracture cases and control). Level of statistical significance was considered at $P < 0.05$. As the sample size was small, the least significant change (LSC) was calculated to determine if differences in individuals were large enough to denote change. The LSC is the smallest change that lies outside the 95% confidence intervals for measurement reproducibility and was calculated as follows: 2.77 x the percent site specific precision error (Baim et al. 2005) (chapter 3, table 3.1).

8.3 Results

Of the 15 participants (8 cases and 7 controls) who were recruited at baseline, four cases and three controls were followed for up to 6-8 months. As shown in figure 8.1, all other participants were lost at follow-up at 6-8 weeks or 3-4 months following the stress fracture.
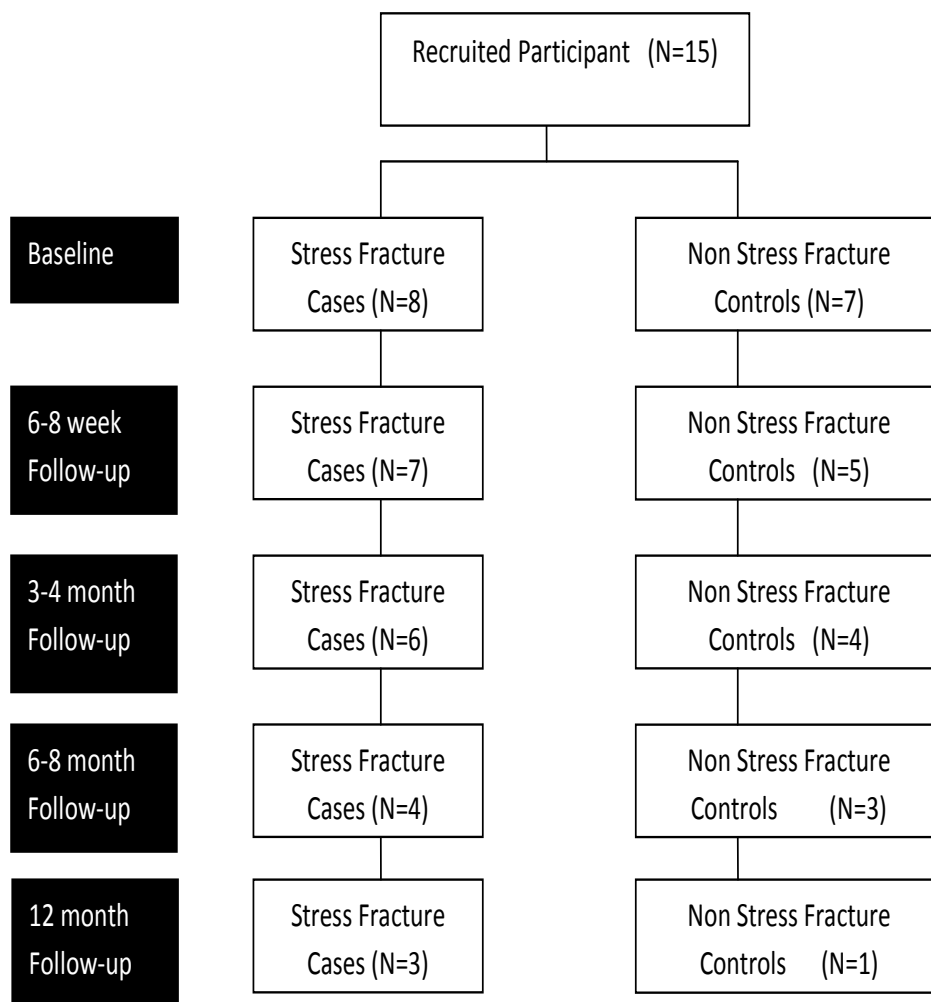
Figure 8.1: Illustrates the baseline recruitment and overall withdrawal rate of the study

8.3.1 Baseline characteristics

All stress fracture cases were matched with a control for age, menstrual status and sporting event. The stress fracture cases were measured at baseline (mean +\-SD) 2 +/- 2 weeks following the stress fracture. Follow up scans were taken 6-8 weeks, 3-4 months, 6-8 months and 12 months from the date the stress fracture was diagnosed with a MRI, CT, or X-ray. The stress fractures were all lower extremity injuries in athletes who were right leg dominant. Four stress fractures were diagnosed on the left, non-dominant side (1 femoral, 1 metatarsal, 2 sacral) and four on the right, dominant side (1 sacral, 1 femoral, 1 proximal tibia, 1 metatarsal). Controls were matched for an injured and healthy limb as stated in the methodology. Of the 15 subjects 6 reported to be currently a/oligomenorrhoeic (3 cases and 3 controls) and 9 eumenorrhoeic, with one matched eumenorrhoeic case and control currently taking hormonal contraceptives.

The comparisons of the means (ANOVA) of the stress fracture cases (n=8) and controls (n=7) revealed no significant differences in age, weight, body fat % and age at menarche, however, stress fractures cases (FX) were significantly shorter than the controls (C) (FX: 1.639 (0.009), C: 1.701 (0.016) m, *p=0.007*). Stress fracture cases reported a higher prevalence of stress fracture history than controls (50% vs 0% respectively). There were no significant differences at baseline in total body, lumbar spine, femoral neck and trochanter BMD or geometric properties CSA, Z, and buckling ratio of the injured and contra-lateral limb between groups. (Table 8.1).

Table 8.1: Comparison of baseline characteristics of stress fracture cases and controls: mean (SE).

	Stress Fracture (N=8)	Control Group (N=7)
Physical characteristics		
Age	19.9 (0.7)	20.7 (1.6)
Body Mass (kg)	56.7 (2.4)	59.1 (2.9)
Height (m)	1.64 (0.01)	1.70 (0.02)*
BMI kg/m^2)	21.1 (0.8)	20.5 (1.1)
Body Fat (%)	20.7 (1.4)	19.5 (3.7)
Age at Menarche	14.5 (0.7)	14.5 (0.2)
Calf Girth (cm) (Injured)	31.7 (1.0)	32.7 (1.0)
Calf Girth (cm) (contra-lateral)	31.5 (1.0)	31.6 (0.7)
History of Stress Fracture (%)	50	0
Bone mineral density (g/cm^2)		
Total body	1.114 (0.027)	1.122 (0.030)
Spine (L1-L4)	1.063 (0.078)	1.144 (0.063)
Femoral neck (Injured)	1.062 (0.051)	1.098 (0.039)
Femoral neck (contra-lateral)	1.041 (0.054)	1.058 (0.036)
Trochanter (Injured)	0.853 (0.047)	0.881 (0.054)
Trochanter (contra-lateral)	0.853 (0.037)	0.926 (0.023)
Femoral neck bone geometry		
CSA (mm^2) (Injured)	145 (9)	162 (9)
CSA (mm^2) (contra-lateral)	141 (7)	154 (8)
Section modulus (mm^3) (Injured)	590 (60)	686 (47)
Section modulus (mm^3) (contra-lateral)	549 (47)	654 (51)
Buckling ratio (Injured)	2.4 (0.3)	2.4 (0.6)
Bucking ratio (contra-lateral)	2.3 (0.2)	3.2 (1.2)

*Significant difference P<0.05

8.3.2 Post injury phase following Stress Fracture

From the completion of the training logs it was identified that progressive weight bearing training resumed at (mean +\-SD) 8.0+/-2 weeks following the stress fracture injury with short durations (10-15 minutes) of low intensity running/walking progressing gradually to normal volumes of training within approximately 4 weeks. Prior to the resumption of weight-bearing activity athletes reported to actively recover from the injury with training consisting of non-weight bearing swimming, aqua jogging, cross training and upper body weight training. From this observation the post injury phase following stress fracture has been identified as the time period between stress fracture diagnoses (baseline) to the 6-8 weeks follow-up assessment.

Due to dropouts only seven stress fracture cases (n=7) and five controls (n=5) were included in the post injury phase of the analysis to determine bone change following stress fracture. Paired T-tests, with a bonferroni correction revealed no significant changes in body mass and fat percent during the post injury phase in stress fracture cases and controls (table 8.2).

No significant mean change was found in BMD of the total body, lumbar spine, femoral neck and trochanter in the stress fracture cases injured and contra-lateral limb. In controls, BMD of the total body was significantly increased with a mean (SE) change of 0.011(0.001), $p < 0.001$, during the post injury phase (figure 8.2). No significant mean changes were found in any of the other BMD or geometric properties of bone in stress fracture case or control groups

Table 8.2: Physical characteristics, BMD and bone geometric parameters during the post injury phase (baseline to 6-8 weeks) following a stress fracture in cases and controls: mean (SE), mean change and p-values

