Energy Balance and Bone Mineral Density in Male and Female Distance Runners

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

K. Hind. Energy Balance and Bone Mineral Density (BMD) in Male and Female Distance Runners. Degree of Doctor of Philosophy, submitted in December, 2004.

Introduction Reports of lumbar spine (LS) skeletal deficits in female athletes with menstrual disorders are common, although it is not clear whether the deficits are confined to this group. The main factor presumed to be responsible is oestrogen deficiency characterised by amenorrhoea, however emerging evidence indicates that energy deficiency can also disturb bone turnover. This thesis aimed to determine whether male distance runners are at a comparable risk for bone loss and whether there was a relationship between reported energy balance and BMD. **Methods** 109 distance runners (18-50 years) participated (65 females, 44 males). A questionnaire assessed menstrual status, performance level and training characteristics. 7-day dietary and exercise records were used to quantify energy balance. LS, dual femur (DF) and total body (TB) BMD were measured using dual-energy X-ray absorptiometry. Bone size was accounted for: bone mineral apparent density (BMAD) =BMD / $\sqrt{}$Bone area. **Results** Male and female LS T-scores were similar (-0.8, -0.8). 41.6% of female and 36.4% of male runners were osteopenic (LS). Age, BMI and body fat- adjusted LS T-scores were lower in male than female runners ($p<0.05$). Adjusted LS T-scores were lower in male compared to eumenorrhoeic runners ($p<0.01$). Female runners who used the oral contraceptive pill had similar BMD to amen/oligo-menorrhoeic runners, which were significantly lower than eumenorrhoeic runners ($p<0.01$). These runners were also more energy deficient ($P<0.01$). Elite runners had greater energy deficits, lower LS T-scores, BMAD and a smaller bone area than club runners ($p<0.001$). DF and TB T-scores were normal, did not correlate with weekly mileage and after adjustment for calcium intake, did not correlate with energy balance. LS T-score negatively correlated with stress fracture incidence ($p<0.0001$). Most runners were energy deficient and the severity of energy deficiency correlated with lower BMI and body fat. Energy balance was the strongest predictor of LS BMD and T-scores. **Conclusions** Low LS BMD in distance runners is not gender-specific; the oral contraceptive pill may be insufficient to protect against bone loss in female runners and elite runners may be more at risk of bone fragility. Energy deficit was the underlying determinant of low BMD in male and female runners, emphasising the importance of energy balance for bone health.

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Chapter 1

Background

"As I see it every day you do one of two things:

build health or produce disease in yourself".

(Adelle Davis, America's first health authoritarian, 1904 – 1974)

The lifestyle choices we make greatly contribute to our health and this is particularly true of osteoporosis. Osteoporosis is a silent but serious disease of bone fragility, ultimately leading to fracture, significant mortality and morbidity. The challenge of osteoporosis is that development of the disease goes unnoticed until skeletal damage has progressed to a most serious state. The prevention of osteoporosis is vital and the most effective strategy to do so is maximising and maintaining bone density during growth and young adulthood. Unfortunately, the incidence of osteoporosis is increasing more than would be expected for the aging population, suggesting that lifestyle changes over the past two decades may be contributing to bone loss in younger generations (IOF 2004). Thus, it is of great importance to investigate and understand the factors which predispose individuals to osteoporosis and to identify vulnerable sub-populations.

Competitive athletes at all levels make lifestyle choices in a quest to become fitter and more successful in their chosen sport. Certain athletes may embark on diets and large volumes of exercise training to attain the ideal body mass, become fitter and improve

performance. It is ironic that such lifestyle choices may indeed produce disease in an individual, so much so that athletes may be fit but also fragile. Skeletal fragility is recognised as a major risk for female athletes who train intensively and experience an absence of menstruation, and this is despite their high levels of weight-bearing exercise. T-scores indicative of osteopenia and osteoporosis have been reported in female runners as young as 20 years of age (Rutherford *et al.,* 1993; Pettersson *et al.,* 1999) and there is concern that such bone loss may be irreversible (Drinkwater *et al.,* 1990; Micklesfield *et al.,* 1998). As bone loss is more rapid after the age of 50 years, a T-score of -2.0 in a 20 year old athlete with osteopenia may provide a worse prognosis for long term skeletal health than a T-score of -2.5 in a 65 year old with osteoporosis. Low bone mineral density (BMD) in young adulthood is a major predictor of later fracture risk (Cummings *et al.,* 1993). Osteoporotic fractures result in pain, loss of movement and significant disability, devastating consequences for the competitive athlete.

Determining how to achieve and maintain good bone density, to prevent its loss and to prevent osteoporotic fracture are the aspirations of osteoporosis-related research. Optimising BMD and preventing osteoporosis in athletes, requires a thorough understanding of the multitude of contributory factors. Aside from menstrual dysfunction and low body mass, are there any other factors associated with low bone density in athletes? Female distance runners are particularly at risk of low bone density and distance running is an increasingly popular sport for men and women of all abilities. Therefore, are male athletes at a similar risk of low BMD? This thesis documents an investigation of BMD in male and female distance runners in an attempt to contribute to the knowledge in this pressing area of research and medical

concern. The first chapter provides a background to the biology of bone, relevant to the understanding of the factors that might affect it. The second chapter reviews the literature concerning the bone health of runners, incorporating the rationale and leading to the main aims of the research conducted.

1.1 Bone anatomy and physiology

1.1.1 Bone composition, function and metabolism

The structural and material properties of bone are functional in that they meet the requirements of mechanical competence by providing strength, adaptation and lightness (Khan *et al.,* 2001; Seeman, 2003).

i) Composition and function of bone

Bone consists of a dense outer shell (cortex) and an internal system of interconnected plates and rods known as trabecular bone. Calcium (37-40%), phosphate (50-58%) and carbonate (2-8%) make up the inorganic component of bone known as hydroxyapatite (Heaney, 1999). The organic bone matrix consists primarily (98%) of type 1 collagen and noncollagenous proteins, including osteocalcin, growth factors (IGF-1 produced by the osteoblasts) and cytokines such as the leading cytokine, transforming growth factor beta which is released during bone resorption and enhances osteoblast activity whilst inhibiting the differentiation of osteoclasts (TGFbeta) (Compston, 1997; Gross *et al.,* 2003). Osteoblasts, osteocytes and osteoclasts comprise around 2% of the bone matrix.

Bone serves both a structural and homeostatic function (Heaney, 1999). Its structural function is to provide strength and flexibility, in order to withstand mechanical loading as well as lightness to enable efficient movement and speed (Heaney, 1999; Seeman, 2001). As different skeletal sites require different structures, bone is heterogeneous and differs throughout the skeleton. For example, the long bones of the appendicular skeleton (such as the femur) are predominantly cortical whereas the bones of the axial skeleton (including the spine and pelvis) are predominantly trabecular (Seeman, 2001). Long bones are typified by a thick cortex and hollow tubes that provide stiffness and lightness, whereas the vertebral bodies of the spine, which are mainly trabecular, provide flexibility during compressive loading by absorbing energy and then returning to their original height (Heaney, 1999; Seeman, 2001). Trabecular bone is also more metabolically active than cortical bone and this is likely to be due to its greater surface area which enables the bone marrow, blood vessels and connective tissue to be in contact with the endosteum, resulting in a more rapid response to homeostatic metabolic alterations (Heaney, 1999). The structure of trabecular bone provides flexibility rather than stiffness therefore there is a higher level of porosity, which may partly explain why the predominantly trabecular axial sites of the spine and pelvis are prone to rapid bone loss when exposed to negative stimuli (Heaney, 1999).

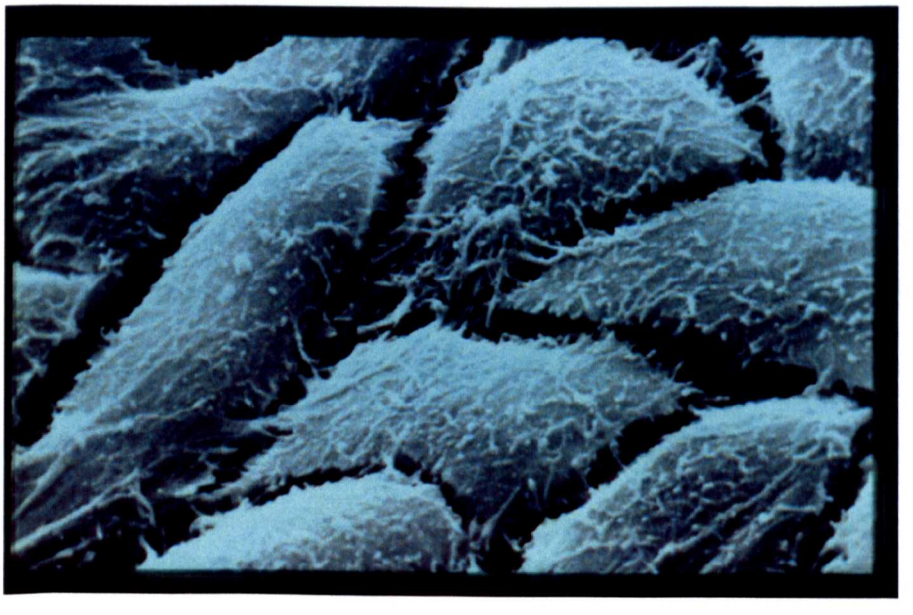
The homeostatic function of bone is to maintain a constant level of both plasma calcium and phosphate (Compston, 1997). With calcium homeostasis, calcium is withdrawn from the skeleton via resorption, when levels of plasma calcium fall (Compston, 1997). Phosphate accounts for nearly 60% of bone mineral and is also necessary for bone metabolism and other life processes, particularly growth, the

metabolism of bone cells, the regulation of 1,25 -dihydroxyvitamin D, energy production and as a buffer (Compston, 1997; Heaney, 1999).

ii) Bone metabolism

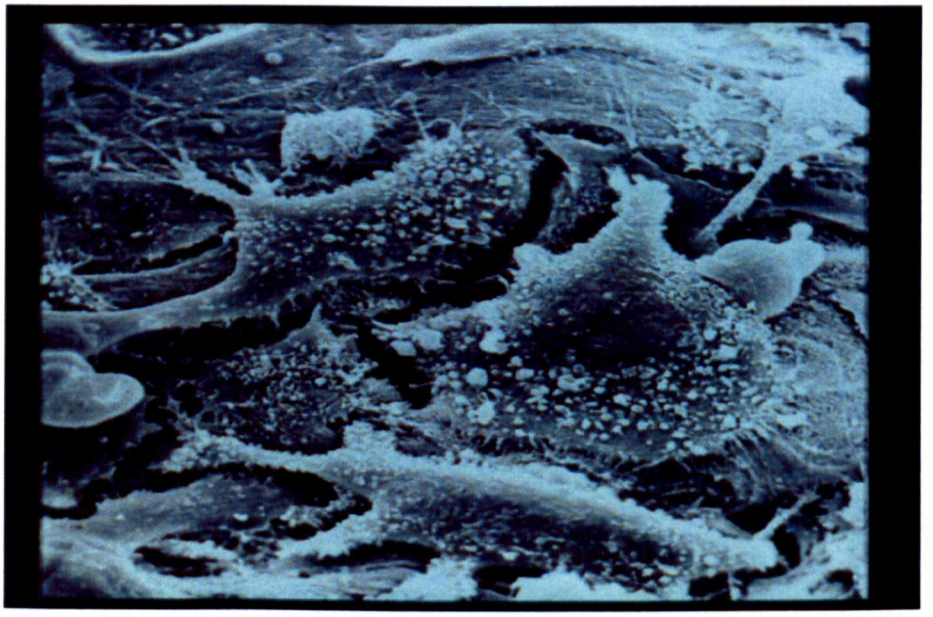
Bone is a living tissue, responding and adapting to changes in its environment by the cellular processes of bone modelling and remodelling (Parfitt, 1984; Compston, 1997). '*Bone modelling*' enables bone growth and adjusts bone strength by increasing mass and expanding the periosteal (outer bone surface) and endocortical (inner bone surface) diameters of bone. It determines the size and structure of bone and can increase bone mass and strength but can rarely reduce them (Frost, 1990; Raisz, 1999). Conversely, bone remodelling maintains or decreases bone density and strength (Frost, 1997). '*Bone remodelling*' defines an organised cellular activity based on the basic multicellular unit (BMU), producing net bone formation or bone resorption.

Figure 1.1: Scanning electron micrograph of a layer of osteoblasts (from Stevenson and Marsh, 1994). *Magnification x 2,500*



Bone cells, notably osteoblasts and osteoclasts, respond to various stimuli and comprise the BMU which coordinates coupling (bone resorption followed by formation) and determines bone turnover (Parfitt, 1984). Osteoblasts are responsible for bone formation and produce the bone matrix whereas osteoclasts are responsible for bone resorption (Parfitt, 1984). Figure 1.1 illustrates a layer of osteoblasts and figure 1.2 is an electron micrograph of osteoclasts.

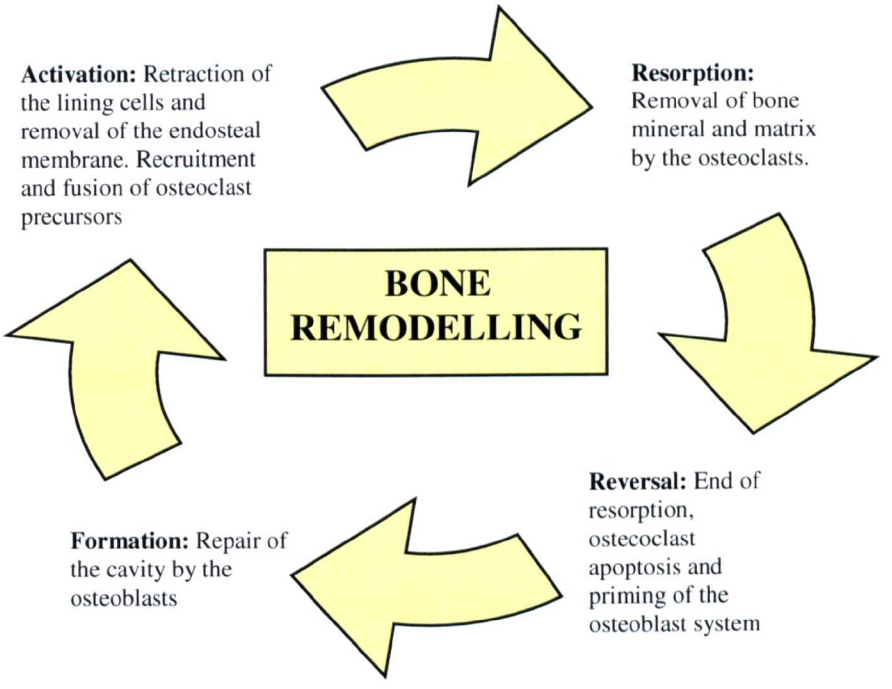
Figure 1.2: Scanning electron micrograph of osteoclasts *in vitro* (from Stevenson and Marsh, 1994). *Magnification x 1,000*



The stages of the bone remodelling cycle are illustrated in figure 1.3. The cycle begins with the attraction of osteoclasts to the target site of microdamage and is characterised by the retraction of the bone lining cells and removal of the thin osteoid (Parfitt, 1984; Compston, 1997; Ralston, 1997). During *resorption*, the osteoclasts remove bone and undergo apoptosis during the *reversal* phase (Parfitt, 1984). Following resorption, matrix components such as osteocalcin, in addition to other components such as

IGF-1, are released and contribute to the recruitment of osteoblast precursors to the bone surface (Raisz, 1999).

Figure 1.3: Bone remodelling cycle



The osteoblast precursors differentiate into mature osteoblasts and form new bone matrix (osteoid), which calcifies to form mature bone. External signals (such as interleukin-1 (Il-1), oestrogen deficiency) to resting osteoblasts cause these cells to release a multitude of cytokines (including Il-1, Il-6), which then enhance the recruitment and differentiation of osteoclasts in order to start the cycle again (Raisz, 1999; Rosen, 2000). In normal circumstances the amount of bone resorbed and formed within each BMU is similar and the entire process takes around 4 months to complete (Parfitt, 1984; Frost, 1997). With this in mind, studies investigating the effects of exposure to certain factors require at least 4 months and may need several remodelling cycles to detect a difference in BMD.

Alterations in activation, resorption, reversal or formation can lead to imbalances in remodelling that can result in bone loss (Raisz, 1999; Rosen, 2000). Two basic cellular mechanisms lead to bone loss:

1) Increased bone turnover, with an increase in the number of remodelling units undergoing resorption on the bone surface in the short term.
2) An imbalance in bone remodelling, where the amount of bone formed in a BMU is less than that resorbed (Compston, 1997).