	Stress Fracture (n=7)				Controls (n=5)			
	Baseline mean (SE)	6-8 weeks mean (SE)	Mean Change	(p-value)	Baseline mean (SE)	6-8 weeks mean (SE)	Mean Change	(p-Value)
Physical characteristics								
Body Mass (kg)	56.0 (2.7)	56.2 (2.8)	0.2	0.863	58.1 (4.1)	57.6 (3.3)	-0.5	0.648
Body fat (%)	19.8 (1.2)	20.2 (1.3)	0.4	0.231	17.3 (4.9)	16.1 (4.4)	-1.2	0.111
Calf circumference (injured)	31.3 (1.1)	31.5 (1.2)	0.2	0.704	32.3 (1.5)	31.5 (1.3)	-0.8	0.128
Calf circumference (contra-lateral)	31.3 (1.2)	31.4 (1.1)	0.1	0.975	31.2 (1.0)	31.9 (1.5)	0.7	0.283
Bone Mineral Density (g/cm^2)								
Total body	1.119 (0.031)	1.111 (0.033)	-0.008	0.067	1.119 (0.040)	1.130 (0.041)	0.011	**<0.001**
Spine (L1-L4)	1.075 (0.089)	1.079 (0.089)	0.004	0.658	1.163 (0.080)	1.167 (0.079)	0.004	0.389
Femoral Neck (Injured)	1.058 (0.59)	1.059 (0.066)	0.001	0.857	1.093 (0.056)	1.081 (0.056)	-0.012	0.182
Femoral Neck (contra-lateral)	1.039 (0.062)	1.034 (0.060)	-0.005	0.676	1.047 (0.056)	1.084 (0.060	0.037	0.089
Trochanter (Injured)	0.846 (0.053)	0.839 (0.051)	-0.007	0.089	0.851 (0.071)	0.86 (0.074)	0.009	0.497
Trochanter (Healthy)	0.839 (0.040)	0.820 (0.040)	-0.019	0.008	0.909 (0.027)	0.859 (0.058)	-0.05	0.338
Femoral neck bone geometry								
CSA (mm^2) (Injured)	146 (10)	147 (11)	1	0.643	166 (12)	164 (13)	-2	0.325
CSA (mm^2) (contra-lateral)	141 (8)	140 (10)	-1	0.671	147 (6)	149 (3)	2	0.703
Section Modulus (mm^3) (Injured)	594 (69)	584 (73)	-10	0.323	646 (22)	618 (3)	-28	0.238
Section Modulus (mm^3) (contra-lateral)	552 (54)	540 (60)	-12	0.333	615 (21)	613 (30)	-2	0.880
Buckling Ratio (Injured)	3.0 (0.6)	2.4 (0.3)	-0.6	0.650	2.2 (0.4)	2.4 (0.6)	0.2	0.459
Bucking Ratio (contra-lateral)	2.9 (0.5)	2.3 (0.2)	-0.6	0.150	3.2 (1.2)	3.2 (1.2)	0	0.761

Bonferroni correction indicates a p<0.003 to be significant
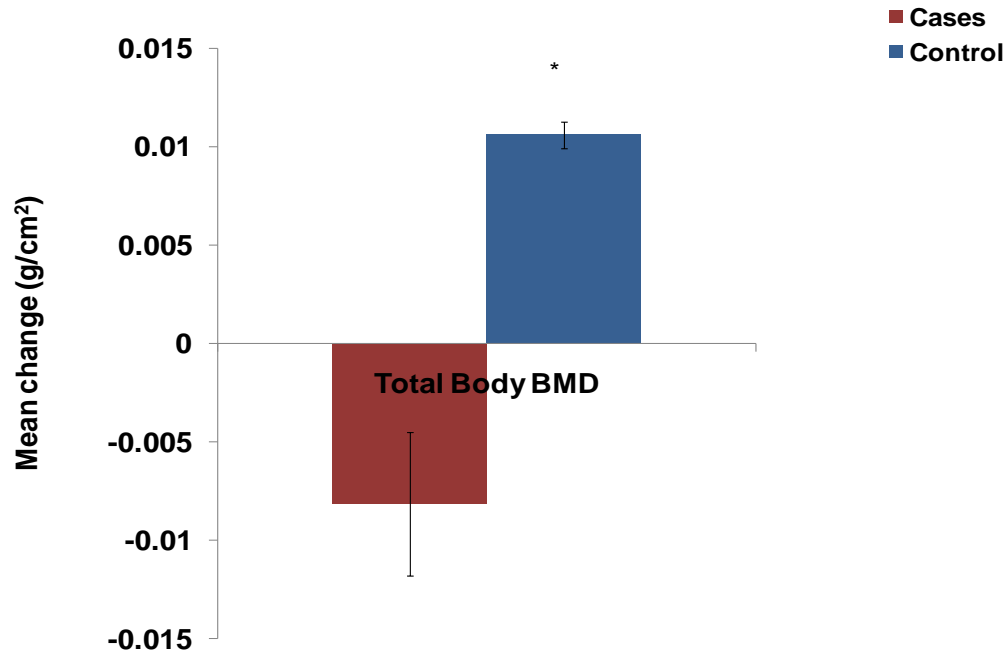
Figure 8.2:Total body BMD mean differences with standard error bars, showing the bone changes during the post injury phase (baseline to 6 to 8 week's) following stress fracture. Bonferroni correction indicates significant p<0.003

8.3.3   Bone changes in retraining phase

A small sub sample of stress fracture cases (n=4) and controls (n=3) who adhered to at least 6-8 months of follow up were analyzed to determine change in the retraining phase following stress fracture. A comparison of baseline means was carried out to determine if sample bias was present in the 4 stress fracture cases and 3 controls that completed the 6-8 month assessment period with those who withdraw from the study (table 8.3).

Table 8.3: Comparisons of means of stress fracture cases and controls who adhered to at 6-8 months follow-up with athletes (cases and controls) who withdrew: mean (SE)

	Analyzed retraining phase		Withdrew	
	Cases (N=4)	Controls (N=3)	Cases (N=4)	Controls (N=4)
Physical characteristics				
Age (years)	20.5 (1.2)	21.7 (3.2)	19.3 (0.6)	20.0 (2.0)
Body Mass (kg)	56.9 (3.0)	54.3 (0.6)	56.5 (4.2)	62.7 (4.3)
Height (m)	1.62 (0.02) *	1.71 (0.01)	1.65 (0.02)	1.700 (0.02)
Body Fat (%)	18.9 (1.8)	14.8 (3.8)	22.5 (1.8)	23.1 (5.6)
History of Stress Fracture (%)	50	0	50	0
Bone mineral density (g/cm^2)				
Total body	1.111 (0.058)	1.090 (0.025)	1.118 (0.015)	1.146 (0.048)
Spine (L1-L4)	1.114 (0.154)	1.112 (0.061)	1.012 (0.055)	1.168 (0.107)
Femoral neck (Injured)	1.052 (0.094)	1.030 (0.045)	1.073 (0.058)	1.150 (0.048)
Femoral neck (contra-lateral)	1.021 (0.102)	0.996 (0.049)	1.061 (0.541)	1.101 (0.039)
Trochanter (Injured)	0.834 (0.094)	0.772 (0.082)	0.873 (0.031)	0.963 (0.041)
Trochanter (contra-lateral)	0.831 (0.066)	0.882 (0.009)	0.875 (0.042)	0.959 (0.030)
Bone Geometry				
CSA (mm^2) (Injured)	148 (18)	152 (9)	142 (4)	169 (14)
CSA (mm^2) (contra-lateral)	138 (14)	145 (8)	144 (6)	162 (12)
Section Modulus (mm^3) (Injured)	629 (125)	634 (25)	551 (10)	725 (78)
Section Modulus (mm^3) (contra-lateral)	552 (96)	612 (30)	545 (28)	686 (89)
Buckling Ratio (Injured)	2.7 (0.4)	2.2 (0.6)	3.2 (1.0)	2.5 (0.1)
Bucking Ratio (contra-lateral)	2.4 (0.1)	3.7 (1.6)	3.3 (0.9)	2.7 (0.4)

*significant difference p<0.05 from the analyzed control athletes

No significant differences were found in physical characteristics, BMD or bone geometric properties in any of the groups. There was no difference in the prevalence of stress fracture history between the stress fracture cases (50%) that completed the study, and stress fracture cases (50%) that did not. Of the 4 stress fracture cases that completed the retraining phase one was currently a/oligomenorrhoeic and 3 reported to be eumenorrhoeic, whereas the 4 cases who withdrew all reported to have current a/oligomenorrhoea.

No significant differences in body mass and fat percentage were found between the stress fracture cases (n=4) and controls (n=3) who adhered to the retraining phase following stress fracture, however as previously shown stress fracture cases were significantly shorter than controls (FX: 1.620 (0.023), C: 1.710 (0.009), m *p=0.029*). No significant bone changes were detected during the post injury phase (baseline to 6-8 weeks) following stress fracture in BMD at the total body, lumbar spine, femoral neck or trochanter in stress fracture cases or controls. Similarly there were no significant differences of the femoral neck CSA, Z, strength index and bucking ratio in stress fracture cases and controls. (Table 8.4).

Repeated measures analysis of variance (ANOVA) showed a significant change over time in BMD at the femoral neck of the stress fracture cases (F=5.925, p=0.016), with no significant change in the control group (F=0.040, p=0.988) (figure 8.3). Multiple paired t-tests, with a bonferroni correction applied revealed that BMD at the femoral neck was significantly increased between 6-8 weeks and 6-8 months in the injured limb of the stress fracture cases (1.042(0.102) to 1.070(0.102) g/cm^2, p=0.004), with no significant change in the contra-lateral limb (1.036(0.102) to 1.054(0.109) g/cm^2, p=0.111) (figure 8.4). Changes in other bone parameters were small and not significantly different in either the stress fracture cases or controls during the retraining phase.

Table 8.4: Mean (SE) changes in BMD and bone geometry in cases and controls over 6-8 months following stress fracture

Scanned area	Cases (N=4)				Controls (N=3)			
	Baseline	6 to 8 weeks	3 to 4 months	6 to 8 months	Baseline	6 to 8 weeks	3 to 4 months	6 to 8 months
Bone mineral density (g/cm²)								
Total body	1.111 (0.058)	1.107 (0.062)	1.106 (0.056)	1.114 (0.058)	1.090 (0.256)	1.100 (0.026)	1.092 (0.028)	1.100 (0.035)
Lumbar spine (L1-L4)	1.114 (0.155)	1.115 (0.154)	1.113 (0.146)	1.135 (0.158)	1.112 (0.061)	1.116 (0.063)	1.108 (0.061)	1.112 (0.069)
Femoral neck (Injured limb)	1.051 (0.094)	1.042 (0.102)	1.045 (0.096)	1.070 (0.102)*	1.030 (0.045)	1.014 (0.057)	1.015 (0.546)	1.019 (0.040)
Femoral neck (contra-lateral Limb)	1.020 (0.102)	1.036 (0.102)	1.046 (0.104)	1.054 (0.109)	0.996 (0.049)	1.012 (0.054)	1.010 (0.398)	0.999 (0.033)
Trochanter (Injured limb)	0.834 (0.094)	0.825 (0.090)	0.823 (0.094)	0.830 (0.100)	0.772 (0.083)	0.773 (0.082)	0.776 (0.080)	0.785 (0.081)
Trochanter (contra-lateral Limb)	0.831 (0.066)	0.808 (0.068)	0.638 (0.219)	0.824 (0.066)	0.882 (0.009)	0.807 (0.070)	0.808 (0.058)	0.820 (0.070)
Femoral neck bone geometry								
CSA (mm²) (Injured limb)	148 (18)	147 (21)	148 (18)	151 (21)	152 (9)	150 (9)	155 (7)	155 (7)
CSA (mm²) (contra-lateral Limb)	139 (14)	139 (18)	138 (17)	143 (17)	145 (8)	147 (4)	147 (4)	144 2)
Section modulus (mm³) (Injured limb)	629 (125)	614 (133)	603 (127)	616 (132)	633 (25)	617 (4)	646 (8)	644 (12)
Section modulus (mm³)(contra-lateral Limb)	552 (97)	546 (109)	543 (95)	572 (111)	612 (30)	611 (42)	629 (42)	598 (65)
Strength Index (Injured limb)	1.7 (0.2)	1.7 (0.3)	1.8 (0.3)	1.8 (0.4)	1.8 (0.1)	2.0 (0.2)	1.9 (0.0)	1.9 (0.1)
Strength Index (contra-lateral Limb)	1.5 (0.1)	1.6 (0.3)	1.4 (0.2)	1.5 (0.2)	1.6 (0.0)	1.7 (0.1)	1.8 (0.1)	1.6 (0.1)
Bucking Ratio (Injured limb)	2.7 (0.4)	2.1 (0.4)	1.9 (0.3)	2.3 (0.6)	2.2 (0.6)	2.6 (0.9)	3.3 (0.8)	2.5 (0.9)
Bucking Ratio (contra-lateral Limb)	2.4 (0.1)	2.0 (0.2)	2.4 (0.3)	2.7 (0.2)	3.7 (1.6)	1.9 (0.1)	2.7 (0.8)	3.1 (0.9)

*Significant bone regain when compared from 6-8 weeks to 6-8 months following stress fracture, Bonferroni correction p< 0.004
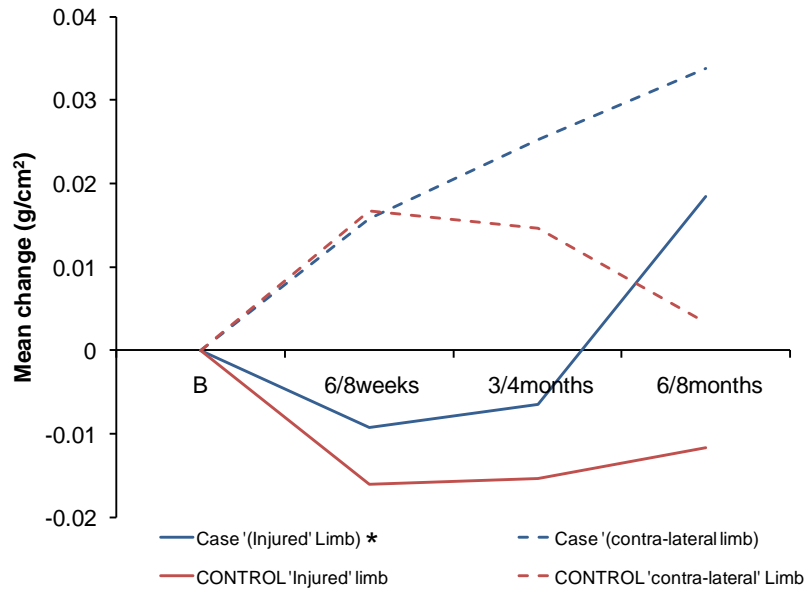
Figure 8.3: Time trend of changes in BMD at the femoral neck following stress fracture in stress cases and controls (injured and contra-lateral leg). *Significance was determined as P< 0.004.*
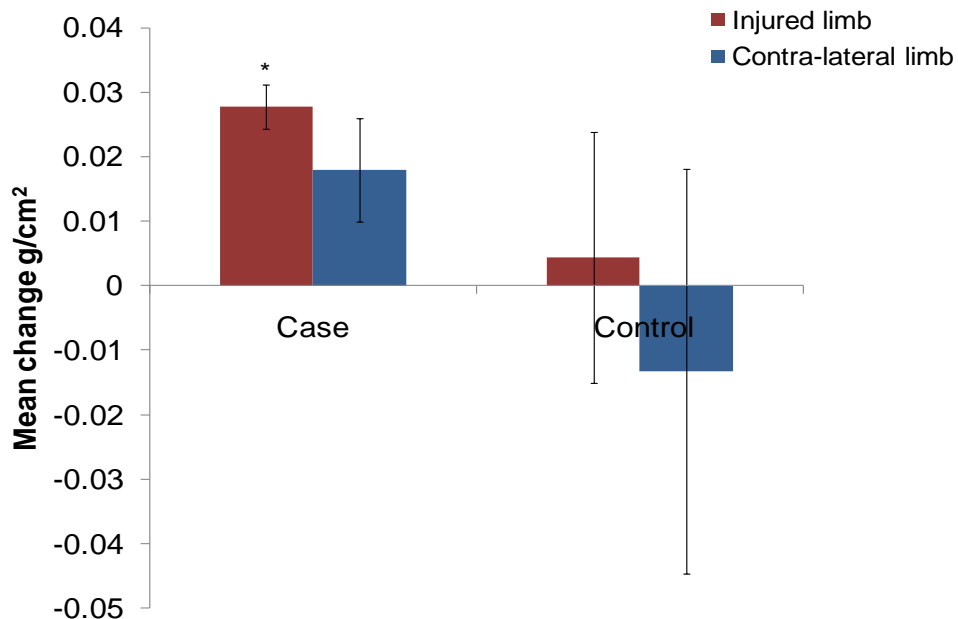
Figure 8.4: Bone change during retraining, illustrated as, mean change (SE) from 6-8 weeks to the 6-8 month of BMD at the femoral neck in case and controls injured and contra-lateral *(* significant mean change P<0.004)*

Calculation of the LSC of femoral neck BMD in the stress fracture cases injured limb to determine clinically meaningful change during retraining revealed that the LSC (2.77x 0.88 = 2.44) was exceeded in case 1 and 4, showing a clinically significant increase in femoral neck BMD. In case 2 and 3 the change in BMD did not exceed the LSC (table 8.5). Repeated measures ANOVA with a between subject factor of case (stress fracture case or control) revealed no significant interaction between the stress fracture cases and controls over the 6-8 month monitoring period in either the healthy or injured leg at any of the measured BMD or geometric sites.

Table 8.5: Changes in femoral neck BMD from post injury (6-8 weeks) to retraining (6-8 months) in the stress fracture cases at an indivdual level: Mean (g/cm2), mean change (g/cm^2) and precent change.

Stress Fracture Cases	Post Injury (6-8 weeks)	Retraining (6-8 months)	Mean change (g/cm^2)	Percent change (%)
1	0.91	0.938	0.028	3.08 *
2	1.329	1.361	0.032	2.41
3	1.047	1.065	0.018	1.72
4	0.883	0.916	0.033	3.74 *

Least significant change at the femoral neck equaled to 2.44% (calculated as 2.77 x 0.88)
*Significant Clinical meaningful change

8.4 Discussion

In the present prospective study, although there was not significant bone loss following a stress fracture in female athletes, there was significant gain in BMD at the femoral neck 6 to 8 months following stress fracture when training had resumed compared with a non-injured controls.

This is the first prospective study to monitor stress fracture at the initial point of injury. It was revealed in the present study that stress fracture cases showed no significant differences at baseline in physical characteristics (body mass and percent fat), age at menarche, calf circumference, BMD and bone geometric properties when compared with controls, therefore supporting previous chapters (4 and 6). These findings however, conflict with past research which have identified risk factors of stress fracture to include low BMD, age at menarche and lower calf circumference (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000). This may reflect a change in stress fracture risk factors, thus supporting chapter 6. It was found that 50% of stress fracture cases had a history of stress fracture which seems to support earlier findings identifying an increased risk of stress fracture in athletes with a past history (Barrow and Saha 1988; Bennell et al. 1996a; Kelsey et al. 2007).

Bone density at the femoral neck changed only by 0.09% in the injured limb and 0.04% in the contra-lateral limb 6-8 weeks following stress fracture, thus indicating a little bone loss following stress fracture. The management of injury in these stress fracture case athletes was consistent with past research (Bennell and Brukner 2005b), requiring only a short period of 6 to 8 weeks of partial/non-weight bearing activity such as cross training (swimming, Aqua jogging, cross training), with full weight-bearing activity resuming at approximately 8 weeks post injury (Bennell and Brukner 2005a; Brukner et al. 1998; Carmont et al. 2009). The short period of non-weight bearing activity therefore, may have

reduced the risk for bone loss following stress fracture in these athletes. A previous study following Achilles tendon rupture has shown loss of BMD at the femoral neck of 1.7% at 6 weeks post injury, with the greatest loss occurring at 3-4 months (3.1%). Following Achilles rupture athletes would require 6 weeks of immobilization by a cast, with full weight bearing walking resuming between 6 weeks and 3 months (Therbo et al. 2003). These extended periods of non-weight bearing and immobilization could therefore explain the larger percentage of bone loss following a short time period.

It is not possible however, to conclude from this study that there is no potential for bone loss following stress fracture due to the small sample size. Sample size estimates were calculated prior to the initiation of this study. To yield a power of 80%, a sample of 28 people in each the case and control group would have been required to detect bone losses of 3% in magnitude, these percentage changes were based on previous findings of bone loss following musculoskeletal injuries (Therbo et al. 2003). Therefore it may be argued that this study is underpowered to detect small changes of BMD at the femoral neck during the post injury phase of training. In this study with an n=8 (stress fracture cases) the size of percentage change that could be detected at the femoral neck would be +/- 6%. This study detected a bone change at 6-8 weeks following stress fracture of +0.09% in injured limb and -0.04% in the contra-lateral limb. While we can be confident that large changes in BMD at the femoral neck are not present following stress fracture, it cannot be concluded from this study that small changes are occurring.