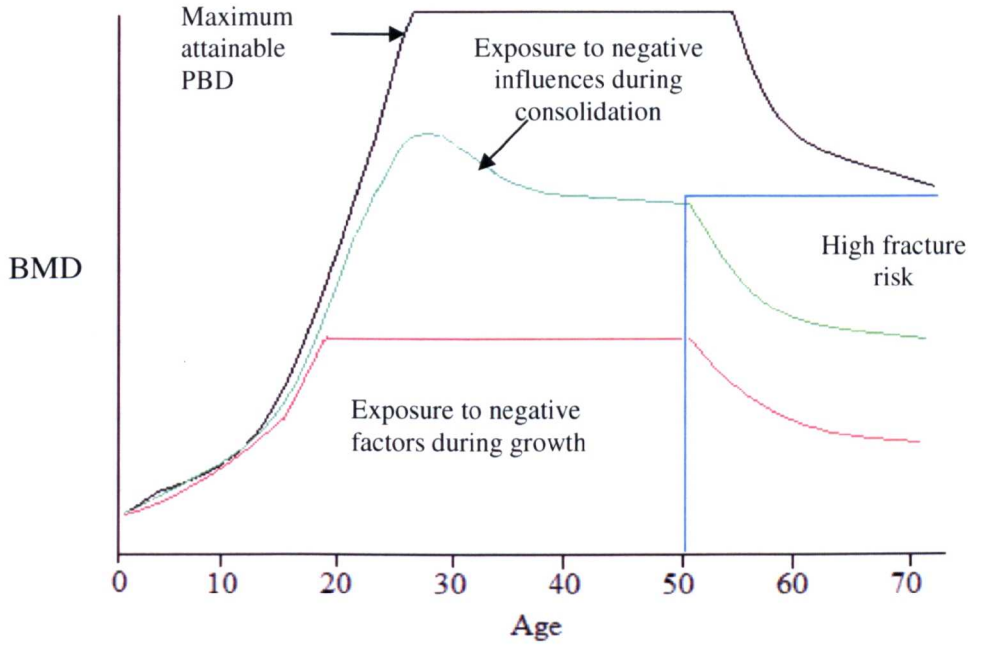
1.1.2 Peak bone density, acquisition and consolidation

i) Peak bone density

Peak bone density (PBD) is defined as the highest amount of BMD achieved during life at a given skeletal site and is based on observations that BMD rapidly increases during puberty, consolidates during young adulthood and declines with age. However, many factors can intervene and affect bone during these life stages such as illness and dietary insufficiencies (Raisz, 1999) Thus, PBD is heterogeneous and varies between individuals depending on heredity and environment. It is possible that the highest bone density ever attained by certain individuals may still be sub-optimum due to prolonged exposure to negative influences. The term *'maximum attainable PBD'* defines the full genetic potential of the individual for bone density without negative interference (of a sufficient duration) from factors such as under-nutrition, calcium deficiency and disease. If low PBD contributes to the reduced BMD observed in fracture patients (Cummings *et al.,* 1993; Marshall *et al.,* 1996), then it would be

hypothesised that the achievement and maintenance of maximum attainable PBD will reduce the risk of later life fragility. This is illustrated in figure 1.4.

Figure 1.4: Schematic representation of the peak bone density concept



ii) Bone acquisition and consolidation

Bone growth and mineral acquisition occurs throughout childhood, adolescence and early adulthood. It is agreed that the most rapid bone acquisition takes place during puberty (Matkovic *et al.,* 1994; Heaney *et al.,* 2000) with the acceleration of trabecular bone accrual corresponding with the secretion of sex steroids (Bailey *et al.*, 1999; Riggs *et al.,* 2002). The sex steroids, particularly oestrogen, are required for normal bone development (Grumbach, 2000). There is also evidence for the importance of IGF-1 in skeletal acquisition (Rosen and Donahue, 1998; Mora *et al.,* 1999).

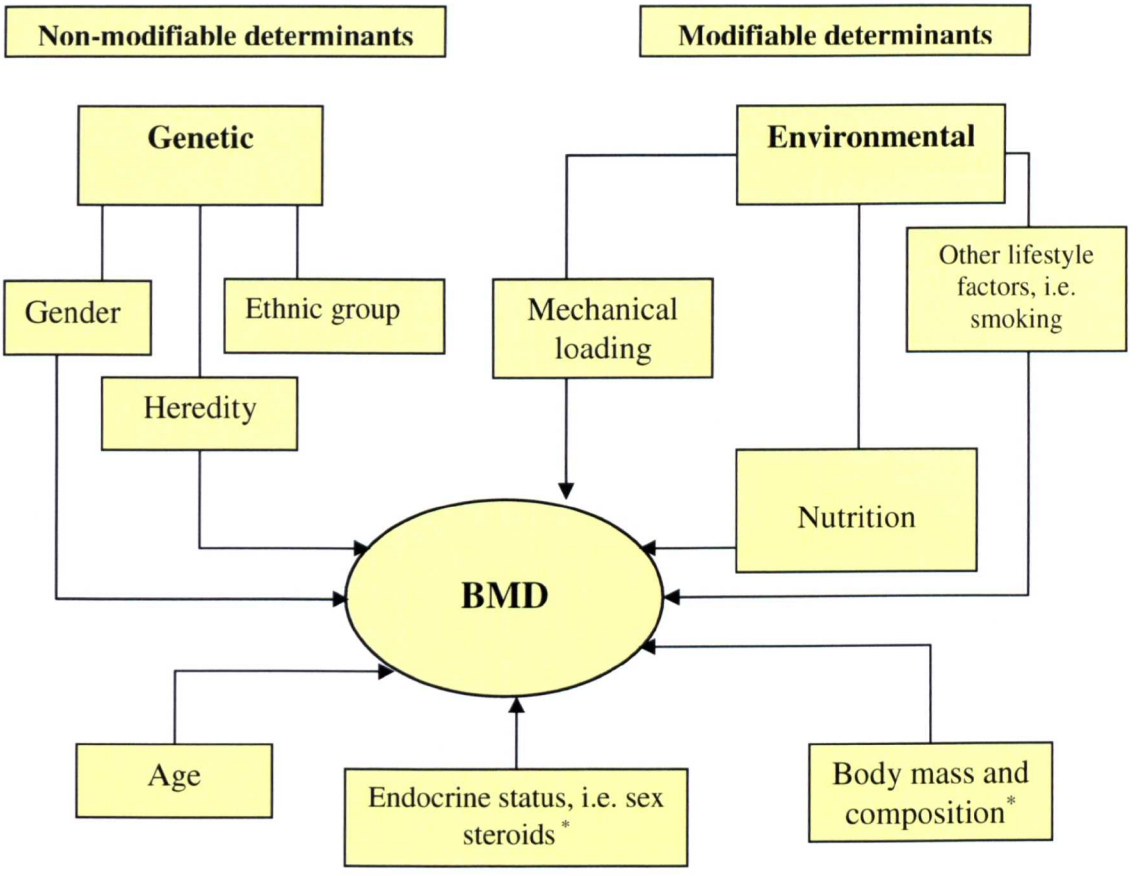
With regard to the nature and tempo of geometrical bone development, recent investigations indicate that peak bone size at the spine and hip are almost fully attained in both sexes by late adolescence (Bradney *et al.,* 2000; Henry *et al.,* 2004). The accumulation of BMD reaches a plateau at a later age and at the spine and the hip this appears to be at around the age of 20 years (Bonjour *et al.,* 1991; Haapasalo *et al.,* 1996; Sabatier *et al.,* 1996; Bainbridge *et al.,* 2002), taking place earlier in women than in men (Bonjour *et al.*, 1991; Rico *et al.*, 1992; Nguyen *et al.*, 2001). Longitudinal work demonstrates that the lumbar spine, hip and total body BMD remain virtually constant between the ages of 20 and 35 years (Slosman *et al.,* 1994). Enhancing BMD, especially before the age of 35, is thus postulated to be the best way to prevent or slow the development of osteoporosis (International Osteoporosis Foundation (IOF), 2004).

1.2 Factors affecting bone mineral density (BMD)

Multiple factors influence bone density throughout the life span and are illustrated in figure 1.5. Maximum attainable PBD is largely dependent on non-modifiable, genetic factors such as gender (Heaney *et al.,* 2000), although environmental factors appear to influence their expression (Kelly *et al.,* 1990).

Figure 1.5: Modifiable and non-modifiable determinants of bone mineral density

* *Environmental determinants that are not necessarily modified by choice*



1.2.1 Non-modifiable determinants

i) Ethnicity

Bone size, quality and BMD vary by ethnic group and this can confound studies if uncontrolled. Variation in BMD by ethnic group becomes prominent during puberty and continues throughout life (*Nelson et al.,* 1997; Gilsanz *et al.*, 1998). Studies consistently report that black ethnic groups have higher BMD than whites, even after

correcting for bone size (Bachrach *et al,* 1999). Using quantitative computed tomography (QCT), Gilsanz *et al* (1998) found that blacks had a greater volumetric trabecular density at the spine than did whites and concluded that increased bone size and density in blacks lower their risk for osteoporotic fracture. Although data regarding the bone mass of Asians and Hispanic populations is limited, it appears that their bone density is similar to or less than the mean for whites (Bachrach *et al.*, 1999). For this reason, any study using a heterogeneous subject group should control for ethnic group variations.

ii) Gender

Gender differences are also apparent during the acquisition and maintenance of BMD (Kelly *et al.,* 1990) and have been postulated to largely reflect differences in bone size (Henry and Eastell, 2000). Men have larger and wider bones than women due to greater periosteal expansion during growth and the attenuation of perisoteal expansion throughout aging (Seeman, 1999; 2001). This perisoteal expansion results in a greater cortical thickness, placing the outer cortical bone mass further from the neutral axis and conferring an increased moment of inertia (Seeman, 2001). In this way men have a biomechanical advantage compared to women, in terms of fracture risk.

One consideration for many studies is that the two-dimensional measurement of bone using dual energy X-ray absorptiometry (DXA), is a function of the size of bone (Seeman, 2001). For example, BMD may be higher in one individual compared to another, due to greater bone size, but in reality their actual bone mineral content within the periosteal envelope may be similar. Reflecting this, studies using DXA to measure BMD consistently report higher values in men compared to women

throughout the life stages (Nguyen *et al.,* 2001) but those measuring volumetric BMD have determined that these discrepancies are due to differences in bone size rather than in actual density (Gilsanz *et al.*, 1994; Zamberlan *et al.*, 1996; Henry and Eastell, 2000). Thus, it has been recommended that studies comparing gender differences in DXA-derived BMD, consider bone size, for failure to normalise BMD for this could lead to misinterpretation of results (Henry and Eastell, 2000). QCT on the other hand, is capable of objectively measuring volumetric BMD. Using this technique, Gilsanz *et al* (1994) found that vertebral cross-sectional area is 25% greater in males than females, but similar volumetric BMD. Naganathan and Sambrook (2003) also found similar lumbar spine volumetric BMD in 82 opposite sex young adult twins, whilst measurements of areal BMD were higher for the male twins. True bone density may not actually differ between men and women and the gender difference in skeletal size is a potential confounder when comparing BMD between males and females.

In response to these findings, several methods have recently been developed to normalise BMD measurements for bone size to allow for an improved representation of true bone density. Some studies have corrected for body size by controlling body mass parameters although this does not correct for bone size. In order to reduce the effect of bone size on BMD estimates of volumetric BMD have recently been developed. This parameter is known as bone mineral apparent density (BMAD) and involves the calculation of estimated volumetric BMD from DXA-derived BMD, BMC and projected bone area values (Henry and Eastell, 2000; Melton *et al.,* 2000; Cvijetic and Korsic, 2004). These three studies used large subject samples of 103, 699 and 541 respectively. All concluded that although men have higher BMD at most sites

compared to women, BMAD is similar, or in some cases, lower in men (Henry and Eastell, 2000).

iii) Age

During childhood, bone density gradually increases until the age of approximately 30 years when skeletal maturity is achieved. In usual circumstances, bone density remains relatively stable throughout young adulthood with bone resorption and bone formation in equilibrium. Thereafter, bone is lost at about 1% per year in men and women due to a thinning of the cortex and demineralisation of bone, although women experience accelerated bone loss for up to 10 years following the menopause due to oestrogen withdrawal (Peel and Eastell, 1995).

iv) Heredity

Heredity exerts a powerful influence on bone density as illustrated in studies of twins (Picard *et al.,* 2001) and a family history of osteoporosis is postulated to be a major risk factor for developing the disease (Ralston, 2003). There are conflicting estimations of the extent of the genetic contribution to BMD. Krall and Dawson-Hughes (1993) studied 40 families and after controlling for lifestyle variables, genetic factors could explain 46-62% of variance in BMD, in men and women. On this assumption, the remaining 38-54% would be attributable to non-hereditable determinants such as lifestyle, body mass and endocrine status. However, it has recently been suggested that genetic factors may contribute to up to 85% (Ralston, 2003). This is in the light of emerging evidence which suggests that several candidate genes may partly underlie the association between environment and adult BMD and that interactions exist between certain genotypes and bone acquisition (Ralston, 2003;

Dennison *et al.*, 2004). Examples of candidate genes include the oestrogen receptor, vitamin D receptor and the growth hormone gene. Thus, it appears to be likely that the extent to which exposure to negative environmental factors affect BMD will in part be determined by the genetic profile of the individual.

1.2.2 Endocrine determinants

Advances in research have uncovered numerous endocrine factors involved in bone metabolism. These can be independent or interacting systemic hormones, cytokines including growth factors that have single or multiple actions on the osteoblasts and osteoclasts (Compston, 1997; Raisz, 1999). Many endocrine disruptions have a negative effect on bone by disturbing normal bone turnover and because interruption at any stage of the remodelling cycle can result in bone loss (Raisz, 1999). Table 1.1 has been compiled according to relatively recent findings and illustrates the effect of several endocrine factors on bone when levels are normal, elevated or reduced.

i) Oestrogen

Oestrogen comprises a group of steroid hormones (oestradiol, oestriol and oestrone) which are produced by the ovaries in women and in small amounts by the male testis and adrenal cortex. It has a necessary role in many life processes, including the development and maintenance of BMD (Raisz, 1999; Rizzoli *et al.*, 2001).

Table 1.1: The effect on bone when selected endocrine levels are normal (↔), increased (↑) or decreased (↓)

	↔	↑	↓
Cytokines			
Interleukin-1 (IL-1)	Induces bone resorption and osteoclastogenesis (Boyce *et al.*, 1989).	↑ resorption, number of OCs and bone turnover (Boyce *et al.*, 1989)	Levels are controlled by oestrogen (Pacifici *et al.*, 1992)
Interleukin-6 (IL-6)	Proliferation of OCs and prepares matrix for formation (Russell *et al.*, 1996)	↑ resorption and hyper-calcemia due to ↑removal of calcium from the matrix (Passeri *et al.*, 1993)	
Growth Factors			
Insulin-like Growth Factor-1 (IGF-1)	Maintenance of bone architecture, proliferation and differentiation of OBs, collagen formator (Chevalley *et al.*, 1998)	↑ bone formation, ↑ anabolic effects of mechanical loading (Gross *et al.*, 2000).	↓ bone formation, turnover and BMD (Grinspoon *et al.*, 1996, 2002).
IGF Binding Protein-3 (IGFBP-3)	Enables circulating IGF-1 to become biologically available (Schwarz *et al.*, 1996).	↑ osteogenesis (Nussey and Whitehead, 2001)	↓ synthesis of IGF-1 (Nussey and Whitehead, 2001)
Hormones			
Oestrogen	Maintains lifespan of OB and down-regulates osteoclastogenesis. Inhibits IL-1 and IL-6 (Girasole *et al.*, 1992; Pacifici *et al.*, 1996). Synergistic effects with calcium and mechanical loading (Heaney *et al.*, 1989; Cheng *et al.*, 1996).		↑lifespan of OC, ↑ resorption and turnover (Mangolagas, 2000). ↓ calcium absorption (Heaney *et al.*, 1989).
Testosterone	Maintain normal bone metabolism when converted to oestradiol	↑ calcium absorption, ↓ osteoclastogenesis and bone turnover (Pacifici *et al.*, 1996).	↓formation (Leder *et al.*, 2003)
Leptin	Facilitates OB differentiation and proliferation (Thomas *et al.*, 1999). Limits osteo-clastogenesis (Cornish *et al.*, 2002). Leptin and OBs derive from the same stem cell (Thomas *et al.*, 1999)	↑osteoblastic activity, facilitates bone growth, determines bone size and cortical thickness. Administration to mice leads to ↑ in BMC, BMD and bone size (Steppan *et al.*, 2000). Administration in humans leads to ↑ in bone formation (Welt *et al.*, 2004).	↓formation (Takeda and Karsenty, 2001)

OB = osteoblasts
OC = osteoclasts
BMC= bone mineral content

Evidence since the 1970's has established that an oestrogen deficiency leads to bone loss in postmenopausal women and also in women with primary ovarian failure (Horsman *et al.,* 1977; Worley, 1981; Turner *et al.,* 1994). A large body of this evidence comprises reports of reduced BMD and altered bone metabolism in postmenopausal women and the improvement or maintenance of BMD with oestrogen treatment (Horsman *et al.,* 1977; Sultana *et al.*, 2002). Oestrogen deficiency can also occur in young women and have a negative effect on their BMD. Secondary hypothalamic amenorrhoea occurs in young women who exercise excessively and /or eat too little, and is an acquired gonadal-releasing hormone (GnRH) deficiency, leading to ovarian suppression and a deficiency of the sex steroids. Secondary amenorrhoea has been frequently linked to osteopenia (Marcus *et al.*, 1985; Rencken *et al.,* 1996) and is present when a female with previously normal menstrual cycles has less than three menstrual cycles per year (Burrows and Bird, 2000).

Bone loss from oestrogen deficiency tends to occur mainly in trabecular bone, and is characterised by accentuated bone turnover with an increase in bone resorption that over-rides the rate of bone formation (Pacifici et al., 1996; Slemenda et al., 1997). Although the exact mechanism whereby oestrogen deficiency causes an increase in bone turnover and resorption is not yet clear, research indicates that it is likely to be mediated via the oestrogen receptors on osteoblasts (Srivastava *et al.,* 2001). The mechanism appears to be characterised by increased osteoclastogenesis (Srivastava *et al,* 2001) due to elevated secretion of local bone resorbing cytokines interleukin 1 (Il-

1), interleukin 6 (Il-6) and tumor necrosis factor TNF; (Girasole *et al,* 1992; Cenci *et al,* 2000).