BMD was gained within 6 to 8 months following a stress fracture with a 2.7% gain at the femoral neck. Femoral neck BMD at 6 to 8 months post injury was above that of the initial baseline measure. Our findings support earlier work by Cattermode el al (Cattermole et al. 1997) who reported BMD to return to baseline measures five months following tibial fracture. The bone gain demonstrated here was more pronounced after 6-8 months

following stress fracture injury than that found in previous prospective studies following lower extremity fractures and musculoskeletal injuries (Kannus et al. 1992; Karlsson et al. 1993; Petersen et al. 1997; Therbo et al. 2003; Veitch et al. 2006), which reported permanent losses in BMD after 13 months of injury. This could indicate therefore, that following a stress fracture injury there is not imbalance between bone resorption and formation, this imbalance which may be present in more severe injuries which require an extended period of immobilization. Due to the small follow-up sample of case athletes in the present study however, the significant findings must be interpreted with caution. One extreme value may influence significance in a small sample and small samples are normally only powered to pick up very large effect sizes as statistically significant. Clinically meaningful change was therefore determined on an individual level in the stress fracture cases to determine the significant response to management. Clinically meaningful change is often used by physicians to determine if a significant response to treatment for osteoporosis has occurred on the individual level (Baim et al. 2005). In the present study, when determining the LSC in the case studies following retraining it was found that in two of the four case athletes the LSC (2.44%) at the femoral neck had been exceeded, allowing the conclusion that there had been a positive response to stress fracture management in these athletes following injury. In athletes with a clinically meaningful change there were no identifiable similarities between the athletes' event, age, or stress fracture location. Due to the crude measure of training it was not possible to determine the similarities between the specific changes in training following injury. Similarly there were no identifiable differences between the athletes who exceeded meaningful change and those who did not.

There were several limitations in this study which will need to be addressed to confirm clear conclusions; the small sample size of stress fracture cases during the study period

resulted in the study being underpowered to detect meaningful bone changes during the post injury phase following stress fracture, further studies are therefore required with larger sample sizes to confirm the present findings. Although the stress fracture cases in this study were matched for age, and menstrual status we were not able to control the point of stress fracture diagnosis and stress fracture location. The baseline assessments on average were taken 2+/-2 weeks following a positive diagnosis of stress fracture; however there was no indication as to the length of time for which pain persisted prior to medical intervention. Therefore some bone loss may have occurred prior to the baseline assessment and been undetected by this study.

Due to time constraints of this study it was not possible to recruit athletes with stress fractures in a similar location. Past research in the recovery of fractures and musculoskeletal injury have identified that bone loss in limbs are determined by the location of the injury, with the greatest bone loss occurring more proximal to the injury site (Petersen et al. 1997; Therbo et al. 2003). The present study therefore, may have reduced the possibility to detect bone loss due to the differing locations of stress fracture. Similarly, the anatomical sites at which BMD was measured using DXA may not have detected the potential bone loss proximal to the stress fracture. Therefore in future studies the location of the stress fracture, sporting event, should be kept constant and the measurement of BMD should include the sites which are proximal to the injury.

The present study, even though under powered is one of the first studies to our knowledge to prospectively monitor changes in BMD and bone geometry following a stress fracture injury, showing bone density and bone geometry is potentially unaffected by short periods of detraining and highlighting that athletes entering back into retaining after 6-8 weeks are not doing so at a point of bone weakness. Bone gains following stress fracture are evident above the initial baseline value within 6 to 8 months following the injury. This would seem

to indicate that the current management guidelines (Brukner et al. 1998; Carmont et al. 2009) for stress fracture are adequate and promote bone healing.   Reoccurrence of stress fracture injury therefore, is not a result of lower BMD during the phase of retraining following stress fracture and therefore seem likely to be a result of the factors which predisposed to the initial injury: potentially other risk factors such as menstrual dysfunction, compulsive exercise or restrictive eating patterns which have been identified previously (chapter 4) (Bennell et al. 1999; Nattiv 2000).

 Although one should be cautious when interpreting these results due to the small sample size and need for further research, it can be concluded from this initial prospective monitoring study that following a stress fracture injury athletes do not seem to lose significant bone density during the post injury phase when partial/non-weight bearing activity is required. Subsequent bone gain above baseline values does seem to occur in the injured leg 6-8 months following the stress fracture which is above the initial baseline measure once training has resumed.

Chapter Nine:
Conclusion

Chapter nine summarises the results of the current research, and provides suggestions for the future directions of bone health research in female endurance athletes.

Conclusion

9.1 Concluding remarks

Prior to this research it was well established that stress fractures were the most common over use injury in athletes with incidence rates and risk factors often greater in female endurance athletes (Bennell et al. 1999). However the previous studies of female endurance athletes to determine incidence and risk factors for stress fracture were limited with small sample sizes (Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000). Exercise increases BMD due to the high impact loads applied to the bone, with athletes often having a higher BMD (15%) than sedentary individuals (Drinkwater 1994; Kannus et al. 1994a; Khan et al. 2001; Marcus et al. 1992; Snow 1996; Uusi-Rasi et al. 1998). However, in amenorrhoeic athletes the benefits of exercise may be counteracted by oestrogen deficiency (Bass 2003; Saxon and Turner 2006). It was unknown whether bone geometry was affected by oestrogen deficiency in the same way as BMD. Finally there was no previous evidence to quantify the seasonal bone changes over a 12-month training phase or the magnitude of bone loss following stress fracture in female endurance athletes. Therefore, this research aimed to address the limitations of previous studies by determining the predictors of bone health and stress fracture risk in female endurance athletes. Specific objectives were fivefold: (1) to determine the correlates of stress fracture history, (2) to compare bone geometry and bone density in female endurance athletes and sedentary controls according to menstrual function, (3) to determine the incidence rate of stress fracture, (4) to quantify the seasonal variation in parameters of bone health, and (5) to determine magnitude and timescale of bone loss and subsequent regain following a stress fracture.

9.2   Key findings

In terms of stress fracture this research can conclude the following: The reported annual incidence of stress fracture (3.3%) in female endurance athletes based in the United Kingdom is substantially lower than previously reported. Furthermore, the incidence of stress fracture seems to be reduced in amenorrhoeic athletes, possibly due to the increased awareness and management of stress fracture risk factors over the past decade. Exercise cognitions, as well as amenorrhoea, were independent predictors of stress fracture history and may have a role in stress fracture etiology. Eating psychopathology, although identified as a potential risk factor of stress fracture history, was not an independent predictor indicating that restrictive eating is possibly contributing to amenorrhoea in female endurance athletes. There were no indications that current BMD, body composition, and training regimens had any association with stress fracture history. Following stress fracture injury no significant bone loss was observed during the post injury phase, indicating that short periods of detraining will not result in BMD loss in athletes. Subsequent bone regain following retraining does seem to occur which is above the initial baseline measure.

The research has shown the following findings in regards to bone health in female endurance athlete: Athletes (particularly those who were eumenorrhoeic) have substantially higher hip BMD compared to the age matched reference data base (mean (SE) femoral neck Z-score in athletes 0.91(0.12) and in eumenorrhoeic athletes femoral neck Z-score 1.14(0.13), both *p<0.001*), despite having a lower body size.   Section modulus was higher in athletes compared to eumenorrhoeic sedentary controls, whose hip BMD was comparable to the age match reference data (z-score mean (SE) femoral neck: 0.07(0.12)). Femoral neck Z and hence strength in bending was relatively maintained in athletes with menstrual dysfunction despite their lower BMD at this site, indicating possible

structural adaptation. Seasonal bone changes in endurance athletes are smaller than those seen in training and detraining studies (Snow et al. 2001; Winters and Snow 2000). However seasonal bone changes are occurring in both eumenorrhoeic and amenorrhoeic athletes despite continuous annual training. Eumenorrhoeic athletes increased their trochanter BMD over the competitive summer season when training intensity is higher. Conversely, amenorrhoeic athletes lost femoral neck BMD (1.1%) over the winter when the percentage of moderate intensity training was higher and this was not recovered during the summer during periods of higher intensity training. Seasonal losses in BMD that are not recovered may contribute cumulatively to bone loss in amenorrhoeic runners.

9.3   Implications

The implications of the present retrospective and prospective research are twofold: (1) it has modernized the past literature on stress fracture injury in female endurance athletes, and (2) it has developed an initial understanding of the effects of bone geometry, and seasonal bone changes in female endurance athletes.

It is important to highlight that within this United Kingdom sample the incidence rate of stress fracture (3.3%) in female endurance athletes was lower than that reported in athletes (male and females) within the United States and Australia. It seems evident that amenorrhoeic athletes are at a reduced risk of stress fracture than previously identified which could be a result of increased awareness of the female athlete triad within the United Kingdom and better preventive management strategies in athletes identified as at risk of stress fracture in the United Kingdom.

This study has clearly identified that stress fracture history is prevalent in athletes who display eating psychopathology and exercise cognitions, independent of menstrual dysfunction. Thus, it is important when working with female athletes to use robust

measures to determine exercise and eating psychopathology. This measure should be examined independently of menstrual dysfunction to identify potential risk for stress fracture development. Prospective monitoring of female athletes with a stress fracture injury identified that BMD was not significantly different from control athletes, suggesting that low BMD in athletes is not a risk factor for stress fracture as previously identified (Bennell et al. 1999; Bennell et al. 1996a; Kelsey et al. 2007; Nattiv 2000). Furthermore, this research, to our knowledge, was the first to address the magnitude and timescale of bone loss and subsequent bone regain following stress fracture management and retraining. Even though this study was under powered it showed no evidence of substantial loss in BMD and bone geometry after short periods of detraining due to a stress fracture injury, and that retraining can potentially increase BMD above the baseline measurement. These findings therefore would not suggest any modifications to current stress fracture management (Brukner et al. 1998)

Potentially the most novel finding of this research indicates that athletes who have lower BMD due to oestrogen depletion may potentially be protected against future osteoporotic fractures as the strength of the bone is relatively maintained due to the participation in loading sports. A/oligomenorrhoeic athletes had no significant differences in BMD when compared to the age match reference data base at the lumbar spine (mean (SE) Z score - 0.13(0.25), *p=0.611*) and femoral neck (0.40 (0.25), *p=0.138*) or the eumenorrhoeic sedentary controls (mean (SE) lumbar spine Z score 0.23(0.11), *p=0.173*, and femoral neck Z score 0.07 (0.12), *p=0.220*). In the present study a/oligomenorrhoeic athletes seemed to maintain Z even though their BMD was significantly lower than eumenorrhoeic athletes, suggesting that lower BMD may be a result of trabecular bone loss (Bagi et al. 1997; Seeman 2002) with endocortical gain (Saxon and Turner 2006), as bone width was not significantly different between groups. Exercise may be maintaining the cortical

strength of the bone at the femoral neck in the presence of oestrogen deficiency, with lower BMD caused by losses in trabecular bone rather than bone at endocorticol surface (Beck et al. 2006).

Finally prospective monitoring of female endurance athletes has initiated an understanding of seasonal bone changes throughout a 12-month training phase. Importantly, this has highlighted that amenorrhoeic athletes lost 1.1% of bone at the femoral neck despite involvement in impact loading, which is believed to benefit BMD. If this continues this may contribute to cumulative bone loss. Although amenorrhoeic athletes did not have particularly low BMD, continued losses may result in an early onset of osteoporosis and it is unclear whether this would be recovered (Hind 2008; Hind 2010; Keen and Drinkwater 1997). The findings continue to emphasize the importance to address the potential cause of long term amenorrhoea in athletes to prevent increased risk of osteoporotic fracture during the menopausal years. .

9.4 Further research

Even though this study has highlighted a number of novel findings which have strong implications for the future medical management of bone health in female athletes it has initiated further research questions and improvements to reduce study limitations.

The prospective monitoring of stress fractures in female endurance athletes is limited with small sample sizes. This was one of the largest studies to date and the first in the United Kingdom to determine the incidence rate of stress fractures. However with a sample size of 70 endurance athletes this study was still under powered. It is likely that studies which focus on the elite athlete population, like the present research, are always going to be hampered by small sample size due to the small pool of elite athletes to recruit from. When

specific events are identified as the focus on the research this recruiting pool becomes even smaller.

As the incidence was low, it was not possible to examine with great power the risk factors for stress fracture. Therefore further research is needed to determine what risk factors are now influencing the incidence of stress fracture in female endurance athletes within the United Kingdom. Further prospective monitoring of athletes with a stress fracture injury is needed to determine the magnitude of potential bone changes post injury. During the present recruitment phase, the number of stress fracture injuries was low and, as such, caution has been applied in the interpretation of results. With an adequately powered study it would be possible to quantify the bone change post stress fracture injury determining in more detail the effects of stress fracture injury on BMD and bone geometry.

In future studies it may be pertinent to examine bone changes following stress fracture and potential risk factors of stress fracture according to the location of the injury. Past evidence has suggested that bone loss following musculoskeletal injury is often proximal to the injury site (Cattermole et al. 1997; Therbo et al. 2003), therefore bone changes following a tibia stress fracture may potentially be different to that of a femur shaft stress fracture. Furthermore risk factors for stress fracture may differ according to location. Athletes with a tibia stress fracture may present with narrow calf circumference whereas as athletes with a femoral shaft stress fracture may not. This could potentially explain the conflicting findings in the literature pertaining to risk factors. Future studies of stress fracture management and potential risk factors for stress fracture may need to consider site specific stress fracture locations.

Longitudinal studies are required in athletes with long term amenorrhoea to determine bone change and the potential consequences. Furthermore, research should consider if bone geometry can compensate for the decrease in BMD in athlete with long term

amenorrhoea. Throughout this research study it was only possible to estimate bone geometric properties using DXA hip structural analysis software. Even though it has been identified in previous studies (Beck 2003; Bonnick 2007) that DXA can provide an estimate of bone geometry a more accurate measure would be to use CT or a pQCT which gives a 3-dimensional image of the bone structure. Therefore, further research is needed to support the findings that Z is maintained despite lower BMD in amenorrhoeic athletes. Future studies should also include an age matched sedentary control and an accurate measure of oestrogen levels throughout the menstrual cycle.

9.5 Overall conclusion

It can be concluded from this research that stress fracture incidence in female endurance athletes in the United Kingdom is substantially lower than previously reported ((Bennell et al. 1996a; Kelsey et al. 2007; Nattiv et al. 2000). The identified risk factors of stress fracture include compulsive exercise behaviours, eating psychopathology as well as amenorrhoea, but BMD and calf girth were not related to risk in this research. There was no substantial BMD loss following stress fracture and this, combined with limited association of BMD with past fracture, suggests other factors may be involved in reoccurrence of stress fracture injury. Findings would therefore indicate that stress fracture injury is well managed although it may be beneficial to pay more attention to eating psychopathology and exercise cognitions

Athletes had higher BMD than controls despite smaller body size. BMD was lower in amenorrhoeic athletes than in eumenorrhoeic athletes, but was still above or no worse than eumenorrhoeic controls. Maintained section modulus in amenorrhoeic athletes suggests structural adaptations are occurring due to mechanical loading. Cortical bone may be maintained in amenorrhoeic athletes at the expense of trabecular bone which could be resulting in the lower BMD. The long term effect in amenorrhoeic athletes on

osteoporosis risk is unclear. Seasonal bone loss was observed in amenorrhoeic athletes. Although amenorrhoeic athletes did not have particularly low BMD, continued losses over time may increase the risk of early onset osteoporosis. It still remains unclear whether bone loss in amenorrhoeic athletes could be recovered, therefore further research is needed.

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APPENDICES

Appendix 1: Ethical Approval Letters

NHS
National Research Ethics Service

Trent Research Ethics Committee
Derwent Shared Services
Laurie House
Colyear Street
Derby
DE1 1LJ

Telephone: 01332 868 905
Amendment/SSI queries 01332 868 842
Facsimile: 01332 868 930

18 April 2008

Miss Rachel Louise Duckham
PhD Research Student
Loughborough University
Human Sciences Department
Brockington Extension
Loughborough University
LE11 3TU

Dear Miss Duckham

Full title of study: **Predictors of bone health and stress fracture in female athletes**

REC reference number: 08/H0405/20

Thank you for your response received on 4 April 2008 , responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). There is no requirement for Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application	V. 5.5	04 February 2008

This Research Ethics Committee is an advisory committee to East Midlands Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the
National Patient Safety Agency and Research Ethics Committees in England

WPH 1370

NHS

National Research Ethics Service

Trent Research Ethics Committee
Derwent Shared Services
Laurie House
Colyear Street
Derby
DE1 1LJ

Telephone: 01332 868 905 Amendment/SSI queries 01332 868 842
Facsimile: 01332 868 930

18 April 2008

Miss Rachel Louise Duckham
PhD Research Student
Loughborough University
Human Science Department
Brockington Extension
Loughborough
LE11 3TU

Dear Miss Duckham

Full title of study:	**Assessment of any bone changes following stress fractures**
REC reference number:	**08/H0405/21**

Thank you for your response received on 4 April 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). There is no requirement for Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application	V. 5.5	04 February 2008
Investigator CV	CI	05 February 2008

This Research Ethics Committee is an advisory committee to East Midlands Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the
National Patient Safety Agency and Research Ethics Committees in England

WPH 1370

Appendix 2: Health Questionnaire to determine exclusion

<u>HEALTH SCREEN QUESTIONNAIRE FOR STUDY VOLUNTEERS</u>

Name/Number   ....

- As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.
- If you have a blood-borne virus, or think that you may have one, please do not take part in this research [*include for projects involving invasive procedures*].

Please complete this brief questionnaire to confirm your fitness to participate:

1. **At present,** do you have any health problem for which you are:

   (a)   on medication, prescribed or otherwise ....   Yes ☐   No ☐

   (b)   attending your general practitioner............................   Yes ☐   No ☐

   (c)   on a hospital waiting list ....   Yes ☐   No ☐

2. **In the past two years,** have you had any illness which required you to:

   (a)   consult your GP...   Yes ☐   No ☐

   (b)   attend a hospital outpatient department....................   Yes ☐   No ☐

   (c)   be admitted to hospital ....   Yes ☐   No ☐

3. **Have you ever** had any of the following:

   (a)   Convulsions/epilepsy ....   Yes ☐   No ☐

   (b)   Asthma ....   Yes ☐   No ☐

   (c)   Eczema ....   Yes ☐   No ☐

   (d)   Diabetes ....   Yes ☐   No ☐

   (e)   A blood disorder ....   Yes ☐   No ☐

   (f)   Head injury ....   Yes ☐   No ☐

   (g)   Digestive problems ....   Yes ☐   No ☐

   (h)   Heart problems ....   Yes ☐   No ☐

(i) Problems with bones or joints .... Yes ☐ No ☐

(j) Disturbance of balance/coordination .... Yes ☐ No ☐

(k) Numbness in hands or feet .... Yes ☐ No ☐

(l) Disturbance of vision .... Yes ☐ No ☐

(m) Ear / hearing problems .... Yes ☐ No ☐

(n) Thyroid problems .... Yes ☐ No ☐

(o) Kidney or liver problems .... Yes ☐ No ☐

(p) Allergy to nuts .... Yes ☐ No ☐

(q) Medical investigations/procedures involving high radiation doses (e.g. radiotherapy) .... Yes ☐ No ☐

4. **Has any,** otherwise healthy, member of your family under the

age of 35 died suddenly during or soon after exercise? .... Yes ☐ No ☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)

....