The efficiency of intestinal absorption of calcium is reduced with oestrogen deficiency and a proportion of calcium that should otherwise be deposited in bone may be lost (Heaney *et al,* 1989; Schutz and Morris, 1999). Reflecting this, high levels of urinary calcium have been reported in postmenopausal women and related to their bone loss (Slemenda *et al,* 1997). There is also evidence to suggest that oestrogen interacts with mechanical strain to exert a synergistic, anabolic influence on bone (Cheng *et al,* 1996; Balasch *et al,* 2003). Thus, without oestrogen the positive effects of exercise on bone may be limited.

ii) Testosterone

Testosterone is an androgenic and anabolic hormone which occurs naturally in men and women, but in men it is the main sex hormone. Adequate levels of testosterone may be necessary for the intestinal absorption of calcium (Heaney *et al,* 1989) and for the stimulation of bone formation (Kasperk *et al,* 1989; Leder *et al,* 2003). There is also evidence to indicate that the bioavailability of oestradiol (the most potent source of oestrogen) may be a determinant of BMD in older men (Szule *et al,* 2001; Leder *et al.,* 2003).

iii) Insulin-like growth factor 1 (IGF-1)

IGF-1 is a growth-promoting polypeptide that is synthesised and released by the liver and tissues, such as the muscles (Rosen and Donahue, 1998; Chicharro *et al.,* 2001). IGF-1 is synthesised by growth hormone (GH) but also mediates the anabolic actions

of growth hormone on various tissues including bone (Yakar and Rosen, 2003). The link between IGF-1 and bone metabolism has been established by *in vitro* and *in vivo* studies. IGF-1 has been shown to have potent anabolic effects on bone by promoting the replication of osteoblasts and increasing the synthesis of type 1 bone collagen and matrix components (Chevalley *et al.,* 1998). As with oestrogen, the ability of mechanical loading to induce a positive response from bone may be enhanced with IGF-1 (Yeh *et al.,* 1994; Bravenboer *et al.,* 2001). In an animal study, mice exposed to IGF-1 have shown an increase in bone formation, twice that of controls (Zhae *et al.*, 2000) and in IGF-1 deficient mice, BMD was 25-40% lower than that of control mice (Mohan *et al.,* 2003).

Observational and experimental work conducted in humans has made significant contributions to the understanding of the role of IGF-1. In humans, serum IGF-1 has emerged as a nutritional marker of energy availability (Clemmons *et al.,* 1985) and deficiencies of the peptide correlate with reductions in bone formation markers in settings of energy deficiency (Grinspoon *et al.,* 1996; 2002). Reduced levels of serum IGF-1 have most frequently been observed in women with anorexia (Grinspoon *et al.,* 1996; Hotta *et al.,* 2000; Gordon *et al.,* 2002; Heer *et al.,* 2004; Miller *et al.*, 2004). An observational study of 19 anorexic adolescent girls, found that serum concentrations of IGF-1 were less than 50% of control levels and that IGF-1 could explain the majority of variation in bone formation (Soyka *et al.*, 1999); $r^2 = 0.72$, $p<0.005$. A recent prospective investigation found that 11 weeks of nutritional therapy in 19 girls with anorexia, led to a three-fold improvement in IGF-1 and a two-fold improvement in bone formation markers, which coincided with a marked improvement in BMI from 14.2 to 17.1 kg m^{-2} (all $p<0.0001$) regardless of menstrual

function (Heer *et al.,* 2002). IGF-1 has also been investigated as a potential treatment strategy against bone loss in women with anorexia. For example, the administration of recombinant human IGF-1 has stimulated a dose-related increase in markers of bone formation over just 6 days (Grinspoon *et al.,* 1996) and 9 months of IGF-1 treatment has led to significant increases in spinal BMD (Grinspoon *et al.,* 2002). In contrast, oral oestrogen treatments have not led to bone status improvements in amenorrheic women with anorexia (Klibanski *et al.,* 1995; Golden *et al.,* 2002; Grinspoon *et al.,* 2002; Munoz *et al.,* 2002). Thus, the current literature indicates that IGF-1 has an anabolic role in bone and that its synthesis and availability is related to nutrition.

iv) Leptin

Leptin is an adipocyte-derived hormone that also reflects adequacy of energy stores (Welt *et al.,* 2004) and there is a consensus that total body fat is a major determinant of leptin secretion (Leal-Cerro *et al.,* 1998; Thong *et al.,* 2000). Low levels of body fat have been associated with reduced BMD (Khosla *et al.,* 1996; Pettersson *et al.,* 1999; Burrows *et al.,* 2003). Until recent years, the candidate responsible for the link between fat mass and bone mass was unknown. However, emerging evidence indicates that leptin is a physiological mediator of bone metabolism (Thomas *et al.,* 1999; Cornish *et al.,* 2002).

The first study to identify a direct link between leptin and bone formation *in vitro,* was conducted by Thomas *et al* (1999) who assessed the effects of human recombinant leptin on a human marrow stromal cell line. Leptin resulted in a dose-dependent increase in osteocalcin and a 59% increase in mineralised matrix. Subsequent work has shown that similar to IGF-1, leptin increases osteoblast

proliferation and inhibits osteoclastogenesis (Cornish *et al.*, 2002). Research conducted in mice has demonstrated increases in bone size, BMC and BMD following leptin administration (Steppan *et al.*, 2000).

In humans, reductions in leptin are related to energy deficiency and have been observed in patients with anorexia nervosa (Weinbrenner *et al.*, 2003; Heer *et al.*, 2004; Miller *et al.*, 2004) and female athletes with a low body mass (Laughlin and Yen, 1997; Warren *et al.*, 1999). Low BMD is commonly reported in both groups therefore it is possible that low levels of leptin may be contributing to their bone loss, arising from a chronic negative energy balance and depletion of fat stores. A recent study investigated the effect of 3 months administration of recombinant human leptin in women runners with hypothalamic amenorrhoea and low body mass (Welt *et al.*, 2004). Leptin led to significant increases in IGF-1, IGFBP-3, bone alkaline phosphatase and osteocalcin, but not urinary N-telopeptide (marker of bone resorption). In summary, novel evidence indicates that leptin, as a marker of energy stores, has an active role in bone formation activity.

1.2.3 Mechanical loading and weight-bearing exercise

In the context of bone health, 'mechanical loading' may be defined as the application of force to bone which is sufficient to induce an anabolic skeletal response. Mechanical loading throughout life is a major determinant of BMD (Frost, 1997; Khan *et al.*, 2001). In the absence of loading, bone loses its function of support and responds by demineralising (Lanyon, 1984); for example, prolonged immobility or

space-flight rapidly leads to increased bone resorption and bone loss (Kannus *et al.*, 1996; Goodship *et al.*, 1998). On the other hand, bone exposed to regular, diverse impact loading adapts its structure to maintain mechanical competence (Lanyon, 1984; Frost, 1997). As weight-bearing exercise generates impact loading, it is recommended as a lifelong strategy to prevent the development of osteoporosis in later life (Genant *et al.*, 1999; Rutherford, 1999; Kai *et al.*, 2003).

'Weight-bearing exercise' is defined as a structured force-generating activity that provides stimulus loading to skeletal regions, above that provided by activities of daily living (Khan *et al.*, 2001). The association between regular weight-bearing activity and increased BMD is most effectively illustrated by the large number of cross-sectional studies which have shown that athletes in weight-bearing sports have higher bone mass at loaded sites compared to non-athletes, in females (Risser *et al.*, 1990; Alekel *et al.*, 1995; Heinonen *et al.*, 1995; Dook *et al.*, 1997) and males (Block *et al.*, 1989; Andreoli et al., 2001). More robust and causal inferences between weight-bearing exercise and BMD have been made in several randomised controlled trials of resistance and running training programmes, although these have mainly been conducted in women (Davee *et al.*, 1990; Snow-Harter *et al.*, 1992; Bassey and Ramsdale, 1994[1]; Heinonen *et al.*, 1996). The anabolic skeletal response to weight-bearing exercise appears to be 'site-specific' for example, numerous within-subject studies have reported that volleyball, squash and tennis players have significantly greater BMD in their dominant playing arm compared to their non-dominant arm (Haapasalo *et al.*, 1994; Kontulainen *et al.*, 2002).

[1] Using a cross-over study design

Cross-sectional, longitudinal and retrospective studies demonstrate that running is associated with greater BMD at the lower limbs (Etherington *et al.*, 1996; Bennell *et al.*, 1997; Dook *et al.*, 1997). Runners have been found with higher BMD at lower skeletal sites compared to cyclists (Stewart and Hannon, 2000) and non-active controls (MacDougall *et al.*, 1992), but they have frequently been found to have reduced spine BMD (Gremion *et al.*, 2001; Burrows *et al.*, 2003). Forces to the lower limbs during running are around 3 times body weight (Dook *et al.*, 1997) whereas those at the lumbar spine are approximately 1.75 times body weight (Cappozo, 1983). Thus, the spine receives less loading stimulus. Conversely, athletes from sports such as kayaking and gymnastics, have superior spine BMD compared to controls (Flodgren et al., 1999). In addition to site-specificity, several principles underlie the optimal loading environment for bone.

1.2.3.1 Principles of mechanical loading

Adaptation of bone to mechanical loading is dependent on the bone strain generated. Bone strain is deformation of bone in response to an applied load and may be defined as the ratio of the change in length of the bone to the original length of the bone. Research has established that the optimal adaptation of bone to mechanical loading will occur with generated bone strains that are *(i) of a sufficiently high magnitude, (ii) unusual distribution and (iii) high rate.* It is also apparent that only a few cycles of the appropriate loading are required to generate an anabolic effect in bone.

(i) Strain magnitude

In his early review of animal studies, Lanyon concluded that highly repetitive, lower peak loads so do not exert as great an osteogenic response as high strain loads (Lanyon, 1984). In agreement Frost (1987; 1990; 1997) claims that there is a minimum force required to elicit an osteogenic response and that forces above this threshold, induce remodelling at skeletal sites resulting in a net bone gain. This is known as the 'mechanostat theory' and in order to switch on the mechanostat, strains must be above the physiological loading zone (Frost, 1987; 1990; 1997). The magnitude of strain required is dependent on the initial status of bone prior to the dose of loading, for example bone can become accustomed to constant loading of a similar magnitude and will not increase its strength until a higher magnitude load is applied (Frost, 1987; 1990; 1997).

Strains in the 'physiological loading zone' maintain bone strength and strains in the 'overload zone' result in increased bone strength (Forwood and Turner, 1995; Frost, 1997). Thus, larger loading magnitudes elicit a greater bone response than lower magnitudes (Lanyon, 1984; 1996; Jiang *et al.,* 1999). Frost developed this theory to explain why weight-lifters have greater bone density than marathon runners (Frost, 1997). Weight-lifters continually increase their work load as they improve and it is this increase in magnitude which results in superior BMD at those sites receiving the strain. These strains usually occur throughout the body and the resultant greater muscle mass will also continue to maintain bone strength (Frost, 1997). Long distance running, on the other hand, generates highly repetitive strain, but because this strain is of a relatively low and consistent magnitude, bone density does not continue to increase, but reaches a plateau. Frost hypothesised that distance running would

increase BMD in the lower extremity bones, but not at the upper extremities (Frost, 1997) and it is likely that due to the consistent magnitude, this density remains relatively constant.

In agreement with this theory, results from numerous human studies favour strength and high impact training, such as weight-lifting and gymnastics, over lower impact sports such as distance running and non-impact sports such as swimming (Heinrich *et al.,* 1990; Kelly *et al.,* 1995; Creighton *et al.,* 2001). Swimming is non-weight-bearing as the body is supported by water and the skeleton receives no impact loading. Reflecting this, swimmers tend to have significantly lower BMD than basketball and volleyball players (Lee *et al.,* 1995; Creighton *et al.,* 2001).

(ii) Unusual strain distribution

Unusual loading of uneven distribution generates a more positive bone response than continuous, repetitive loading (Lanyon, 1996). In this way, gymnastics is particularly effective as it demonstrates novel strain distributions and high magnitude. On the other hand, sports such as running generate homogeneous strains that are localised to the same skeletal regions over again. Reflecting this, research has shown that gymnasts have higher BMD than runners and controls, despite menstrual disorders and inadequate calcium intakes (Kirchner *et al.,* 1995; Snow *et al.,* 2000).

(iii) Strain rate, duration and frequency

High strain rates are more effective than low strain rates in stimulating bone formation (Chilibeck *et al.,* 1995; Lanyon, 1996; Judex and Zernicke, 2000). Research conducted using animal models supports this concept. An intervention study in

roosters compared the mechanical milieux produced by drop-jumping compared to walking and running (Judex and Zernicke, 2000). Drop-jumps produced large peak strain rates (+740%) and resulted in significantly increased bone formation at periosteal (+40%), but mainly endocortical bone surfaces (+370%). Treadmill running had no effect on bone and it was concluded that high strain rates are required to enhance bone formation. Thus, it would be expected that in athletics, sprinting would produce a more favourable effect on bone compared to distance running. In support of this, Bennell *et al* (1997) reported that male and female sprinters gained significantly more BMD at multiple sites over a period of 12 months, compared to distance runners and non-active controls.

It has also been postulated that shorter, multiple exercise sessions as more osteogenic than long, uninterrupted exercise sessions (Umemura *et al.,* 1997; Robling *et al.,* 2002). In rats, 5 jumps per day resulted in the same level of bone formation that was observed following 20, 50 and 100 jumps (Umemura *et al.,* 1997). Furthermore, the insertion of recovery periods in between loading sessions enhances bone adaptation (Robling *et al.,* 2001; Srinivasan *et al.,* 2002). Recovery periods are recommended to restore the mechano-sensitivity of the bone cells, which has been shown to decline after only a few loading cycles (Robling *et al.,* 2001).

Concerning frequency, bone appears to respond more to alternate-day loading rather than consecutive daily loading (Raab-Cullen *et al.,* 1994). A study of national-league footballers found that bone benefits are achieved with 6 hours of activity per week and that additional exercise confers no higher BMD (Karlsson *et al.,* 2001). A similar threshold has recently been identified in 462 non-elite, healthy young adult men and

women (Bakker *et al.,* 2003). This 10-year longitudinal study analysed two aspects of exercise participation; the mechanical component (sum of ground reaction forces) and the metabolic component (weighted energy cost) in relation to lumbar spine and total body BMD (Bakker *et al.,* 2003). The authors concluded that there may be a metabolic 'threshold' at which extra exercise becomes deleterious to BMD. In summary, bone functionally adapts to its loading environment. The optimal adaptation of bone to mechanical loading will occur with dynamic strains of unusual distribution, high rate and in particular, high magnitude.

1.2.4 Body mass and composition

(i) Body mass

The World Health Organisation (WHO) and the recent National Institute for Clinical Excellence (NICE) guidelines recognise that a body mass index (BMI) less than 19.0 kg m^{-2} is an independent risk factor for osteoporosis (Genant *et al.,* 1999; NICE, 2004). In support, numerous studies have reported significant positive correlations between body mass, BMI and BMD. The Framington Health Study (Felson *et al.,* 1993) conducted over 4 years, found body mass explained 20% of the total variance in lumbar spine, femoral neck and radius BMD in men and women. Similarly, the Northern Ireland Young Hearts Project (Neville *et al.,* 2002) clarified a significant positive relationship between body mass and BMD at all skeletal sites in men and women aged 20-25 years.

The positive effect of body mass on bone may reflect the influence of mechanical loading. As the load applied must be of a sufficient magnitude to induce an osteogenic response in bone (Frost, 1987; Lanyon, 1996), a higher body mass is more likely to result in greater skeletal loading compared to a lower body mass. Alternatively or additionally, the attainment of low body mass may be the result of dietary energy restriction which may independently lead to certain endocrine and bone metabolic alterations (Thong *et al.,* 2000).

(ii) Lean body mass

It has been proposed that lean body mass (LBM) contributes to strain magnitude and muscular contractions contribute to the strains that maintain bone structure (Marcus, 2001; Ferretti *et al.,* 2002; Reid, 2002). In 40 eumenorrhoeic female runners and weight-lifters, LBM was the best predictor of lumbar spine, femoral neck and radius BMC with LBM and BMC greater in the weight-lifters (Heinrich *et al.,*1990). Supporting this theory, both lean body mass and muscle strength are positively associated with bone density (Snow-Harter *et al.,* 1992; Ilich-Ernst *et al.,* 2002). The relationship between muscle strength and BMD has been demonstrated in men (Snow-Harter *et al.,* 1992) and in women (Snow-Harter *et al.,* 1990; Khosla *et al.,* 1996; Emslander *et al.,* 1998). In a cross sectional study of female runners and weight-lifters, lean body mass was the best predictor of lumbar spine BMC (Heinrich et al., 1990). A larger study of 225 men and 241 women, found that lean body mass was the most robust predictor of BMD at 9 measured sites over a 10-year period (Bakker *et al.,* 2003). Thus, in contributing to the strain generated through their insertion in bone, higher muscle mass would be expected to reflect greater BMD.

(iii) Body fat

Numerous studies demonstrate positive associations between body fat and BMD (Pettersson *et al.,* 1999; Burrows *et al.*, 2003). As with lean body mass, fat mass contributes to total skeletal load, therefore is likely to add to the loading magnitude on bone. Additionally, several hormones link fat to bone tissue and activity (Reid, 2002). Body fat is associated with the secretion of oestrogen, leptin and IGF-1 (Warren, 1999; Bollag *et al.,* 2001; Ong *et al.,* 2002; Reid, 2002), thus low body fat may infer a reduction in these bone active hormones and as a consequence, an alteration in bone remodelling.