....

5. **Are you taking any of the following medications that may affect bone?**

(a) Alendronic Acid .... Yes ☐ No ☐

(b) Cyclical Etidronate.. Yes ☐ No ☐

(c) Ibandronate .... Yes ☐ No ☐

(d) Risedronate .... Yes ☐ No ☐

(e) Zoledronic Acid.. Yes ☐ No ☐

(f) Raloxifene .... Yes ☐ No ☐

(g) Strontium Ranelate.. Yes ☐ No ☐

(h) Parathyroid Hormone .... Yes ☐ No ☐

(i) Calcitonin.. Yes ☐ No ☐

(j) Calcitriol... Yes ☐ No ☐

(k) Other .... Yes ☐ No ☐

6. Additional questions for female participants

(a) Are your period's normal/regular? .... Yes ☐ No ☐

(b) Are you on "the pill"? .... Yes ☐ No ☐

(c) Could you be pregnant? .... Yes ☐ No ☐

(d) Are you taking hormone replacement therapy Yes ☐ No ☐
(HRT)?

Demographic Information

Name:

Address:

Tel:

Email:

Appendix 3: Recruitment Letters

Appendix one displays one of the recruitment letter used to recruit athletes into the current study.

Human Science Department
Loughborough University
Loughborough
LE11 – 3TU

To Whom It May Concern:

Research on stress fractures

We are recruiting participants to take part in a major research study examining the predictors of bone health and stress fracture in female athletes. As stress fractures are common in events such as running and triathlon this is an area of great importance to a number of athletes and coaches, and we hope that the study will provide information to aid in the future prediction and prevention of stress fractures.

Who are the investigators?
Dr. Katherine Brooke-Wavell, Lecturer, Dept Human Sciences, Loughborough University
Rachel Duckham, PhD research student, Dept Human Sciences, Loughborough University
Dr. Nick Peirce, MD English Institute of Sport and QMC, Nottingham
Dr. Greg Summers, Consultant rheumatologist, Derbyshire Royal Infirmary

Who is needed?
We are recruiting female endurance athletes that are at a high standard within England and surrounding areas. All participants must be aged 18 or over and not pregnant.

What is required?
Athletes will be asked to visit the laboratory a maximum of three times for approximately one hour. During these sessions the athletes will fill out a questionnaire that will give information on menstrual cycle, training, and stress fracture history. A DXA scan will determine bone mineral density, bone mineral content, bone geometry and body composition. A small sample of venous blood will be collected to determine bone formation and resorption. Some isometric muscle strength tests will be carried out. From this visit each participant will receive a report giving details of their body composition (including fat and fat-free mass), bone health, and muscle strength.
After the laboratory visit athletes will be asked to keep a monthly training, menses and stress fracture incidence diary and wear a small device (accelerometer) for a week during training to provide an objective measure related to impact forces.

Who should I contact if I am interested and want more information?
Dr. Katherine Brooke-Wavell: email: K.S.F.Brook-wavell@lboro.ac.uk Tel 01509 222749
Rachel Duckham: email: R.L.Duckham@lboro.ac.uk, Tel: 01509 228159
Dr Nick Peirce: email: Nick.Peirce@eis2win.co.uk
We hope this information will be of interest to you and hope to hear from you very soon
.
Regards

Rachel Duckham

Version 1 Recruitment Letter Feb 2008

Appendix 4: Posters

Appendix two displays one of the poster used to recruit athletes into the current study/

Loughborough University
Department of Human Sciences
in collaboration with the English Institute of Sport

Stress fracture
of the tibia
(shin bone)

Runners and Triathletes
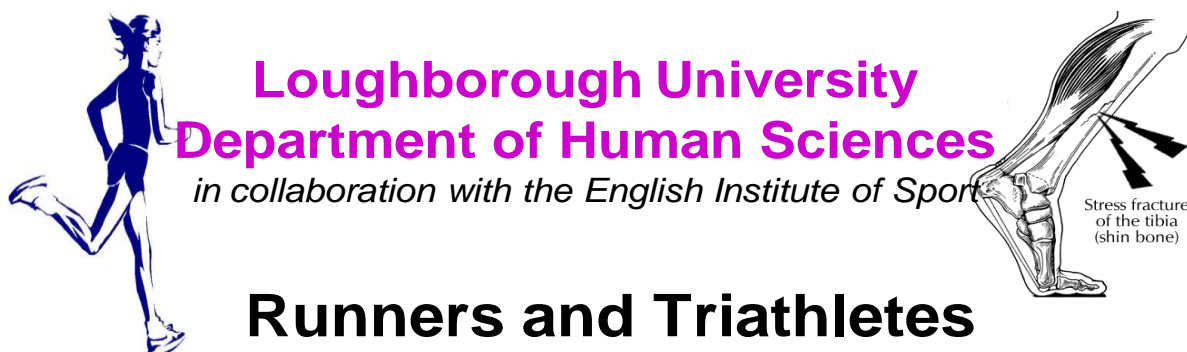
There is a 20% incidence of stress fracture each training year. Will it be your next injury?

We are conducting a study to determine the predictors of stress fractures and bone health in female athletes.

PARTICIPANTS NEEDED!
•*Are you a female runner or triathlete?*
•*Are you 18 years of age or over?*
•*Do you want FREE information on your:*
– *Body Composition*
– *Bone Health*
•*Are you willing to give up a couple of hours to help our understanding of stress fractures and bone health?*

If so this will be a study you will not want to miss.

For more information without commitment please contact:
Rachel Duckham
Tel: 01509 228159
Email: R.L.Duckham@lboro.ac.uk

ENGLISH
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University

Version 1 Poster Feb 2008

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Appendix 5: Press release for recruitment

Female endurance athletes needed for bone health study

New research by Loughborough University and the English Institute of Sport is hoping to discover why elite athletes are more susceptible to stress fractures.

Although athletes are extremely fit they are more likely to sustain injuries, with stress fractures being amongst the most common. These types of fractures typically occur in weight-bearing bones, such as the tibia/fibula (bones of the lower leg) and metatarsals (bones of the foot). As many as one in five athletes in some sports can be affected by such injuries each year.

The study, being led by Dr Katherine Brooke-Wavell and Rachel Duckham from the University's Department of Human Sciences and Dr Nick Peirce from the English Institute of Sport, East Midlands, is focussing on female athletes. They are looking for female endurance runners and triathletes of a high athletic standard aged between 18 and 45 to take part in the research. Volunteers would have to attend three one hour visits to the University over a 12 month period, where they would fill out questionnaires and undergo bone scans and muscle function tests.

Speaking about the research Rachel Duckham said: "We see this research as a means to help fully understand the factors which cause stress fractures in athletes, and to do this we need the help of the country's top class and developing endurance athletes.

"We would not ask athletes to make any changes to their training or lifestyle; in fact we want them to continue as normal. In return for taking part, we will provide athletes with information on their bone health and body composition, and can analyse blood haemoglobin levels on request."
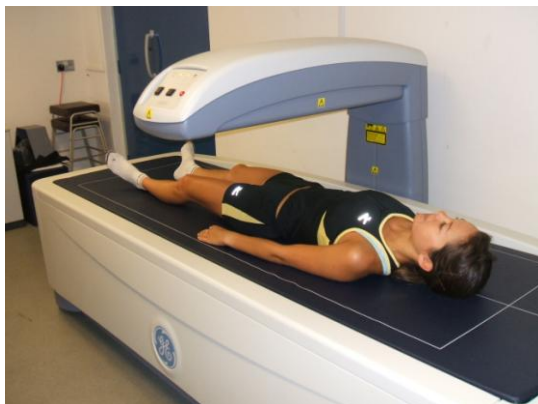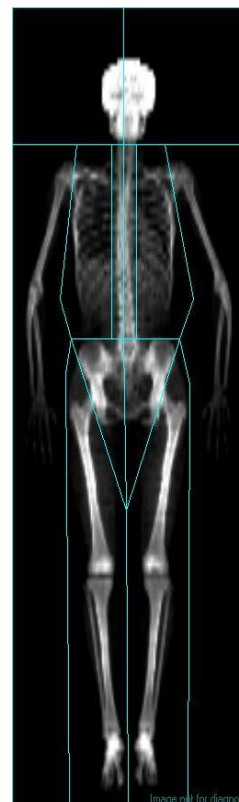
Anyone interested in taking part in the study should contact

Rachel Duckham, by emailing R.L.Duckham@lboro.ac.uk, or calling 01509 228159. Alternatively you can email Dr Katherine Brooke-Wavell at K.S.F.Brooke-wavell@lboro.ac.uk

For all media enquiries contact:
Judy Wing, Senior Public Relations Officer, Loughborough University, T: 01509 228697, E: J.L.Wing@lboro.ac.uk

Appendix 6: EPIC Nutritional questionnaire

Appendix 3 illustrates an example page of the EPIC nutritional questionnaire Bingham (Welsh 2005). Permission from EPIC (University of Cambridge) was sort prior to the use of this question. This questionnaire was given to athletes at each assessment. Please request full questionnaire.

1. **YOUR DIET LAST YEAR**
    For each food there is an amount shown, either a "medium serving" or a common household unit such as a slice or teaspoon. Please put a tick (✓) in the box to indicate how often, **on average**, you have eaten the specified amount of each food **during the past year**.

    EXAMPLES:

    For white bread the amount is one slice, so if you ate 4 or 5 slices a day, you should put a tick in the column headed "4-5 per day".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls								✓	

For chips, the amount is a "medium serving", so if you had a helping of chips twice a week you should put a tick in the column headed "2-4 per week".

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
POTATOES, RICE AND PASTA (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Chips				✓					

For very seasonal fruits such as strawberries and raspberries you should estimate your average use when the fruits are in season, so if you ate strawberries or raspberries about once a week when they were in season you should put a tick in the column headed "once a week"

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
FRUIT (1 fruit or medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Strawberries, raspberries, kiwi fruit			✓						

Please estimate your average food use as best you can, and please answer every question – do not leave ANY lines blank. PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
MEAT AND FISH (medium serving)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Beef: roast, steak, mince, stew or casserole									
Beefburgers									
Pork: roast, chops, stew or slices									
Lamb: roast, chops or stew									
Chicken or other poultry eg. turkey									
Bacon									
Ham									
Corned beef, Spam, luncheon meats									
Sausages									
Savoury pies, eg. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls									
Liver, liver paté, liver sausage									
Fried fish in batter, as in fish and chips									
Fish fingers, fish cakes									
Other white fish, fresh or frozen, eg. cod, haddock, plaice, sole, halibut									
Oily fish, fresh or canned, eg. mackerel, kippers, tuna, salmon, sardines, herring									
Shellfish, eg. crab, prawns, mussels									
Fish roe, taramasalata									
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

Appendix 7: Four-day Diet Diary

Appendix 5 illustrates the first day of the four day diet analysis diary given to athletes at each assessment. Please request full version.

4-Day Diet Analysis Intake Form

Please fill out the following information as accurately as possible. In the analysis please include one weekend day and three week days. This will allow us to gain information that reflects day-to-day variability in food consumption. Please record **_everything_** that you eat and drink over the four days

Date: _____   **Day of the Week:** _____

Before Breakfast			
Food/Drink	**Description and Preparation**	**Amount Eaten**	**Added Salt/sugar/ fat**
Breakfast			
Food/Drink	**Description and Preparation**	**Amount Eaten**	**Added Salt/sugar/ fat**

Mid Morning - Between breakfast and lunch time			
Food/Drink	**Description and Preparation**	**Amount Eaten**	**Added Salt/sugar/ fat**

Lunch			
Food/Drink	**Description and Preparation**	**Amount Eaten**	**Added Salt/sugar/ fat**

Mid Afternoon - between lunch and dinner			
Food/Drink	**Description and Preparation**	**Amount Eaten**	**Added Salt/sugar/ fat**

Dinner			
Food/Drink	**Description and Preparation**	**Amount Eaten**	**Added Salt/sugar/ fat**

Late Evening Snack -Between dinner and bedtime.			
Food/Drink	Description and Preparation	Amount Eaten	Added Salt/sugar/ fat

Appendix 8: Eating psychopathology and Exercise cognition questionnaires

Appendix 6 illustrates the first page of 1) the Eating Disorder Examination questionnaire-version 5, and 2) the Compulsive Exercise Test (CET).

1) EDE-Q version 5 – for full version please request

EDE-Q: Instructions
The following questions are concerned with the **PAST FOUR WEEKS ONLY (28 days)**. Please read each question carefully and circle the appropriate number on the right. Please answer all the questions.

ON HOW MANY DAYS OUT OF THE PAST 28 DAYS …….		No days	1-5 days	6-12 days	13-15 days	16-22 days	23-27 days	Every day
1.	Have you been deliberately trying to limit the amount of food you eat to influence your shape or weight?	0	1	2	3	4	5	6
2.	Have you gone for long periods of time (8 hours or more) without eating anything in order to influence your shape or weight?	0	1	2	3	4	5	6
3.	Have you tried to avoid eating any foods which you like in order to influence your shape or weight?	0	1	2	3	4	5	6
4.	Have you tried to follow definite rules regarding your eating in order to influence your shape or weight; for example, a calorie limit, a set amount of food, or rules about what or when you should eat?	0	1	2	3	4	5	6
5.	Have you wanted your stomach to be empty?	0	1	2	3	4	5	6
6.	Has thinking about food or its calorie content made it much more difficult to concentrate on things you are interested in; for example, read, watch TV, or follow a conversation?	0	1	2	3	4	5	6
7.	Have you been afraid of losing control over eating?	0	1	2	3	4	5	6
8.	Have you had episodes of binge eating?	0	1	2	3	4	5	6
9.	Have you eaten in secret? (Do not count binges.)	0	1	2	3	4	5	6
10.	Have you definitely wanted your stomach to be flat?	0	1	2	3	4	5	6
11.	Has thinking about shape or weight made it more difficult to concentrate on things you are interested in; for example read, watch TV or follow a conversation?	0	1	2	3	4	5	6
12.	Have you had a definite fear that you might gain weight or become fat?	0	1	2	3	4	5	6
13.	Have you felt fat?	0	1	2	3	4	5	6
14.	Have you had a strong desire to lose weight?	0	1	2	3	4	5	6

2) CET – For full version please request

Instructions: Listed below are a series of statements regarding exercise.
Please read each statement carefully and circle the number that best indicates how true each statement is of you.
Please answer <u>all</u> the questions as honestly as you can.

	Never true 0	Rarely true 1	Sometimes true 2	Often true 3	Usually true 4	Always true 5

1) I feel happier and/or more positive after I exercise.	0	1	2	3	4	5
2) I exercise to improve my appearance.	0	1	2	3	4	5
3) I like my days to be organised and structured of which exercise is just one part.	0	1	2	3	4	5
4) I feel less anxious after I exercise.	0	1	2	3	4	5
5) I find exercise a chore.	0	1	2	3	4	5
6) If I feel I have eaten too much, I will do more exercise.	0	1	2	3	4	5
7) My weekly pattern of exercise is repetitive.	0	1	2	3	4	5
8) I do not exercise to be slim.	0	1	2	3	4	5
9) If I cannot exercise I feel low or depressed.	0	1	2	3	4	5
10) I feel extremely guilty if I miss an exercise session.	0	1	2	3	4	5
11) I usually continue to exercise despite injury or illness, unless I am very ill or too injured.	0	1	2	3	4	5
12) I enjoy exercising.	0	1	2	3	4	5
13) I exercise to burn calories and lose weight.	0	1	2	3	4	5
14) I feel less stressed and/or tense after I exercise.	0	1	2	3	4	5
15) If I miss an exercise session, I will try and make up for it when I next exercise.	0	1	2	3	4	5
16) If I cannot exercise I feel agitated and/or irritable.	0	1	2	3	4	5
17) Exercise improves my mood.	0	1	2	3	4	5
18) If I cannot exercise, I worry that I will gain weight.	0	1	2	3	4	5
19) I follow a set routine for my exercise sessions e.g. walk or run the same route, particular exercises, same amount of time, and so on.	0	1	2	3	4	5
20) If I cannot exercise I feel angry and/or frustrated.	0	1	2	3	4	5
21) I do not enjoy exercising.	0	1	2	3	4	5
22) I feel like I've let myself down if I miss an exercise session.	0	1	2	3	4	5
23) If I cannot exercise I feel anxious.	0	1	2	3	4	5
24) I feel less depressed or low after I exercise.	0	1	2	3	4	5

Appendix 9: History questionnaire

Appendix 7 illustrates the history questionnaire given to the athletes at baseline and 12 month assessments.

Researcher: _____   Date: _____
Questionnaire Number: _____   Subject Number: _____

Questionnaire

The aim of this questionnaire is for the investigator to collect history of your menstrual cycle, past stress fractures, training, training surface, and past and current shoe wear. We ask that you fill this questionnaire with as much accuracy as possible. If you have any questions please do not hesitate to ask the investigator.

Demographic Information

Date of birth:

Age:

Ethnicity:

Weight History

1 What is your ideal weight?   Kg

2 Have you ever been at your ideal weight?   Yes   No

3 Last time you were at your ideal weight?   Years   Months

4 Do you control your weight during a competitive season   Yes   No

5 Do you control your weight in the off season?   Yes   No

Musculoskeletal/Health History

1 Is there a history of osteoporosis in your family?   Yes   No

2 Have you ever been diagnosed with or treated for any of the following?

 Low bone density   Anorexia Nervosa   Bulimia

3 Have you ever suffered a stress fracture as a result of training or competition?

          Yes   No

 If YES how many stress fractures have you had in your lifetime? _____

Nutritional History

1 How many meals do you eat in a day (ie breakfast, lunch, dinner)

          1   2   3   4   5   6

2 How many times a day do you snack (i.e. candy, sports bars, fruit)

          1   2   3   4   5   6

3 Do you skip meals?   Frequently   Occasionally   Never

4 Are you Vegetarian?   Yes   No

 If Yes: What kind of Vegetarian are you?   _____

          Version 2 Questionnaire Mar 2008

5 Do you restrict the amount of food you eat to control your weight   Yes   No

6 Do you restrict the types of food you eat?   Yes   No

7 Do you think your diet is nutritionally adequate?   Yes   No

8 Have other people indicated that you have an abnormal or unusal eating behaviour?

   Yes   No

9 Do you take Vitamin supplements?

   Daily   Sometimes   Never

   Please indicate the brand and type of vitamin you take.   _____

10 Do you take sports supplements?

   Daily   Sometimes   Never

   Please indicate which product you use: _____

History of Menstrual Cycle

1 How old were you when you had your first menstrual period?

            Years   _____   Months   _____

2 How frequent is your menstrual period currently? *(times per year)*

      0–3   3-6   6-10   10-13

3 What date did your last menstrual period start?

      Day   _____   Month   _____   Year   _____

4 What is the typical length of your monthly bleed in days?

   1-3 days   4-5 days   5-7 days   > 7 days

5 Have you ever had a period of time when you had no mentraul bleed for more than 3 months?

            Yes   No

6 IF YES: How many months did the absence of menstrual bleed last?

   < 6months   6- 12 months   > 12 months

7 Are you using Oral Contraceptives?

            Yes   No

   If yes: How long have you been using oral contraception?

   _____

   _____

   If yes for what reason are you using oral contraceptive?

   A.Regulate absent periods   B. Regulate menstrual flow   C. Other

   If yes what is the name, brand name and dose of the oral contraceptive pill you are taking?