1.2.5 Nutrition

Nutrition has a pivotal role in bone health and it is well known that calcium and vitamin D are mandatory for optimal bone mass. This has been identified in the WHO Task Force nutritional strategy for the prevention of osteoporosis, which recommends,

> *"a diet that maintains normal body weight throughout life and provides a calcium intake of some 1000mg/day from late-childhood to midlife-at least in the developed countries;* (and) *...promotion of vitamin D supplementation and/or regular time spent outdoors"*

(Genant et al., 1999; p262).

In the UK the Nutrition Forum led by Dr. Francis (Newcastle) and consisting of expert panel members meet regularly to produce guidelines, comment on proposed

EU legislation and provide advice. The majority of work appears to focus on calcium and vitamin D, vitamin K, protein and more recently, vitamin A. While these nutrients and micro-nutrients are essential to bone health, as yet, little emphasis has been given to the role of energy balance, despite established associations between nutritional markers and bone turnover in men and women.

i) Calcium and vitamin D

It is known that calcium is the dominant mineral in bone apatite with 98% of total body calcium existing within bone (Heaney, 1999). As the bone stores of calcium are called upon when levels of plasma calcium fall, a constant supply of calcium is recommended to maintain calcium homeostasis and prevent the loss of bone mineral (Weaver, 2000).

Numerous studies support the positive role for calcium intake on BMD (Recker *et al.,* 1992; Welton *et al.,* 1995). In a large meta-analysis of cross-sectional and intervention studies dating back to 1975, Heaney (2000) deduced that 75% (64) of 86 cross-sectional studies, found a positive association between calcium intake and bone density. From the 52 eligible controlled intervention trials, all but 2 demonstrated a relation between calcium supplementation and improved or retained BMD.
Calcium retention is higher in children than in adults (Matkovic and Heaney, 1992; Heaney, 2000). In order for adults to attain the 40mg day $^{-1}$ of net calcium retention recommended for bone health, at least 960mg day $^{-1}$ of calcium is required (Heaney et al., 2000). Welton *et al* (1995) reviewed 33 well-designed studies in men and women aged 18-50 years and concluded that calcium intakes of 1000mg day $^{-1}$ could prevent the loss of 1% BMD. The optimal calcium intake for young adults appears to be

around 1000mg day $^{-1}$ (Heaney, 2000), although the current UK RDA for adults currently stands at 700mg day $^{-1}$ (NOS, 2002).

It was illustrated in figure 1.5 that several lifestyle factors can contribute to BMD. Some of these factors work together in attaining the genetic potential of an individual for BMD. One such interaction is between calcium and weight-bearing exercise (Weaver, 2000). An early cross-sectional study conducted by Kanders *et al* (1988) found a synergistic effect on BMD of calcium intake (800-1000mg day $^{-1}$) and weight-bearing exercise in a group of 60 eumenorrhoeic women aged 24-34 years. Other prospective and cross-sectional studies agree with Kanders (Halioua *et al.*, 1989; Valimaki *et al.*, 1994) and in 1996, Specker *et al* reviewed 16 exercise and calcium intervention trials and confirmed the relationship. The authors found that interventions of increasing calcium intake without weight-bearing exercise were associated with only a small gain in BMD, whereas calcium plus weight-bearing exercise were associated with significant increases at the lumbar spine (Specker *et al.*, 1996). Moderate levels of weight-bearing exercise do appear to enhance the effect of calcium on BMD. However, the transport and absorption of calcium may largely depend on vitamin D status (Szule et al., 2003).

Vitamin D is a major determinant of calcium absorption (Arden, 1997) and a deficiency stimulates PTH release, leading to an increase in bone turnover that is not often matched with an increase in bone formation (Rosen, 2000). It has been predicted that around 15 to 30 minutes of daily sunlight exposure is sufficient for most individuals to synthesise the vitamin D they need (Atkinson and Ward, 2001). Vitamin D is a current topic of interest throughout the literature. It has been suggested

that greater body fat levels can increase the risk of vitamin D deficiency, for when vitamin D is synthesised through the skin, it becomes blocked by the fat tissue and is unable to enter the body (Holick, 2004). Positive relationships between dietary intake of vitamin D and BMD have been found in young women (Fehily *et al.,* 1992) men (Szule *et al.,* 2003). However, it is likely that these results have been confounded by sunlight exposure which is difficult to account for.

(ii) Energy

In addition to a diet adequate in calcium, the WHO Task Force for the prevention of osteoporosis also recognise the importance of,

> *"a diet that maintains normal body weight throughout life",*

(Genant *et al.,* 1999; p262).

The importance of energy balance to BMD has received less attention and this most likely reflects the need for more research in this area although several good studies are emerging (Zanker and Swaine, 1998; 2000; Irle and Loucks, 2004; Zanker, 2004). It is known that in order to maintain body mass, a diet should be energy balanced, thus dietary energy intake should match energy expenditure. When energy deficit occurs, the body slips into an 'energy conservation' mode and certain hormonal and metabolic changes occur in order to facilitate this energy conservation (Williams et al., 1995; 2000; Loucks and Thuma, 2003) originating from central hypothalamic disruption. The hypothalamus is a complex neurological organ which controls menstrual function, endocrine production and homeostasis (De Cree, 1998).

Energy drain is implicated in the disruption or cessation of normal menses (Williams *et al.,* 1995; Loucks and Thuma, 2003) and this is discussed further in chapter 2. It is possible that the scarce metabolic fuels in women with negative energy balance are prioritised to enable locomotion during exercise and as a consequence, less energy is available for maintaining reproductive function (De Souza *et al.,* 2003; Loucks, 2004). Lutenising hormone suppression occurs between menstrual regularity and amenorrhoea (Loucks, 1990; Loucks and Thuma, 2003) and the abrupt exposure to significant energy deficiency can lead to the suppression of luteinising hormone pulsatility in non-athletic women (Loucks and Thuma, 2003). The results of Loucks and Thuma's recent study (Loucks and Thuma, 2003) are presented in table 1.2 and demonstrate that greater energy deficits have a more severe effect on menstrual function.

Table 1.2: Dose-dependent effects of energy availability on menstrual function (results from Loucks and Thuma., 2003)

Energy availability (kcal kg LBM $^{-1}$ day $^{-1}$)	**Menstrual function response**
45	No change
20	16% reduction in LH pulsatility ($p<0.01$) and a non significant reduction in oestradiol.
10	39% reduction in LH pulsatility ($p<0.01$) and 15% reduction in oestradiol ($p<0.01$).

However, conflicting data has been provided by a recent study showing that regular menstruation can be maintained despite severe energy restriction in women with anorexia-nervosa. Miller *et al* (2004) found that 42 women with anorexia, from a sample of 116, had regular menstrual cycles and normal levels of oestradiol, despite severe under-nutrition and a low BMI (Miller *et al.,* 2004). Serum IGF-1 and leptin

were slightly higher in the regularly menstruating women with anorexia, although they were still relatively low (41.8 *v* 30.8 nmol litre $^{-1}$ and 3.7 *v* 2.8 ng litre $^{-1}$ respectively). Furthermore, reduced BMD was observed in both the regularly menstruating and the amenorrhoeic women. Thus, nutritional and hormonal status may be independent contributors to BMD in women with anorexia or with low body mass (Miller *et al.,* 2004). Moreover, although the severity of energy deficiency may determine whether menstrual function is disrupted it is also possible that genetics may determine the hormonal response to under-nutrition.

Negative energy balance has been implicated in the aetiology of menstrual dysfunction, which would suggest it indirectly affects bone by instigating a suppression of the reproductive hormones, most notably oestrogen (Aulin, 1995; Tomten *et al.,* 1998; Rumball and Lebrun, 2004). However, accumulating evidence suggests that energy balance has a role in bone metabolism that is beyond that associated with reproductive hormonal status. Energy conservation arising from energy deficiency may result in less energy availability for bone formation and bone turnover. Indeed, as discussed earlier IGF-1 is an anabolic peptide which is involved in bone formation but depressed under settings of energy deficiency (Grinspoon *et al.,* 1996; 2002; Zanker and Swaine, 2000; Heer *et al.,* 2002). Short and long-term energy restriction in men and women, results in metabolic changes including reductions in IGF-1 (Grinspoon *et al.,* 1996; Laughlin and Yen, 1997), regardless of the carbohydrate, protein and fat composition of the ingested calories (McMurray *et al.,* 1991). Levels of serum IGF-1 are low in women with anorexia nervosa regardless of menstrual status, increase with weight gain and correlate with markers of bone formation (Grinspoon *et al.,* 1996; Hotta *et al.,* 2000). Oestrogen administration to

women with anorexia effectively halts bone loss but does not restore lost bone unless overall nutrition and body mass improves (Abrams *et al.*, 1993). Moreover, Klibanski *et al* (1995) showed that BMD rises in patients recovering from anorexia, who improve nutrition and gain weight even before menses resume. These results suggest that in young adults, nutrition may be a more fundamental determinant of bone health than oestrogen deficiency characterised by amenorrhoea. However, it is clear that more evidence is required.

Research demonstrates that weight loss attained by energy restriction, is accompanied by significant reductions in BMD (Bassey and Ramsdale, 1994; Pritchard et al., 1996). In young adult women without anorexia but who wanted to lose weight, weight loss has resulted in bone loss. Six months of dietary restriction resulted in a loss of 3.4kg in body weight and 0.7% and 0.5% of total body and lumbar spine bone BMD respectively (Bassey and Ramsdale, 1994). In men, a randomised controlled trial found that 6.4kg loss of body weight resulted in a 1.5% loss of bone density (Pritchard *et al.*, 1996). Bone loss has also occurred in women who lost weight, despite partaking in regular, weight-bearing exercise (Ryan *et al.*, 1998). This suggests that even mechanical loading can not protect against bone loss associated with dietary energy restriction. It is possible that weight loss leads to a loss of bone due to decreased magnitude during load-bearing or from the hormonal and metabolic changes associated with hypometabolism during energy.

(iii) Protein

It is recommended that men and women have a dietary protein intake of 0.8 g kg^{-1} day^{-1} (Wilmore and Costill, 1999). An adequate protein intake is required to obtain the

9 essential amino acids that can only be acquired from dietary sources. Such amino acids are required for the growth, repair and maintenance of body tissues, including bone. Protein is necessary to maintain production of growth factors that modulate bone synthesis (Atkinson and Ward, 2001; Ginty, 2003). Experimentally induced protein deprivation has led to reductions in serum IGF-1 (Oster *et al.,* 1995) which has been established as having a role in bone metabolism. Although more research is required, adequate protein appears to be needed for anabolic processes that are likely to include those involved in the maintenance of bone mass.

Summary

Of the nutrients, calcium and vitamin D are established as having a fundamental role in bone health. However, accumulating evidence such as that provided by Zanker and Swaine (1998; 2000) and by Grinspoon *et al* (1995; 1996; 1999; 2002) indicates that an adequate intake of energy may be equally as important for good bone health.

1.3 Low BMD, osteoporosis and fracture risk

Osteoporosis is a serious, debilitating disease associated with bone loss and diminished bone strength, ultimately resulting in fracture. Although it was once assumed that osteoporosis only occurred in postmenopausal and elderly women, it is now recognised that accelerated bone loss and osteoporosis can occur at any age, in men as well as women. Maximising peak bone density is the most effective strategy

for preventing osteoporosis as indicated above. However, the incidence of the disease is increasing more than would be expected for the aging population, which suggests that lifestyle changes over the past two decades may be contributing to bone loss in younger generations (IOF, 2004). Such negative factors have been addressed earlier (see page 22), for example physical inactivity, smoking, excessive alcohol, low calcium intake and dieting, and together with low BMD, are useful for predicting osteoporosis and future fracture risk (Cummings *et al.,* 1993). If a greater number of young people develop osteopenia, this will lead to further increases in the incidence of osteoporosis and fracture, for these individuals are most likely to develop osteoporosis later in life (Johnston and Longcope, 1990). Not only is there significant personal cost with osteoporosis, but healthcare for osteoporotic-related fractures currently cost the UK National Health Service (NHS) between £1.4 billion and £1.8 billion per year (NICE, 2004).

1.3.1 Osteoporosis and osteopenia

i) Definitions

Osteopenia is a progressive condition that places individuals at risk of osteoporosis, increased morbidity and mortality (Derman, 2003).

Osteoporosis is a systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue, with a consequential increase in bone fragility and susceptibility to fracture (WHO, 1994). The differences between the

micro-architecture of normal compared to osteoporotic trabecular bone can be seen in figures 1.6 and 1.7 respectively.

Osteoporosis occurs from an imbalance in bone remodelling that leads to cortical and trabecular thinning and perforation. The reduced bone mass and changes in micro-architecture result in bone fragility and susceptibility to low trauma fracture. Fragility fractures are costly clinical consequences of osteoporosis, produce serious morbidity and tend to occur in older age (Keen, 1999). The diagnosis of osteoporosis and osteopenia is made by measuring BMD by dual energy X-ray Absorptiometry (DXA). This technique is reviewed in Chapter 3.

Figure 1.6: Scanning electron micrograph of normal trabecular bone (Stevenson and Marsh, 1994) *Magnification x 50*

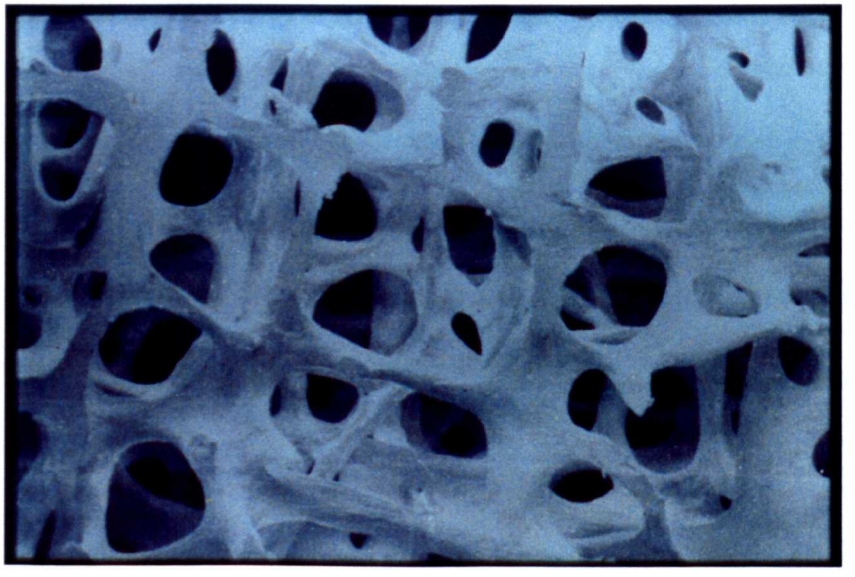
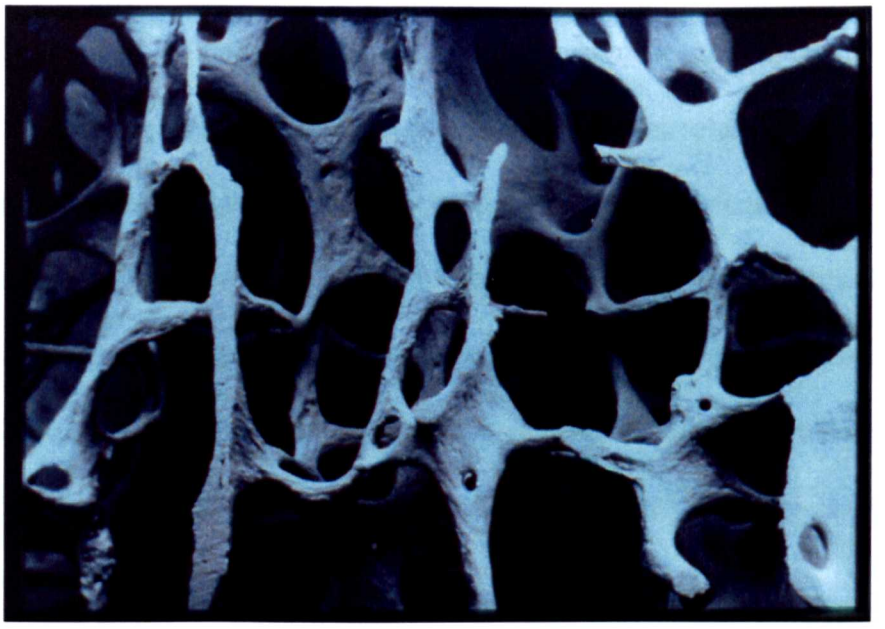


Figure 1.7: Scanning electron micrograph of osteoporotic trabecular bone (Stevenson and Marsh, 1994) *Magnification x 50*



ii) Low BMD and fracture

Fracture is the structural failure of bone, occurring from a material loss of bone mineral and the architectural thinning of cortices (Seeman, 2003). Prospective studies show that low BMD is a determinant of future osteoporotic fracture (Cummings *et al.,* 1993) and there are indications that the risk of fracture approximately doubles for every SD reduction in BMD below the mean value for age (Cummings *et al.,* 1993; Marshall *et al.,* 1996).

Reduced spinal BMD is associated with an increased risk of vertebral fracture. In a large cross-sectional study, premenopausal and postmenopausal women with spine fractures were found to have significantly reduced BMC, BMD and volume-adjusted BMD as measured by DXA and expressed in T- and Z-scores (Duan *et al.,* 1999). Women with spinal fractures were also found to have smaller vertebrae with less bone

within the vertebral bodies, thus representing inferior structural competence. A more recent study investigated the risk of spinal fracture with regard to BMD in men and women (Cauley *et al.,* 2004). Although the men were significantly under-represented compared to women (n=317 *v* 2,067), the subject sample was relatively large and the authors partially corrected for gender differences in bone size by calculating BMAD. Using measures of BMD and BMAD it was shown that low values in both of these measures at the total hip, spine and femoral neck were associated with increased odds of spinal fracture in men and women (Cauley *et al.*, 2004). Thus, research indicates that BMD can predict fracture risk, although it cannot identify individuals who will have a fracture (Marshall *et al.*, 1996).

1.3.2 Diagnostic criteria

In the recent International Society for Clinical Densitometry (ISCD) special report, it was clarified that the

> *" measurement of BMD by DXA is the 'gold standard' method for the non-invasive diagnosis of osteoporosis"*

(Lewiecki *et al.,* 2004),

and it has subsequently been verified that the preferred measurement sites for diagnosis are the anterior posterior (AP) lumbar spine (lumbar vertebrae 2-4) and total hip (NOS, 2002; Lewiecki *et al.,* 2004). These skeletal sites are the most clinically relevant because most osteoporotic fractures occur here and cause considerable

morbidity, reduced quality of life and mortality (Genant *et al.*, 1999; NOS, 2002), although due to an absence of clinical symptoms, a considerable proportion of spine fractures go undetected (NOS, 2002). Although total body DXA tests provide useful information on overall distribution of BMC, BMD and body composition, they cannot be used to diagnose osteoporosis (NOS, 2002). Figure 1.8 presents the universally recognised diagnostic criteria for osteoporosis and osteopenia of the 1994 World Health Organisation (WHO).

Figure 1.8: WHO diagnostic criteria for osteoporosis and osteopenia

Normal-- BMD greater than -1 SD from the young adult reference mean
(T-score >-1)

Osteopenia (low BMD)-- BMD between 1 and 2.5 SD below the young adult reference mean
(T-score <-1 and >-2.5)

Osteoporosis-- BMD 2.5 SD or more below the young adult reference mean
(T-score <-2.5)

Established osteoporosis-- T-score <-2.5 and the presence of one or more fragility fractures

These are based on BMD measurements expressed in standard deviation (SD) units known as T-scores. The T-score definition of osteoporosis was originally designed to quantify the extent of the osteoporosis problem for policymakers (Melton *et al.*, 2000) and was formulated to document the prevalence of the problem in postmenopausal

women. A T-score result indicates the difference between an individual's BMD and the ideal BMD of healthy young adults (20-40 years) matched for gender and ethnic group, and is as follows:

$$\text{T-score} = \frac{\text{Measured BMD} - \text{Young Adult Mean BMD}}{\text{Young Adult SD}}$$

T-scores depend on the BMD of the individual and the BMD of the young adult population (Levasseur *et al.,* 2003). The choice of reference population is important because BMD for an individual of a given age and weight varies by gender (Nguyen *et al.,* 2001), ethnic group (Gilsanz *et al.,* 1998) and country (Levasseur *et al.,* 2003).

According to the WHO guidelines (figure 1.6), a T-score of less than 2.5 SD indicates osteoporosis and the lowest T-score of AP (anterior posterior) spine or total hip should be selected. However, this definition is primarily intended for postmenopausal women up to the age of 75 years (NOS, 2002). In response to the lack of guidelines regarding indications for testing in populations other than postmenopausal women, the ISCD established comprehensive indications for bone density testing that consider all adults (Lewiecki *et al.,* 2004). According to the ISCD report, the WHO criteria should not be applied in their entirety to children, adolescents, premenopausal women or adult men under the age of 50 years (Lewiecki *et al.,* 2004).

i) Premenopausal women (age 20 years to menopause)

In accordance with the ISCD, the diagnosis of osteoporosis in premenopausal women should not be made on the basis of densitometric criteria alone (Lewiecki *et al.,* 2004). Osteoporosis can be diagnosed in premenopausal women so long as low BMD

is accompanied by secondary causes such as hypogonadism (oestrogen deficiency, ovarian failure, amenorrhoea) or hyperparathyroidism, or with risk factors for fracture (Lewiecki *et al.,* 2004). In the young adult healthy population, it has been estimated that approximately 15% of women have a T-score indicative of osteopenia and 0.5% had a T-score within the osteoporotic range (Kanis *et al.,* 1994).

ii) Men (age 20 years and older)

The definition of male osteoporosis is similar to that used for women, so long as male-specific reference data is used to calculate the T-score (Kanis *et al.,* 2000; Byers *et al.,* 2001; Blinkley *et al.,* 2002; Lewiecki *et al.,* 2004). However, it has been argued that this may underestimate the size of the male population with osteoporosis because once adjusted for bone size men may have similar or even lower BMD than women (Faulkner and Orwoll, 2002). Nonetheless, the larger bone size and greater cortical thickness of bone in men represents a biomechanical advantage against fracture risk (Seeman, 1999; 2002). As in women the presence of secondary causes of bone loss and/or risk factors for fracture are required in order to diagnose osteoporosis (Lewiecki *et al.*, 2004).

iii) Z-scores

Z-scores are useful for assessing BMD in patients compared to that expected for their age and sex. The Z-score is calculated in a similar way to the T-score, but the patient's BMD is compared with the mean BMD expected for their age, gender and ethnic origin.

$$\text{Z-score} = \frac{\text{Measured BMD} - \text{Age-matched Mean BMD}}{\text{Aged-matched SD}}$$

A low Z-score indicates that BMD is lower than expected and this requires a search for a pathological cause (NOS, 2002, Watts, 2004). Z-scores are useful as they express the individual's risk of sustaining a fracture compared to their age-matched counterparts. An in-depth meta-analysis of prospective studies on fracture risk concluded that for every 1 SD decrease in the Z-score, there is a 1.5 to 2.5 increase in the relative risk of fracture (Marshall *et al.*, 1996).

1.3.3 Epidemiology of osteoporosis

Osteoporosis is one of the most debilitating diseases in the world associated with significant mortality and morbidity (WHO, 1999; NICE; 2004). As indicated previously it is a major burden for the National Health Service, currently costing the UK approximately £1.7 billion per year and this figure is likely to rise to £2.1 billion by 2010 (NICE, 2004). Maximising the peak bone density is the most effective approach for the prevention of osteoporosis. As indicated before, the incidence of osteoporosis is increasing more than would be expected for the aging population which suggests that lifestyle changes over the past two decades may be contributing to bone loss in younger generations (IOF, 2004). Osteoporosis was also once viewed as a disease occurring in postmenopausal or elderly women and this stereotypical connotation has lead to the underestimation of the risk in men by General Practitioners and indeed by men themselves. However, in the UK, osteoporosis affects one third of women and at least 1 in 12 men over the age of 50 (IOF, 2004), although a recent report produced on behalf of the IOF states that osteoporosis affects 1 in 5

men over the age of 50 years (Seeman, 2004). Thus, it appears that osteoporosis is neither an age- nor gender-dependent disease.

Summary

Bone is a living tissue that responds and adapts its structure and density according to its environment. Many factors contribute to BMD in men and women and although genetics is a strong determinant of BMD numerous endocrine, mechanical and nutritional determinants are modifiable contributions to the BMD. Exposure to certain nutritional deficiencies and lack of weight-bearing exercise, can lead to bone loss in young adults. A low BMD for age is a serious issue for long-term skeletal health and the evidence has established that a particular group of young people at risk of bone loss and osteoporosis are female athletes who have amenorrhoea (absent menses). The following chapter examines the numerous reports of BMD in distance runners and the factors that may contribute to a low BMD. The chapter reviews the evidence that energy balance may be implicated in the bone health of this population and develops the rationale underlying the work conducted for this study.

Chapter 2

Bone mineral density in distance runners

2.1 Introduction

Distance (endurance) running has been associated with both positive and negative skeletal effects (Gibson *et al.*, 2000). As a weight-bearing exercise, 'running' subjects certain skeletal sites to mechanical loading and numerous studies provide evidence to support its positive effects on bone density (Snow-Harter *et al.,* 1992; Etherington *et al.*, 1996; Heinonen *et al.*, 1999; Duncan *et al.,* 2002; Gustavssan *et al.*, 2003). On the other hand, distance running has been associated with significant reductions in BMD, particularly in female runners who have menstrual disorders (Drinkwater *et al.*, 1984; Marcus *et al.*, 1985; Myerson *et al.*, 1992; Micklesfield *et al.*, 1995; Tomten *et al.*, 1998; Gibson *et al.*, 2000). This emphasises the need to understand the interaction of mechanical, nutritional and hormonal factors on bone health in athletes.

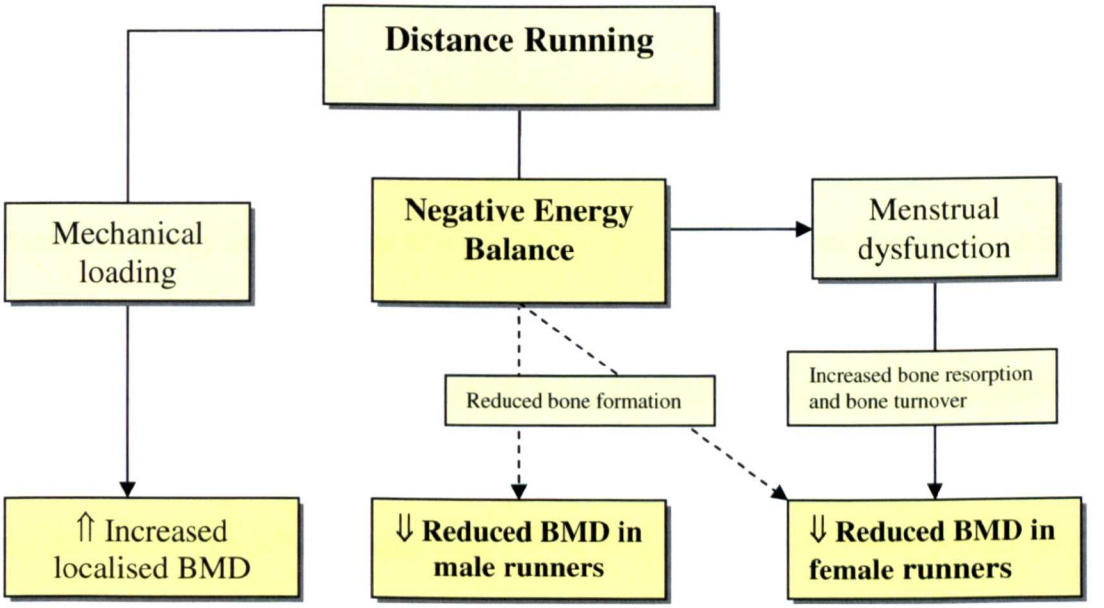
The true prevalence of low BMD in distance runners is unknown. Due to their age and athletic profile they are unlikely to be targets for clinical osteoporosis screening, with the exception of female runners who have persistent amenorrhoea (absence of menses; NOS, 2002). The majority of research into the bone health of distance runners has focused upon female athletes and the problem was first reported in the early 1980's when female amenorrhoeic runners were found to have serious bone mineral deficits compared to their eumenorrhoeic counterparts. Since that time, most investigations of BMD in female athletes have focused upon the effect of oestrogen

deficiency (Myerson *et al.*, 1992; Myburgh *et al.*, 1993; Micklesfield *et al.*, 1995; Pettersson *et al.*, 1999). Indeed, oestrogen deficiency is an established cause of bone loss as demonstrated by the vast research conducted in postmenopausal women. However, it seems that the common assumption that female runners lose bone solely because of oestrogen deficiency may be somewhat arbitrary, for emerging evidence suggests that energy deficit may have an equal, or perhaps more fundamental role in bone loss than sex hormone deficiency (De Souza *et al.*, 1991; Zanker and Swaine, 1998; Grinspoon *et al.*, 2002; Heer *et al.*, 2002; Cobb *et al.*, 2003; Ihle and Loucks, 2004). Although most research has been conducted in women with anorexia (Grinspoon *et al.*, 1996; 2002; Heer *et al.*, 2002; Hotta *et al.*, 2002; Mika *et al.*, 2002), accumulating evidence is offering new insights into the pathogenesis of bone loss in athletes involved in high energy-demanding sports (Zanker and Swaine, 1998; 2000; Ihle and Loucks, 2004), who also have a high potential to be in negative energy balance.

As discussed in the previous chapter, low energy availability is linked to metabolic disturbances such as lowered IGF-1 and leptin (Grinspoon *et al.*, 1996; Hotta *et al.*, 1998; 2002; Weinbrenner *et al.*, 2003), which are subsequently associated with negative effects on bone remodelling (Holloway *et al.*, 2002; Welt *et al.*, 2004). It is possible that negative energy balance is particularly common amongst distance runners due to their exceptionally high energy expenditures and may be physiologically manifested in their low BMI, low body fat and potentially linked to low BMD. Although oestrogen deficiency can contribute to bone loss in female athletes, negative energy balance may have a separate, independent role and may also apply to male athletes.

Figure 2.1 places the potential link between negative energy balance and low BMD within the context of established factors that can affect bone density in distance runners. The relationship between energy balance and BMD in male and female runners has not yet been investigated and as a contemporary focus of enquiry, provides the direction of the current study.

Figure 2.1: Flow chart representation of the potential role for negative energy balance in the aetiology of reduced bone mineral density in distance runners



2.2 Energy balance and BMD in distance runners

'Distance' or 'endurance' sports are defined as sports which involve high energy expenditures and create a catabolic environment that is prolonged and elevated for a substantial period of time. Prime examples are sports such as distance running,

cycling and swimming. For this study, the focus is on distance runners, defined as male and female runners who regularly participate in running competitions. The distances include 3-10 kilometres (km) on the track and 3 km and above on the road and country. It is not only the events and competitions that require high energy stores, but also the daily training, which predominantly determines performance outcome and overall energy expenditure.

2.2.1 Energy status of distance runners

The term 'energy deficiency' may be defined as a state of catabolism when energy expenditure is greater than dietary energy intake. It is possible that many distance runners are energy deficient due to high energy requirements from frequent exercise training that is often twice-daily and from prolonged, high intensity competitions (King *et al.*, 1997). Furthermore, research indicates that the incidence of sub-optimum energy intakes, eating disorders and purposeful energy restriction are particularly high amongst distance runners (Tanaka *et al.*, 1995; Beals and Manore, 1998; Hulley and Hill, 1999; Sundgot-Borgen, 1999).

In addition to energy and carbohydrate requirements, athletes have greater protein requirements for the repair and maintenance of body tissues and as an auxiliary fuel source (Wilmore and Costill, 1999). It has been recommended that these athletes consume 1.2 to 1.4 g $kg^{-1}day^{-1}$ of protein (Economos et al., 1993; Wilmore and Costill, 1999).

With regard to energy intake, recommended calorific requirements in the UK for normally active men and women are 2500 and 2000 kcal respectively. As distance runners have greater energy needs due to exercise demands their daily energy requirements are well above average (Economos *et al.*, 1993; Loucks, 2004). The calorific needs of athletes are primarily dependent on metabolic efficiency, body mass and exercise training (intensity, duration and frequency of daily exercise). General energy requirements[1] for male athletes have been estimated to be 50 kcal kg^{-1} day^{-1}; and for female athletes, 45-50 kcal kg^{-1} day^{-1} (Economos *et al.*, 1993). For a male runner weighing 67kg, this equates to 3350 kcal day^{-1} and for a female runner weighing 53kg, 2385-2650 kcal day^{-1}. Carbohydrate is a principle energy-providing nutrient, and nutritional recommendations for athletes are intakes of 5 to 7g kg^{-1} day^{-1} for general training needs and 7 to 10g kg^{-1} day^{-1} for high levels of endurance training (Burke *et al.*, 2001).

Despite the importance of energy for exercise training and performance (Wilmore and Costill, 1999) many distance runners may not consume adequate calories and thus be chronically energy deficient (Deuster *et al.*, 1986; Sugiura *et al.*, 1999; Hassapidou and Manstrantoni, 2001). There are several reasons for this:

- **High energy expenditure:** Training and competition in distance running requires high amounts of energy which some runners may be unable to compensate for through their diet alone. Thus, their energy deficit may be involuntary.
- **Performance:** Low body mass is often viewed as advantageous to running performance, thus many athletes follow restrictive diets that are potentially

[1] Based on exercise training for at least one hour per day

destructive to their health and performance (Brownell *et al.*, 1987). Dietary records have indicated many runners are energy deficient (Marcus *et al.*, 1985; Micklesfield *et al.*, 1995). It is possible that many of the athletes partaking in these studies may have under-reported their dietary intakes. However, this is difficult to determine.

- **Appetite suppression:** High intensity exercise training can suppress hunger and contribute to insufficient energy intake (Blundell and King, 1998; Loucks, 2004).
- **Weight-loss strategy:** Distance running is attractive to those who wish to lose weight, due to the expenditure of large cumulative amounts of energy.
- **Eating disorders:** There have been numerous reports of eating disorders in male and female distance runners (Sungot-Borgen et al., 1993; Hulley and Hill, 1999).

i) Energy intakes of runners

Sub-optimum energy and carbohydrate intakes are frequently documented in endurance athletes (Marcus *et al.*, 1985; Edwards *et al.*, 1993; Hawley *et al.*, 1995; Beals and Manore, 1998). Most research has been conducted on female and elite athletes, and there appears to be a global trend of low energy intake amongst female distance runners of Western, Greek and Japanese origin (Deuster *et al.*, 1986; Sugiura *et al.*, 1999; Hassapidou and Manstrantoni, 2001). Several research groups have provided details of estimated energy intakes amongst female runners by using 3-day (Marcus *et al.*, 1985; Cook *et al.*, 1989; Micklesfield *et al*, 1995; Rencken *et al.*, 1996; Winters *et al.*, 1996), 5-day (Gremion *et al.*, 2001) and 7-day (Prior *et al.*, 1990; Pettersson *et al.*, 1999; Burrows *et al.*, 2003) self report dietary records, as well as

providing information on weekly mileage. These records indicate that many runners are consuming too few calories to fuel exercise training. A longitudinal study found a high incidence of skipping meals, eating disorders and decreased energy intake in female runners that did not improve over three years and the prevalence of menstrual irregularities remained high (Wiita and Stombaugh, 1996).