   _____

   _____

Injury History

1 Have you ever had a stress fracture?

Yes   No

If Yes please complete the table below

For each stress fracture	1	2	3	4	5	6
Age when stress fracture occurred						
What time of year (IE month) the stress fracture occurred						
Location of the stress fracture						
Diagnosis (MRI, Bone scan, x-ray or other)						

Training History

1 What is the primary sport you participate?

Running [   ]   Triathlon [   ]

2 Years of Participation in the Sport club level and up?

Years [   ]   Month [   ]

3 Please complete the following chart below giving the last 12 months of training phases.

Training Season (Dates)	Event training for	Frequency (sessions per week)	Hours per week	Intensity % at L/M/H	Training Surface
Winter (Dec-Mar)	*5 km x country*	*10*	*20*	*L:25, M:50, H: 25*	*Grass*

Version 2 Questionnaire Mar 2008

4 What are your personal best times?

_____
_____
_____

5 What are your seasons best times?

_____
_____
_____

Appendix 10: Abstract

Duckham R, Peirce N, Meyer C, Brooke-Wavell K. Stress fracture history, eating and exercise behaviours and menstrual dysfunction. BASEM Annual Congress 2009,(In press) (Oral presentation)

STRESS FRACTURE HISTORY, EATING AND EXERCISE BEHAVIOURS AND MENSTRUAL DYSFUNCTION IN FEMALE ENDURANCE ATHLETES

R. Duckham[1], N. Peirce[2], C. Meyer[1], K. Brooke-Wavell[1]

[1].Loughborough University, Loughborough, Uk [2.] Nottingham University Hospitals Trust and English Institute of Sport, UK

This study aimed to determine whether eating psychopathology, exercise behaviours and cognitions, menstrual function and bone density differ according to stress fracture history in female endurance athletes.

Participants were female competitive (elite-county level) endurance athletes (runners and triathletes). Seventy athletes (58 runners and 12 triathletes) aged 26.0 ± 7.4 years completed self reported measures to ascertain stress fracture history, menstrual history and athletic training. Attitudes towards eating and exercise were assessed using the eating disorders examination questionnaire (EDE-Q) and the compulsive exercise test (CET). BMD, content and hip geometric parameters were assessed using dual x-ray absorptiometry (DXA). Variables were compared between athletes with and without a history of stress fracture using chi-squared, ANOVA and Mann Whitney U tests.

Nineteen (27%) athletes had been clinically diagnosed with one or more stress fractures on average 4.2 ± 5.6 years ago. The most common stress fracture sites were the metatarsals (46%) and the tibia/fibula (38%). Athletes with a history of stress fracture had a significantly higher prevalence of current (47% vs 27%, $p=0.008$) and past (79% vs 53%, $p=0.035$) menstrual dysfunction and higher global scores on the EDE-Q ($p=0.049$) and the CET ($p=0.016$). Bone parameters by DXA, training duration, age, age at menarche and anthropometric measurements did not differ between groups.

Athletes with a history of stress fracture reported higher levels of disordered eating and compulsive exercise as well as having higher prevalence of menstrual dysfunction. Longitudinal research is required to determine the impact of eating psychopathology and compulsive exercise stress fracture risk

Appendix 11: Abstract

BONE GEOMETRY AND BONE DENSITY IN ATHLETES AND SEDENTARY CONTROLS ACCORDING TO MENSTRUAL FUNCTION.

Rachel L.Duckham, Christine A. Bailey and Katherine Brooke-Wavell

Loughborough University, UK

Introduction: Athletes have higher bone mineral density (BMD) compared with non-athletes. In amenorrhoeic athletes BMD may be compromised by oestrogen deficiency but it is unknown whether this is accompanied by structural differences. **Purpose:** To compare bone geometry and density of a/oligomenorrhoeic athletes (AA), eumenorrhoeic (EA) athletes and eumenorrhoeic controls (EC). **Methods:** 158 women (70 endurance athletes and 88 controls) were recruited. BMD was measured at the femoral neck and lumbar spine (L1-L4) using dual x-ray absorptiometry (DXA), and femoral neck section modulus (Z) was estimated. Menstrual function was assessed by questionnaire and classified as eumenorrhoeic ($\geq$ 10 periods/year) or a/oligomenorrhoic ($\leq$ 9 periods/year). Bone variables were compared between groups with ANOVA and analysis repeated with age, height and weight included as covariates. **Results:** 30 athletes were AA and 40 EA. EC were significantly older, heavier and shorter than EA and AA who did not differ significantly. Femoral neck BMD was significantly higher in EA than AA and EC [mean (SE) 1.117 (0.015); 1.036 (0.020) and 0.999 (0.014) g cm^{-2} respectively; $p<0.001$]. Z was significantly higher in EA than EC, [EA: 657 (20), AA: 639 (20), and EC: 592 (10) cm^3 $p=$ 0.004] although AA did not differ significantly from EA and EC. Lumbar spine BMD was significantly lower in AA than EC [EA: 1.141 (0.019), AA: 1.105 (0.026) and EC: 1.188 (0.014) g cm^{-2}, $p=0.007$]. All differences persisted after adjustment for height, age and body mass. **Conclusion:** Eumenorrhoeic athletes had significantly higher femoral neck BMD and Z than controls, consistent with previous research. Femoral neck Z and hence strength in bending was relatively maintained in athletes with menstrual dysfunction despite their lower BMD at this site, indicating possible structural adaptation.

Appendix 12: Abstract

INCIDENCE OF STRESS FRACTURE IN FEMALE ENDURANCE ATHLETES IS HIGHER IN ATHLETES WITH NO IDENTIFIABLE RISK FACTORS.

Duckham R.L [1], Peirce N [2], Summers G [3], Cameron N [1], Brooke-Wavell K [1].
[1] Loughborough University [2]. Nottingham University Hospitals [3]. Derby Hospitals

It is well established that stress fracture injuries are the most common over use injury in athletes. There are only two studies which have prospectively monitored stress fractures in female athletes reporting annual incidence rates between 8.7% and 21.1%. Small sample sizes (37 and 49 female track and field athletes respectively), stress fracture definition of diagnosis, inconsistent methods of reporting incidence rates and athlete geographic location could explain the substantially different annual incidence rates. The primary purpose of this study was to determine prospectively the incidence of stress fracture in female endurance athletes based in the United Kingdom. A secondary objective was to determine whether previously identified risk factors were present in stress fracture cases. Following ethical approval, 61 female endurance athletes aged between 18-45 years were monitored for a 12-month period. At baseline, athletes completed questionnaires to assess history of stress fracture, menstrual history, training history, eating psychopathology and compulsive exercise. Bone mineral density (BMD) of total body, spine, hip and radius was assessed using dual x-ray absorptiomertry (DXA). Stress fracture and training were monitored prospectively. Among the 61 athletes, two sustained a stress fracture diagnosed by MRI, giving an annual incidence rate of 3.9%. The stress fracture cases were 800m runners aged 19 and 22 years, training on average 14.2 hours a week, eumenorrhoeic with no history of amenorrhoea. BMD, energy intake and scores of eating psychopathology and compulsive exercise similar to mean values in the non-stress fracture group. Those athletes who did sustain stress fracture would not have been identified based on previously recognised risk factors. It can be concluded the incidence of stress fracture in this sample of female endurance athletes is lower than previously reported, possibly due to increased awareness of stress fracture diagnosis, risk factors and athlete management.

Appendix 13: Abstract

SEASONAL BONE CHANGES IN EUMENORRHOEIC AND AMENORRHOEIC ENDURANCE RUNNERS

Duckham R.L [1], Peirce N [2], Summers G [3], Cameron N [1], Brooke-Wavell K [1].

[1]. Loughborough University, [2] Nottingham University Hospitals, [3] Derby Hospitals

Introduction: Athletes display seasonal gains and losses in BMD associated with changes in training. In amenorrhoeic athletes, it is possible that any seasonal losses may not be recovered thus contributing to their lower BMD. **Purpose:** To quantify seasonal variations of bone parameters in eumenorrhoeic (EA) and amenorrhoeic (AA) endurance runners. **Methods:** Following ethical approval, 52 female endurance runners aged 18-45 years were measured at the beginning of the summer (April to June) and winter (October to December) seasons over 12-months. BMD was measured at the femoral neck, trochanter, and lumbar spine (L1-L4) using dual x-ray absorptiometry (DXA), and femoral neck section modulus (Z), and minimal neck width were estimated. Menstrual function was assessed by questionnaire and classified as EA ($\geq$10 periods/year) or AA (<9 periods/year). Training was prospectively monitored. Seasonal variation was detected with a repeated measure ANOVA (between-subject factor of menstrual function) and Bonferroni post hoc comparisons. **Results:** 28 athletes were EA and 24 AA. There were no significant differences at baseline or seasonal variation in height, weight, and body fat percentage. In EA, trochanter BMD increased over the summer (mean (SE) 0.885(0.019) to 0.947(0.177) g/cm^2, p=0.002) with no significant change over the winter (0.880 (0.018) to 0.885 (0.018), g/cm^2 p=0.153). In AA, femoral neck BMD decreased in over the winter (1.065(0.021) to 1.052(0.020) g/cm^2, p=0.030) with no significant change over the summer (1.050 (0.020) to 1.052 (0.020), p=0.770). Minimal femoral neck width increased in the group as a whole over the winter (28.4(0.3) to 28.7(0.3) mm, p=0.039) with no significant change over the summer 28.8(0.3) to 28.7 (0.3), mm p=0.333). There were no significant seasonal variations in other bone parameters, season x group interactions or annual changes. **Conclusion:** EA increased trochanter BMD over the summer, and this was maintained over the winter. Conversely, AA lost femoral neck BMD over the winter and this was not recovered during the summer, although the increase in width of the femoral neck may partly compensate BMD loss to maintain strength in bending. Seasonal losses in BMD that are not recovered may contribute cumulatively to bone loss in amenorrhoeic runners.