Although it has been suggested that nutritional inadequacies separate female athletes with menstrual disorders from those with regular menses, Table 2.1 illustrates the potentially large negative energy balance of runners regardless of menstrual status, engaged in demanding training schedules. In order to allow comparison between studies it is useful to use energy intake per kg of body mass. However, most authors have not provided such data. For this reason, table 2.1 includes an estimation of these values based on the mean results from the studies. However, these are only estimations and may differ from actual values based on the body mass and energy intake of individual subjects (such data has not been published). As a reminder, energy requirements for male athletes have been estimated to be 50 kcal kg^{-1} day^{-1} and for female athletes, 45-50 kcal $kg^{-1}day^{-1}$ (Economos *et al.*, 1993).

Table 2.1: Estimated daily energy intakes and exercise/energy expenditure (kcal) of female distance runners

Study	Subjects (menstrual status)	Mean body mass (kg) of subjects	Mean daily energy intake and kcal kg $^{-1}$*	Mean weekly mileage, weekly hours, or daily energy expenditure
Marcus *et al.*, 1985$^{\Omega}$	A	49.7	**1272 (25.6)**	58 miles week^{-1}
	E	53.8	**1715 (31.9)**	58 miles week^{-1}
Cook *et al.*, 1989$^{\Omega}$	A	55.3	**2000 (36.2)**	43.2 miles week^{-1}
	E	53.4	**1800 (33.7)**	30.1 miles week^{-1}
Micklesfield *et al.*, 1995	A	57.2	**1546 (27.0)**	3260 kcal day^{-1} $^{\delta}$
	E	58.3	**1710 (29.3)**	3043 kcal day^{-1} $^{\delta}$
Winters *et al.*, 1996$^{¥}$	E	58.7	**2013 (34.3)**	2673 kcal day^{-1}
Pettersson *et al.*, 1999	A	52.4	**1842 (35.2)**	10 hours week^{-1}
	E	59.0	**2091 (35.4)**	9 hours week^{-1}
Gremion *et al.*, 2001$^{¥\Omega}$	A	49.9	**2131 (42.7)**	57 miles week^{-1}
	E	51.4	**2200 (42.8)**	61 miles week^{-1}
Cobb *et al.*, 2003	A	58.1	**2219 (38.2)**	39 miles week^{-1}
	E	58.6	**2241 (38.2)**	33 miles week^{-1}

A = amenorrhoeic
E = eumenorrhoeic
* *estimated from the mean body mass and energy intake values*
$^{\delta}$ *original data reported in mega joules (MJ) per day*
$^{\Omega}$ *original data reported in km per week*
$^{¥}$ *excluded runners with clinical eating disorders*

Information regarding energy balance in male athletes is limited, but it has been suggested that they may also ingest too few calories to match energy expenditure (Hawley *et al.*, 1995; Tanaka *et al.*, 1995). A recent study of both male and female Australian elite athletes found that athletes from 'weight-conscious' sports, including distance running, reported low energy intakes and were least likely to consume sufficient carbohydrate for training (Burke *et al.*, 2003). Similarly in 291 male and 56 female marathon runners, caloric intakes were significantly lower than required

amounts (Niemen *et al.,* 1989). In athletes from other sports, low energy intakes have been reported by female endurance cyclists (Burke, 2001) and elite female volleyball players (Beals, 2002). Male and female gymnasts and male wrestlers have reported daily energy intakes as low as 400 kcal whereas footballers have reported intakes as high as 5,270 kcal (Short and Short, 1983; Jonnalagadda *et al.,* 1998; Rico-Sanz *et al.,* 1998). It is likely that the lower intakes from athletes in sports such as running, gymnastics and wrestling reflect the sport-specific emphasis on low body weight and body fat for performance and appearance. Self-selected daily energy intakes in anorexic patients have been carefully observed and reported at 1,017 kcal. When expressed as kcal per kg body mass, this intake appeared sufficient to maintain their low body mass (Gwirtsman *et al.,* 1989). Although lower than the dietary energy intake reported by female runners, it is possible that the energy deficit would be similar or perhaps greater in the athletes when accounting for their high exercise energy expenditures.

ii) Under-reporting

The dietary energy intakes of the female runners in figure 2.1 are considerably lower than the energy intake recommendations for endurance athletes (Economos *et al.,* 1993) with some intakes even lower than BMR (Marcus *et al.,* 1985; Micklesfield *et al.,* 1995). However, the majority of research has relied on self-reported measures of energy intake and these are fully dependent upon the honesty and accuracy of the participants. Many investigators advise that self-report dietary record data from female athletes should be interpreted with caution, as under-reporting or under-eating during the recording period may be common practice (Edwards *et al.,* 1993;

Schoeller, 1995; Burke, 2001). The issue of under-reporting is addressed in more detail in the methodology chapter (Chapter 3.1).

iii) Metabolic indications of energy deficit

Anorexia-nervosa is a condition characterised by severe chronic calorific deprivation and excessively low body mass. Patients with anorexia are consistently found with endocrine abnormalities depicting under-nutrition and energy deficit (Grinspoon *et al.*, 1996; Hotta *et al.*, 2002; Weinbrenner *et al.*, 2003; Heer *et al.*, 2004). Although the potential for under-reporting by female runners cannot be ruled out, there is objective evidence for an energy deficit in these athletes, arising from nutritionally-related endocrine alterations that are strikingly similar to those observed in anorexic patients (Marcus *et al.*, 1985; Myerson *et al.*, 1991; Laughlin and Yen, 1996; 1997; Zanker and Swaine, 1998). These are shown in table 2.2.

The studies cited in table 2.2 report metabolic reductions in amenorrhoeic (Ding *et al.*, 1988; Zanker and Swaine, 1998; Lebenstedt *et al.* 1999; Thong *et al.*, 2000) and in regularly menstruating (Laughlin and Yen, 1996; Thong *et al.*, 2000; De Souza *et al.*, 2003) runners. Indeed, comparisons have previously been made between amenorrhoeic athletes and women with anorexia (De Souza and Metzger, 1991). Both groups of women have a low body mass, a high incidence of amenorrhoea that arise from hypothalamic dysfunction and share similar endocrine abnormalities. Furthermore, both male and female endurance athletes have been found to display comparable behavioural profiles as individuals with clinical anorexia (Yates *et al.*, 1983; Klocks and De Souza, 1995). These are described in chapter 2.2.2.

Table 2.2: Hormonal and metabolic disruptions indicative of chronic energy deficiency in exercising females/runners and in women with anorexia nervosa

Hormonal and metabolic disruptions	**Exercising females**	**Women with anorexia**
Low levels of 3,5,3 triiodothyronine (T_3)	Marcus *et al.*, 1985; Myerson *et al.*, 1991; Loucks and Callister, 1993; Zanker and Swaine, 1998; Lebenstedt *et al.*, 1999; Thong *et al.*, 2000; De Souza *et al.*, 2003	Munoz and Argente, 2002; Weinbrenner *et al.*, 2003
Low insulin	Laughlin and Yen, 1996; Thong *et al.*, 2000; De Souza *et al.*, 2003	Grinspoon *et al.*, 1996; Munoz and Argente, 2002; Weinbrenner *et al.*, 2003
Low IGF-1 and IGF BP-1	Laughlin and Yen, 1996; Zanker and Swaine, 1998; De Souza *et al.*, 2003	Grinspoon *et al.*, 1996; Hotta *et al.*, 1998; Soyka *et al.*, 1999; Heer *et al.*, 2004
Low plasma glucose concentrations	Laughlin and Yen, 1996 De Souza *et al.*, 2003	Munoz and Argente, 2002; Weinbrenner *et al.*, 2003
Low leptin	Laughlin and Yen, 1997; Thong *et al.*, 2000; De Souza *et al.*, 2003	Weinbrenner *et al.*, 2003; Heer *et al.*, 2004; Miller *et al.*, 2004
Elevated cortisol	Ding *et al.*, 1988; Loucks *et al.*, 1989; De Souza *et al.*, 1991; Laughlin and Yen, 1996;	Newman and Halmi, 1989; Munoz and Argente, 2002; Weinbrenner *et al.*, 2003

The importance of the endocrine and behavioural similarities between certain athletes and individuals with anorexia is that these two groups may also share the same mechanism of bone loss. Both groups display reductions in IGF-1 and leptin which have emerged as likely candidates underlying bone loss arising form energy deficit (Thomas *et al.*, 1999; Grinspoon *et al.*, 2002; Welt *et al.*, 2004; Zanker, 2004).

iv) Reduced metabolic rate

The ability of some runners to maintain a steady body mass whilst ingesting so few calories (Marcus *et al.*, 1985; Beidleman *et al.*, 1995; Winters *et al.*, 1996) may reflect energy conservation and the adaptation of basal metabolic rate (BMR) as an attempt to conserve energy. This has been confirmed by investigations of biochemical markers that have consistently identified 'low T_3 syndrome' in female runners (Loucks and Callister, 1993; Laughlin and Yen, 1996; 1997; Zanker and Swaine, 1998; Thong *et al.*, 2000; De Souza *et al.*, 2003). Lowered T_3 concentrations signify a reduction in BMR, reflecting the conservation of metabolic fuels in settings of energy deficiency (Myerson *et al.*, 1991; Thong *et al.*, 2000; De Souza *et al.*, 2003). Again, similar metabolic disturbances are documented in women with anorexia (Grinspoon *et al.*, 1999; Hotta *et al.*, 2000). In contrast, Wilmore *et al* (1992) failed to find any differences in BMR, energy cost of running or the thermic effect of food between amenorrhoeic and eumenorrhoeic runners. However, in this study, there were no differences in energy balance between the two groups.

v) Appetite suppression

Research indicates that exercise performed at greater than 60% VO_2 max (maximum volume of oxygen consumed per minute) may suppress appetite (Blundell and King, 1998), at least in the short term (Scheurink *et al.*, 1999). Scheurink *et al* (1999) describe this as the,

> *"anorexic-effect of exercise"*,
>
> (Scheurink *et al.*, 1999; pp51)

and argue that this may explain involuntary energy deficit in endurance athletes. Twice-a-day training at 70% VO_2 max (King *et al.*, 1997) and a 20% increase in marathon training volume over 40 weeks (Westerterp., *et al*, 1991) has shown to encourage no increase in energy intake in male athletes. Furthermore, although energy deficit caused by food restriction increased hunger, energy deficit caused by increased energy expenditure did not (Hubert *et al.*, 1998). Therefore, it would appear that athletes are at increased risk of negative energy balance, even without purposefully restricting dietary intake.

2.2.2 Restrictive and disordered eating

Distance running is a sport in which a low body weight is often assumed to be advantageous for successful performance. Consequently, many athletes are over-concerned with reducing body mass and body fat to levels that are detrimental to long-term health. The problem of prolonged energy restriction in male and female athletes has recently been termed,

> *"anorexia athletica"*

(Sudi *et al.*, 2004; pp657).

Highly trained runners share similar personality traits to individuals with anorexia nervosa (Yates *et al.*, 1983; Klock and De Souza, 1995). Female runners have scored in extreme ranges of depression and have eating disorder inventory (EDI) scores denoting anorexia nervosa, bulimia, binge eating and sub-clinical eating disorders (Klock and De Souza, 1995). In addition, elite runners have scored higher on eating disorder and running addiction measures compared to lower level runners (Estok and

Rudy, 1996). The same study demonstrated that 25% of those who ran over 30 miles per week had EDI scores indicating high risk for anorexia-nervosa. More recently, a study conducted from Leeds in the UK found that amongst a sample of 181 competitive female distance runners, 29 were either anorexic or bulimic and a further 20 suffered from sub-clinical eating disorders (Hulley and Hill, 2001). These runners did not differ in training regimen but had a significantly lower BMI, lower self-esteem and reported a higher frequency of dieting.

The prevalence of eating disorders appears to be higher in male and female athletes than in the general population (Sundgot-Borgen and Torstveit, 2004) and represents what is potentially the instigating factor of the Female Athlete Triad (disordered eating, amenorrhoea and low BMD)-although due to its very name, this obviously does not apply to male athletes! The Female Athlete Triad is discussed in Section 1.5.4. Male athletes who share the similar competitive drive for leanness may also be affected by eating disorders, although this area of research has received less attention. An early study of 60 male marathon and trail runners found that they displayed extremely high self-expectations, compulsive behaviour, a high tolerance of physical discomfort, a tendency towards depression and an emphasis on lean body mass (Yates *et al.*, 1983). The investigators also identified the runners as having a,

> *"bizarre occupation with food"*

(Yates *et al.*, 1983, pp308).

A large study of elite Norwegian athletes aged 15-40 years, found that 8% met the diagnostic criteria for anorexia compared to only 0.5% of the general male population

(Sungot-Borgen et al., 1999). The same study found that 20% of the female athletes met the criteria for anorexia nervosa, compared to 9% of the general female population. Sundgot-Borgen's most recent study of the entire population of Norwegian elite male and female athletes (n=1620) compared to controls (n=1696) showed that the prevalence of eating disorders is higher in leanness-dependent sports such as endurance and in athletes who competed at elite level (Sundgot-Borgen and Torstveit, 2004). Figure 2.3 presents the findings of studies investigating the prevalence of disordered eating amongst distance runners.

Table 2.3: Eating disorders in distance runners

Study	Subjects	% with eating disorders (anorexia, bulimia or sub-clinical)
Rosen *et al.*, 1986	182 collegiate female runners	32%
Prussin and Harvey, 1991	174 female runners	19%
Estok and Rudy, 1996	130 moderate-high level female runners	25%
Sungot-Borgen *et al.*, 1999	Population study. Male and female Norwegian elite runners	Female: 20% Male: 8%
Hulley and Hill, 2001	181 female UK competitive runners	16% with clinical eating disorders 11% with sub-clinical eating disorders
Cobb *et al.*, 2003	91 competitive female runners	25% EDI scores to indicate high risk of anorexia

Dieting behaviours may be frequently encouraged by coaches even at the highest level. A recent study surveying 55 Brazilian coaches of Olympic athletes, found that 27% of coaches recommended deleterious weight loss practices to athletes whilst the

majority encouraged excessively low fat diets and held incorrect food beliefs (Juzwiak and Ancona-Lopez, 2004).

It is possible that some athletes may have a type of eating disorder that does not meet any clinical diagnostic criteria, by being over-concerned with reducing fat intake and keeping their weight to what appears to be optimal for performance or to achieve the 'right' look. These cases are difficult to distinguish yet are likely to result in energy and nutrient intakes well below recommended amounts (Beals and Manore, 1998; Sungot-Borgen *et al*, 1993).

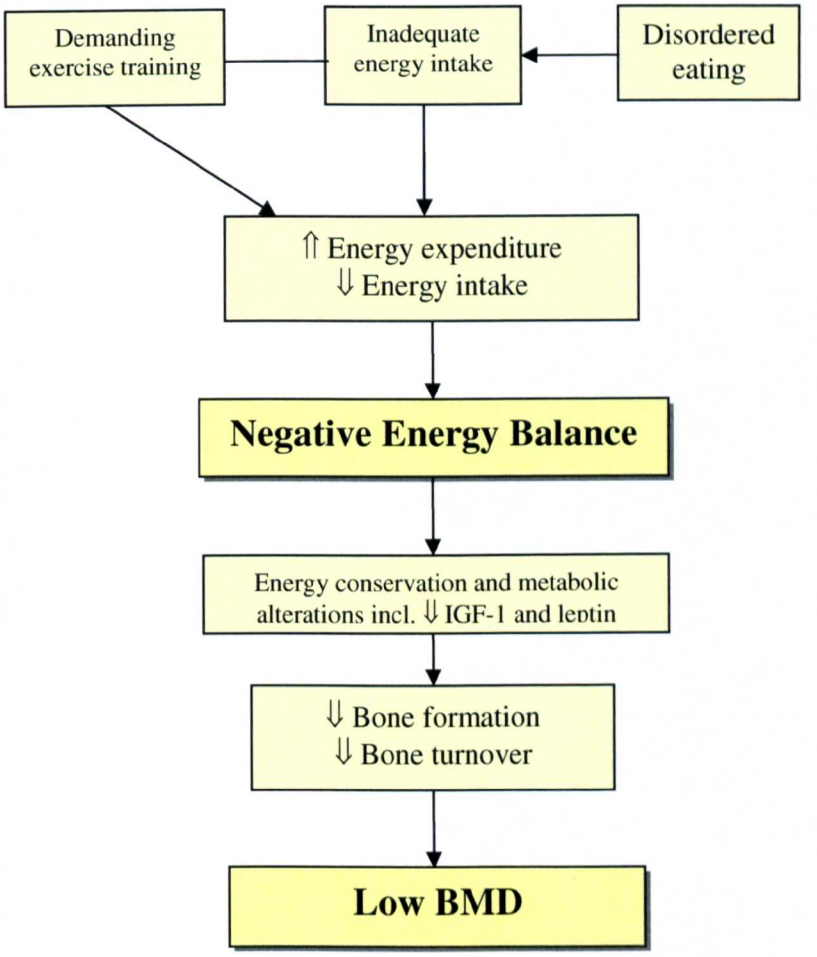
2.2.3 Distance running, energy balance and bone

A major observation to be drawn from the literature is that the large number of studies reporting sub-optimum energy intakes amongst distance runners also report that these runners have significantly compromised BMD (Marcus *et al.*, 1985; Drinkwater *et al.*, 1984; Bilanin *et al.*, 1989; Pettersson *et al.*, 1999). Could it be that the arising negative energy balance is linked to their bone loss? Unfortunately, the majority of investigations into the BMD of distance runners have focused on associations with amenorrhoea and have not estimated energy balance (Marcus *et al.*, 1985; Cook *et al.*, 1989; Micklesfield *et al*, 1995; Rencken *et al.*, 1996; Pettersson *et al.*, 1999).

As discussed earlier, low energy availability can induce metabolic and endocrine aberrations such as low IGF-1 and leptin, which can disturb the bone remodelling balance (Loucks *et al.*, 1998; Zanker and Swaine., 1998; 2000). Based on this

evidence, figure 2.2 proposes the link between negative energy balance and reduced bone density. Furthermore, several endocrine alterations that occur during endurance training may also have the potential to negatively affect the bone remodelling balance and contribute to bone loss.

Figure 2.2: Model depicting the potential relationship between negative energy balance and bone loss



i) Energy balance, IGF-1 and bone formation

As discussed in chapters 1.2.2 and 1.2.5, serum IGF-1 has emerged as a sensitive marker of nutritional status, decreasing in settings of energy restriction and increasing in response to energy repletion (Clemmons *et al.*, 1985; Grinspoon *et al.*, 1996).

Short and long-term energy restriction results in metabolic changes that are reflective of energy conservation, including reductions in IGF-1 (Grinspoon *et al.*, 1996; Laughlin and Yen, 1997). Levels of IGF-1 are low in patients with anorexia nervosa and have shown to increase with weight gain (Grinspoon *et al.*, 1996; Hotta *et al.*, 2000). Low IGF-1 has also been observed in amenorrhoeic runners (Zanker and Swaine, 1998; Snow *et al.*, 2000) and in energy deficient, exercising male runners (Zanker and Swaine, 2000). Negative energy balance leads to a reduction in IGF-1 synthesis in bone (Ammann *et al.*, 1993; Chevalley *et al.*, 1998) and the link between energy balance, IGF-1 and bone formation has been verified by a number of well-designed investigations (Zanker and Swaine, 1998; 2000; Snow *et al.*, 2000).

As the first to investigate the influence of energy balance on bone remodelling in energy-deficient amenorrhoeic runners, Zanker and Swaine (1998) found low serum IGF-1 and low BMI were associated with reduced bone turnover and formation. The hypothesis that negative energy balance adversely affects bone metabolism (Williams *et al.*, 1995; Loucks *et al.*, 1998) is supported by Zanker's second study (Zanker and Swaine, 2000). The authors investigated bone turnover and IGF-1 in eight male runners, whilst manipulating energy intake and expenditure. The exercise intervention involved running for 60 minutes on a treadmill at an intensity of up to 85% maximum heart rate. In energy balance, there were no alterations to IGF-1 or bone turnover. Under energy restriction (50% less than the energy required), IGF-1 reduced by 17% and levels of the bone formation marker, N-terminal pro-peptide of type 1 collagen (PINP) were reduced by 15%. Both changes were statistically significant ($p=0.007$ and $p=0.008$ respectively) indicating that bone turnover is not negated due to strenuous exercise except in settings of energy deficiency. Thus, it

would appear that chronic negative energy balance may be a major factor involved in the genesis of bone loss in both male and female runners.

ii) Energy balance and bone remodelling

Unlike bone loss arising from oestrogen, bone loss arising from chronic negative energy balance may be typified by reduced bone formation and imbalanced bone turnover (Grinspoon *et al.,* 1996; Zanker and Swaine, 1998; 2000). Amenorrhoeic athletes would be expected to exhibit increased bone resorption due to oestrogen deficiency yet they have been found with significantly reduced bone formation and bone turnover, and without any signs of excessive bone resorption (Okano *et al.,* 1995; Zanker and Swaine, 1998; Zanker, 2004).

iii) Energy balance and BMD in runners

Studies investigating the bone density of distance runners have found considerably lower energy intakes than daily recommended amounts, let alone extra energy requirements for exercise training. However, most of these studies have focused on amenorrheic runners and the influence of menstrual dysfunction on bone and energy intake has been allocated a subordinate role or left unaddressed (Myerson *et al.,* 1992; Myburgh *et al.,* 1993; Rencken *et al.,* 1996). The prevalence of low BMD in these runners has been addressed in chapter 2.3.4. On closer inspection of the results from these studies, it appears that even eumenorrhoeic runners report substantially low energy intakes (Marcus *et al.,* 1985; Micklesfield *et al.,* 1995) and many eumenorrhoeic runners have reduced BMD compared to normal data (Myerson *et al.,* 1992; Micklesfield *et al.,* 1998; Pettersson *et al.,* 1999).

The severity of osteopenia is higher in women who are oestrogen-deficient and anorexic compared to those who have hypothalamic amenorrhea alone (Grinspoon *et al.*, 1999) and oestrogen therapy has been unsuccessful in reversing the bone loss in women with anorexia (Munoz *et al.*, 2002) until nutrition is improved (Klibanski *et al.*, 1995). It is possible that the main cause of bone loss in runners and anorexic individuals may share a similar aetiology encompassing energy balance.

Significant bone loss occurs in women who have eating disorders, notably anorexia-nervosa (Rigotti *et al.*, 1984; Grinspoon *et al.*, 1999). Restrictive eating habits have been associated with bone loss in female runners regardless of menstrual status (Cobb *et al.*, 2003). Although the authors did not use biochemical tests of sex steroids, they did report interesting information to associate disordered eating measured using the Eating Disorder Inventory (EDI), with reduced BMD independent of overt menstrual function. The EDI questionnaire is used to assess for the risk of disordered eating-high scores indicate a high risk for eating disorders. Cobb *et al* (2003) found that both menstrual dysfunction and disordered eating independently predicted low BMD. Runners with menstrual disorders had a 5% reduction in lumbar spine BMD compared to eumenorrhoeic runners. However, regardless of menstrual status, runners with elevated EDI scores had 11% lower spine, 5% lower hip and 5% lower total body BMD, than runners with normal EDI scores. These findings suggest that nutrition factors may have more of an influence on BMD than menstrual disorders. There is also data to suggest that eumenorrhoeic runners with restrictive eating behaviours are more likely to suffer a bone-related injury, but whether this is related to low BMD is not known (Beals and Manore, 2000).

(iv) Endurance exercise, endocrine alterations and bone remodelling

In addition to energy deficiency *per se*, endurance exercise is also associated with endocrine alterations that have the potential to negatively affect bone. Research has shown that prolonged endurance exercise, such as the marathon, leads to significant reductions in IGF-1 and IGFBP-3 (Koistinen *et al.*, 1996). Other endocrine alterations include an increase in the secretion of interleukin-6 (Pederson *et al.*, 2001), tumour necrosis factor (Moldoveanu *et al.*, 2000), growth hormone (Ronson *et al.*, 2001) and cortisol (Viru, 1992). These alterations are associated with energy metabolism and conservation and as discussed in chapter 1, can exert negative effects on bone.

In summary, distance running and negative energy balance can result in metabolic and endocrine disturbances such as reductions in sex steroids and IGF-1, which in turn leads to negative alterations in bone remodelling (Loucks *et al.*, 1998; Zanker and Swaine, 1998; 2000). Evidence indicates that distance runners are a particular sub-set of athletes at risk for energy deficiency. However, no study has primarily investigated the relationship between reported energy balance and BMD in this population.

2.3 Factors associated with low BMD in runners

Over the last two decades, explanations for the problem of low BMD in endurance athletes and indeed, distance runners, have included low calcium intake, low body mass and body fat and the most popular explanation dominating the literature has been a deficiency in the reproductive hormones, notably oestrogen. This section

reviews the evidence concerning the current explanations and develops the rationale for this thesis.

2.3.1 Low calcium intake

Although the UK RDA for calcium is 700mg day $^{-1}$, the NOS recommend that female athletes consume at least 1000mg day $^{-1}$ (NOS, 2002). This is because research in male and female runners suggests they may require higher calcium intakes due to additional sweat calcium losses (Klesges *et al.*, 1996), muscular contractions (Heaney, 2000) and elevated levels of parathyroid hormone (PTH; Grimston *et al.*, 1993) which triggers the release of calcium from bone (Rosen, 2000). It is possible that these factors may contribute to bone loss particularly if calcium intake is low. It is recommended that amenorrhoeic athletes consume adequate calcium since oestrogen deficiency reduces calcium absorption (Schutz and Morris, 1999).

It has been hypothesised that athletes who restrict their energy intake will consume insufficient calcium (Sundgot-Borgen *et al.*, 1993; Clarkson and Haymes, 1995) and that calcium deficiency may contribute to the observed low bone density in many runners (Dalsky, 1990; Wolman et al., 1992). However, the majority of studies to date have found that calcium intakes in male and female runners are above and beyond the UK RDA (Bilanin *et al.*, 1989; Pettersson *et al.*, 1999; Gremion *et al.*, 2001). These are shown in table 2.4.

Table 2.4: Previously reported calcium intakes amongst distance runners

Study	Subject status (n=)	Calcium mg day $^{-1}$
Drinkwater *et al.*, 1984	amenorrhoeic † (14)	960 (98)
	eumenorrhoeic (14)	1100 (153)
Marcus *et al.*, 1985	amenorrhoeic † (11)	738 (98)
	eumenorrhoeic (6)	1129 (300)
Bilanin *et al.*, 1989	male runners† (13)	1373 (486)
	non-runners	1267 (236)
Micklesfield *et al.*, 1995	amen/oligomenorrhoeic† (10)	774 (350)
	eumenorrhoeic (15)	705 (459)
Rencken *et al.*, 1996	amenorrhoeic† * (29)	1062 (37)
	eumenorrhoeic (20)	981 (32)
Winters *et al.*, 1996	female runners† (10)	1090 (592)
	active controls (10)	641 (207)
Pettersson *et al.*, 1999	amenorrhoeic† (10)	1107 (758-1888)
	eumenorrhoeic (10)	1024 (408-1808)
Gremion *et al.*, 2001	amen/oligomenorrhoeic† (11)	904 (121)
	eumenorrhoeic (10)	765 (126)
Burrows *et al.*, 2003	amenorrhoeic and eumenorrhoeic† (52)	831 (257)
Cobb *et al.*, 2003	female normal eating habits (67)	1467 (96)
	high eating disorder score (23)†	1300 (147)

† *low lumbar spine BMI*
* *low at weight-bearing sites*

In summary, although many runners have reported substantially low energy intakes, this has not compromised their intake of calcium (Nelson *et al.*, 1986; Marcus *et al.*, 1985; Bilanin *et al.*, 1989). As bone density is compromised in these runners, it appears that even recommended levels of calcium intake cannot counteract their bone loss.

2.3.2 Low body mass and body fat

Distance runners are typically of small frame and lean physique with a lower body mass and body fat than non-runners (Bennell *et al.*, 1997; Neville *et al.*, 2003). Positive relationships have been made between percentage body fat and BMD in female (Pettersson *et al.*, 1999; Burrows *et al.*, 2003) and male runners (Lima et al., 2001). Although not all studies have found associations between body fat and BMD in runners (Myerson *et al.*, 1992; Gremion *et al.*, 2001), this may reflect the similarities in body composition between the subjects. The low levels of body fat in runners may be resultant of the combination of intensive exercise training coupled with low energy and low fat diets. The chronic catabolic state arising from energy deficiency, particularly when accompanied by prolonged endurance exercise will result in a reduction in body mass and body fat. Although runners generally have greater lean body mass which may exert beneficial effects on bone density (Khosla *et al.*, 1996; Ferretti *et al.*, 2002) the overall effect of low total body mass may be negative (Munoz and Argente, 2002).

Although it is possible that genetically smaller and lighter athletes self-select into running as it fits their body type, some athletes may maintain low body mass by training and it is possible that low body mass and body fat are physiological signs of under-nutrition (Pritchard *et al.*, 1996; Ryan *et al.*, 1998). In addition to the metabolic association, low body mass independent of energy balance would result in a lower magnitude of loading to skeletal sites during running. As discussed in chapter one, in order for mechanical loading to generate a positive skeletal response, it must be of sufficient magnitude (Frost, 1992; 1997). Thus, a lower body mass may be expected to result in a lower strain magnitude during running compared to a higher body mass.

This has been supported in several cross-sectional studies demonstrating a positive effect of body mass and BMI on BMD at the lumbar spine, femoral neck, trochanter, total femur and tibia in distance runners (Drinkwater et al., 1984; 1990; Goodpaster *et al.,* 1996; Rencken *et al.*, 1996; Pettersson *et al.,* 1999; Burrows *et al.,* 2003).

Variations in body mass may confound BMD results in athletes. Drinkwater *et al* (1984) found reduced lumbar spine and femoral shaft BMD in their group of amenorrhoeic distance runners. However, after using analysis of covariance (ANCOVA) with body mass as the covariate they found that lower BMD remained at the lumbar spine but not the femoral shaft. Several studies have presented results in terms of both observed BMD and weight-adjusted BMD (Myerson *et al.*, 1992; Cobb *et al.*, 2003). However, a number have not (Rencken *et al.,* 1996; Burrows *et al.,* 2003; Gibson *et al.,* 2004). In their methods section, Rencken *et al* (1996) defended their decision not to weight-adjust BMD for two reasons. First, body mass was not a significant predictor of BMD at all sites and second, at sites where body mass was influential, the adjusted means as determined by ANCOVA varied from the actual means by less than 1.0%.

2.3.3 Site-specificity of mechanical loading

Although high energy expenditures characterise all endurance sports, distance running differs from sports such as swimming and cycling, in that it generates mechanical loading from impact ground-reaction forces. Indeed, running is highly repetitive with many cycles of foot strike and exposure to force application.

The most common site for bone loss in runners is the lumbar spine (Drinkwater *et al.*, 1984; Marcus *et al.*, 1985; Myerson *et al.*, 1992; Hetland *et al.*, 1993; Gremion *et al.*, 2001) which suggests that the mechanical strain from running cannot counteract whatever is causing their bone loss. As the spine is further from the area of force application, it would be expected to receive substantially less impact from the mechanical strain generated during running activity, compared to the lower extremity sites. Frost (1997) proposed that BMD would be elevated in the lower extremity skeletal sites of distance runners, based on the principle that strain must be localised and of a sufficient magnitude for a positive bone response to occur. However, studies in distance runners have reported conflicting results.

A number of studies have provided evidence to indicate that BMD at weight-bearing sites is normal or above normal in distance runners. In a study of male and female track and field athletes, Bennell *et al* (1997) found that male and female distance runners had significantly greater BMD at the femur, tibia, fibula and foot compared to non-athletic controls. In athletes with normal or irregular menstrual cycles, Gremion *et al* (2001) found that Z-scores at the femoral sites were significantly above zero in all runners regardless of menstrual status. Several studies have indicated that although BMD at weight-bearing sites was normal, it was not as high as would be expected when considering their large training volume (Bilanin *et al.*, 1989; MacDougall *et al.*, 1992; MacKelvie *et al.*, 2000).

Moderate levels of running have been associated with high or improved BMD at various skeletal sites (Snow-Harter *et al.*, 1992; Mussolini *et al.*, 2001). A population-based study in men found that those who jogged at least 9 times per month had greater

total femur BMD than men who did not jog or who jogged 1 to 8 times per month (Mussolini *et al.,* 2001). In women, 3-4 sessions of jogging per week (totalling 10.5 miles per week) resulted in a 1.3% increase in lumbar spine BMD over 8 months (Snow-Harter *et al.,* 1992). However, such levels of running training are low in comparison to the volume of training performed by more competitive runners.

In highly trained athletes running at least 6 days per week (totalling over 45 miles per week), negative associations have been found between running mileage and lumbar spine BMD (MacDougall *et al.*, 1992; Hetland *et al.,* 1993; Winters *et al.,* 1996; Burrows *et al.,* 2003). The evidence has mainly been provided in the few studies conducted in male athletes and may reflect the wider range of training volume in men compared to women. One study in male runners concluded that there was no benefit to be gained at weight-bearing sites in those who ran over 58 miles per week (95km) (MacKelvie *et al.*, 2000). Similarly, MacDougall *et al* (1992) identified a running threshold of 20 miles per week in male runners, above which no extra skeletal benefits at the lower limbs were gained (MacDougall *et al.,* 1992). In both studies, high mileage runners (60-75miles per week) tended to have lower BMD than non-active controls. An earlier study, also in highly trained male runners observed a significant inverse correlation between weekly running mileage and lumbar spine BMD (Bilanin *et al.,* 1989).

In female runners, Winters *et al* (1996) observed a negative correlation between weekly running mileage and lumbar spine BMD in female runners. Burrows *et al* (2003) computed regression analyses and their results indicated for every 6 mile (10km) increase in weekly running mileage, there was a 2% decrease in femoral neck

BMD. From the research to date, it appears that running 15-40 miles per week may be optimum and that higher mileages may have a detrimental affect (Macdougall *et al.*, 1992; MacKelvie *et al.*, 2000; Burrows *et al.*, 2003).

Several studies have reported compromised BMD at weight-bearing sites such as the tibia and femoral shaft in female runners with menstrual disorders (Myerson *et al.*, 1992; Rencken *et al.*, 1996; Pettersson *et al.*, 1999). Myburgh *et al* (1993) found that BMD values at the femur were 13 to 19% lower than age-matched reference data. However, unlike running, it appears that the exercise training undertaken by gymnasts is optimal for preserving or increasing BMD at various skeletal sites including the lumbar spine, despite inadequate dietary intake, a history of menstrual dysfunction, and/or current menstrual dysfunction (Robinson *et al.*, 1995; Taaffe *et al.*, 1997; Snow *et al.*, 2000; Zanker, 2004). One study found significantly greater BMD in gymnasts compared to cross-country runners aged 18 to 22 years and there were no associations with menstrual status and BMD (Bemben *et al.*, 2004). Thus, it is possible that the forces generated by running are insufficient to protect from bone loss at lower skeletal sites, particularly in those engaged in high volume training. However, the results to date have been conflicting and continue to present a challenge to researchers in bone and sports medicine, emphasising that further research is required.

2.3.4 Low BMD and stress fractures

Stress fractures are overuse injuries that are particularly common amongst distance runners (Bennell *et al.,* 1996b; Korpelainen *et al.,* 2001), and develop from micro-cracks that result from excessive repetitive loading beyond the strain micro-damage threshold level (Chilibeck *et al.,* 1995). Micro-cracks reduce the energy-absorption capabilities of bone by approximately 40% and can develop into stress fractures when vulnerable sites are exposed to continuous loading (Reilly and Currey, 2000). Thus, even small repetitive strains can result in fracture.

In normal circumstances BMU-based remodelling successfully repairs micro-damage, resulting in the deposition of bone that can withstand the new mechanical strain (Frost, 1987). By this process, the osteoclasts remove fatigue-damaged bone to enable the osteoblasts to deposit new bone matrix and mineral, preventing micro-damage accumulation (Frost, 1997). However, if adequate time is not allowed for new bone to accumulate or if there is an alteration to remodelling, the continuous application of stress (such as in running) can result in a site-specific stress fracture (Bennell *et al.,* 1996; Boden *et al.,* 2001).

Stress fractures in runners occur most frequently in the predominantly cortical, lower extremity sites, where overload is most likely to occur. Common sites are the tibia, metatarsals, pelvis and sacrum (Bennell *et al.,* 1996; Nattiv, 2000; Korpelainen *et al.,* 2001). Compared to sprinters, jumpers and ball game players, distance runners appear to be at a greater risk for multiple stress fractures (Korpelainen *et al.,* 2001) and

research has found the most common site of fracture to be the tibia (Bennell *et al.*, 1996; Korpelainen *et al.*, 2001).

It has been suggested that BMD is a major determinant of stress fracture risk,

> *"for the same stress, less dense bones bend to a greater degree than do more massive bones, and hence they incur more fatigue damage",*

(Heaney *et al.*, 2000; p988).

Consistent with this hypothesis, low BMD is a risk factor for stress fractures in athletes (Myburgh *et al.*, 1990; Johnson *et al.*, 2001). A cross-sectional study of male and female athletes revealed that those who had suffered a stress fracture had significantly lower spine BMD (Myburgh *et al.*, 1990). In a prospective cohort study of track and field athletes, the subgroup of women who developed tibial stress fractures had 8% lower BMD at the tibia compared to those who did not (Bennell *et al.*, 1996). The same study did not find any relationship between stress fracture incidence in men and BMD. However, a more recent study conducted in male athletes with comparable training regimens, found that those with long-term medial tibial stress syndrome had 23% lower tibial BMD than athletes without (Magnusson *et al.*, 2001). Other studies have found no relationship between stress fracture incidence and bone density (Crossley *et al.*, 1999; Korpelainen *et al.*, 2001). Conflicting results are likely to indicate that factors aside from low BMD may also contribute to the development of stress fractures. In addition to BMD, bone strength is a function of its structural properties (Seeman, 2001) and thus, geometric qualities are likely to contribute to stress fracture risk. The amount of load bone can withstand is

proportional to the cross-sectional area of bone. Bones with a larger cross-sectional area and with bone tissue distributed further away from the central neutral axis are stronger and more resilient (Seeman, 2001). Research in athletes is required to investigate the role of bone structure and geometry in male and female athletes.

Numerous studies have reported correlations between menstrual irregularity, osteopenia and the incidence of stress fractures among runners (Drinkwater *et al.*, 1984; Marcus *et al.*, 1985; Barrow and Saha, 1988; Myburgh *et al.*, 1990; Pettersson *et al.*, 1999). This may reflect the effect of low BMD, for runners with menstrual disorders have been found to have lower BMD than their eumenorrhoeic counterparts. Dietary restriction and abnormal eating habits are also related to stress fracture incidence in female athletes (Bennell *et al.*, 1995; Nattiv *et al.*, 1997). Again, these runners may also have lower BMD and a higher prevalence of menstrual disorders.

In summary, repetitive mechanical loading from distance running, may increase microdamage to bone, particularly when BMD is low. If this damage is not adequately repaired, then stress fractures are likely to result.

2.3.5 Sex hormone suppression

As discussed in chapter 1, oestrogen has a fundamental role in bone metabolism and an oestrogen deficiency can lead to bone loss via augmented bone turnover and resorption. The following section reviews the evidence to date, concerning the link

between sex hormone deficiency and bone loss in female distance runners with and without menstrual disorders, as well as the evidence concerning bone loss in men.

i) Delayed Menarche

There are uncertainties as to whether delayed menarche has a negative effect on adult bone health for no longitudinal investigation has controlled confounding variables and followed subjects from puberty to the fourth decade. From studies conducted in female athletes, most indicate that menarchal age has a relatively minor role, if any on BMD. Myburgh *et al* (1993) found a significantly later age of menarche in amenorrhoeic runners compared to eumenorrhoeic runners (14.2 *v* 12.4 years) but this was found to be unrelated to BMD. Rencken *et al* (1996) also reported a later age of menarche in amenorrhoeic compared to eumenorrhoeic runners (14.4 *v* 13.1 years) although this was only predictive of BMD in the amenorrhoeic group.

Although age of menarche appears to occur later in athletes than in non-athletes (Bennell *et al.,* 1997; Warren, 1999) there is no evidence to suggest that exercise *per se* is the cause. It may be argued that young people who are lighter and smaller may self-select into distance running in keeping with their body type. Self-selection into sport according to genetic predisposition, later maturation and smaller body type may reflect the susceptibility of runners to a later age of menarche. However, Bennell *et al* (1997) reported that power athletes (shot putters, javelin throwers, sprinters) had a similar age of menarche to the endurance group (distance runners, walkers) (14.3 *v* 14.3 years). Both groups had a significantly later age of menarche than non athlete controls (13.2 years) and the power group had significantly greater BMD and muscle mass than the endurance group. Thus, self-selection into sport does not appear to be a

major determinant of age of menarche and age of menarche does not appear to be a major determinant of BMD in athletes. It is likely that confounding variables following menarche including nutrition, body mass, exercise and total months of oestrogen exposure (Micklesfield *et al.*, 1995; 1998) are more influential.

ii) Menstrual disorders and BMD

Menstrual disorders in female athletes occur through a reduction in sex hormones which result from the suppression of the hypothalamic pulsatile release of the GnRH pulse generator (Nussey and Whitehead, 2001; Warren and Perlroth, 2001). This results in decreased secretion of luteinising hormone (LH) and to a lesser extent follicle-stimulating hormone (FSH), which in turn, limits the production of the gonadotropic hormones- oestrogen and progesterone, resulting in menstrual dysfunction (Chilibeck, 2000; Warren and Perlroth, 2001). Mild suppression of the menstrual cycle arises from a prolonged follicular phase or the absence of an adequate surge of LH or oestrogen during mid-cycle (Warren and Perlroth, 2001). Delayed menarche or secondary amenorrhoea occurs due to very low levels of LH (Loucks *et al.*, 1989; Warren and Perlroth, 2001). Menstrual disturbances that commonly occur in athletes include primary and secondary functional hypothalamic amenorrhoea, oligomenorrhoea and luteal phase deficiency (Loucks *et al.*, 1990; De Souza *et al.*, 1998). These are defined in figure 2.3.

Figure 2.3: Menstrual disorders in female athletes

Eumenorrhoea refers to the state of general menstrual cycle regularity of ten to thirteen menstrual cycles annually (Rencken *et al.*, 1996; Keen *et al.*, 1997).

Primary amenorrhoea is the failure to attain menarche by the age of 16 years (Warren, 1999). It has been suggested that the reproductive axis is more vulnerable during puberty and cases of primary amenorrhoea may exist in girls who exercise heavily at a young age (Warren, 1999).

Secondary amenorrhoea is present when a female with previously normal menstrual cycles has less than three menstrual periods per year (Rencken *et al.*, 1996; Burrows and Bird, 2000), although the definition may vary according to different sources (De Cree, 1998).

Oligomenorrhoea is menstrual cycle irregularity, with the occurrence of between four and nine menstrual cycles per year (Micklesfield *et al.*, 1995; Burrows and Bird, 2000).

Luteal phase deficits occur without overt menstrual disturbance, despite a shortened luteal phase of less than 10 days (normal length is 14 days) and decreased progesterone secretion (Prior *et al.*, 1990; De Souza *et al.*, 2003).

An early theory for menstrual disorders in athletes was low body mass and body fat. The theoretical basis for this theory originates from the work of Frisch and McArthur (1974) who estimated that there is a critical percentage of body fat required for normal reproductive function and development. It was estimated that in order for menarche to occur, 17% body fat is required; for the maintenance of menstruation it

was suggested that 22% body fat is required. However, there are several problems with this hypothesis. Firstly, due to genetic variations between individuals it cannot be assumed that all women will experience a loss of menses when their body fat levels fall below 22%. Secondly, subsequent research has shown that female runners with normal menstrual cycles have body fat levels below these thresholds, for example, Gremion *et al* (2001) found a mean body fat percentage of 15.0 (SD-1.1) in their group of eumenorrhoeic runners. In contrast, amenorrhoeic runners have been reported with body fat percentages as high as 22.7% (SD-1.0) (Cobb *et al.,* 2003). Both of these studies used dual-energy X-ray absorptiometry (DXA) to quantify body composition, thus, the observed variation cannot be attributed to different measurement techniques. Secondly, the 'body composition hypothesis' is based wholly on observational rather than on experimental evidence (Linnell *et al.,* 1984); and the majority of studies have found no significant differences in body composition or BMI between amenorrheic and eumenorrheic distance runners (Nelson *et al.,* 1986; Myerson *et al.,* 1992; Tomten *et al.,* 1998).

Based on accumulating evidence, a more convincing theory is that athletic menstrual dysfunction occurs as a metabolic effort by the body to conserve energy during periods of inadequate energy availability (Williams *et al.,* 2001; De Souza *et al.,* 2003; Loucks and Thuma, 2003). Anorexia-nervosa, even without excessive exercise, is associated with hypothalamic amenorrhoea and bone loss in this population is particularly severe (Rigotti *et al.,* 1984; Grinspoon *et al.,* 1999). This is likely to reflect the cumulative effects of both reduced bone formation arising from under-nutrition, and increased bone resorption from oestrogen deficiency. Many observational studies have found that amenorrhoea is particularly prevalent amongst

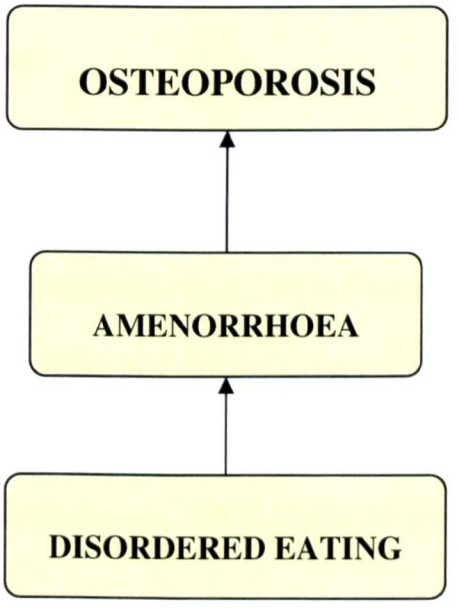
runners who report low energy intakes (Marcus *et al.*, 1985; Tomten *et al.*, 1996; Pettersson *et al.*, 1999; Cobb *et al.*, 2003). Causal evidence for a link between energy deficiency and menstrual dysfunction in exercising women has been provided by Williams *et al* (1995). Following a four-day exercise trial of intensive running, a 21% reduction in LH pulsatility was induced in women consuming a low energy diet compared to women consuming sufficient calories to meet exercise energy requirements (Williams *et al.*, 1995). In a subsequent study, the authors experimentally induced and reversed amenorrhoea in monkeys by altering energy availability (Williams *et al.*, 2001). In agreement, Loucks *et al* (1998) experimentally disrupted LH pulsatility in regularly menstruating women by restricting their calorific intakes and concluded that low energy availability not exercise stress, suppresses LH pulsatility and causes menstrual dysfunction.

The exact prevalence of menstrual disorders amongst female runners is unknown, although the rate appears to be higher than in the general population (Warren and Perlroth, 2001). Tomten *et al* (1998) found that in 187 Norwegian female runners aged 16 to 40 years, 14.6% were amenorrhoeic and 9.5% were oligomenorroheic, although these runners were performance-matched at elite level. A study of 205 Danish female runners of various performance levels, revealed that the prevalence of amenorrhea was 1% in recreational runners and 11% in elite runners (Hetland *et al.*, 1993a) suggesting elite runners may be more at risk of menstrual disorders.

To date, menstrual dysfunction has generally been regarded as the primary risk factor for bone loss in female athletes and the associated oestrogen deficiency as the major cause (Tomten *et al.*, 1998; Gibson *et al.*, 2000). In response to the problem of

compromised BMD in female athletes who should be aiming to maximise peak bone density, the American College of Sports Medicine (ACSM) devised the '*Female Athlete Triad*' model, shown in figure 2.4. The ACSM defines the female athlete triad as a syndrome occurring in physically active girls and women, and its interrelated components are illustrated in figure 2.4, comprising of disordered eating, amenorrhoea and osteoporosis (Yeager *et al.,* 1993). The triad infers that osteoporosis in female athletes is caused by amenorrhoea (lack of oestrogen) and that amenorrhoea is caused by disordered eating, in the sequence shown in figure 2.4.

Figure 2.4: The Female Athlete Triad



The purpose of the Triad is to promote understanding of the factors underlying bone loss in female athletes, to enable the identification of those at risk and to provide the direction for physician guidelines, educational materials and research (Yeager *et al.,* 1993).

In 2001, Khan *et al* produced a review of the Triad's disorders and their prevalence in female athletes, entitled *"New criteria for female athlete syndrome?"* (Khan *et al.,* 2001). The authors argued that rather than 'osteoporosis' as the defining criteria for the Triad, 'osteopenia' or low bone mass would be more relevant to the young population and identify better those at risk for serious skeletal problems.

Indeed there have been many reports of reduced BMD in amenorrhoeic runners including cases of osteoporosis and osteopenia (Drinkwater *et al.,* 1984; Rutherford *et al.,* 1993; Pettersson *et al.,* 1999). However, there have also been several indications of osteopenia in eumenorrhoeic runners and reports of reduced BMD in male runners (Grimston *et al.,* 1993; Hetland *et al.,* 1993a). These have received considerably less attention in the literature.

The majority of studies have investigated BMD in amenorrhoeic runners relative to eumenorrhoeic runners (Drinkwater *et al.,* 1990; Pettersson *et al.,* 1999) or sedentary controls (Myerson *et al.,* 1992) and deficits of up to as 16% have been reported (Petterson *et al.,* 1999). These BMD decrements are most often reported at the lumbar spine (Cann *et al.,* 1980; Compston, 1997). Table 2.5 presents these studies and their main findings.

Table 2.5: Low lumbar spine bone mineral density in female distance runners

A=amenorrhoeic runners
O=oligomenorrhoeic runners
E=eumenorrhoeic runners
O/A=oligo-amenorrhoeic runners
DPA=dual photon absorptiometry
DXA=dual-energy X-ray absorptiometry
CT=computed tomography
C=controls

Study	Subjects (age, yrs)	Technique	Lumbar Spine BMD (g cm^{-2})			
			E	A/O	% difference *(A/O v E)*	C
Drinkwater *et al.*, 1984	A=14 E=14	DPA	1.30	1.12*	-14%	
Marcus *et al.*, 1985	A=11 E=6 (19-29)	CT	182 (mg/cm^3)	151* (mg/cm^3)	-13%	
Cook *et al.*, 1987	O=19 E=17 (15-44)	DPA Lunar	1.226	1.131*	-8%	
Myerson *et al.*, 1992	A=13 E=13 C=12 (21-35)	DPA Lunar	1.049 *(-4% than SC)*	0.942*	-10%	1.085
Myburgh *et al.*, 1993	A=12 E=12 (20-36)	DXA Norland	1.050	0.928*	-12%	
Micklesfield *et al.*, 1995	O/A=10 E=15 (20-39)	DXA Hologic	1.088	0.946*	-13%	
Micklesfield *et al.*, 1998 Follow-up	O/A=7 E=12 SC=8 (29-46)	DXA Hologic	1.043 *(-5% than SC)*	0.936*	-10%	1.094
Pettersson *et al.*, 1999	A=10 E=10 C=16 (16-30)	DXA Lunar	1.25 *(-7% than AC)*	1.05*	-16%	1.34
Gremion *et al.*, 2001	O=11 E=10 (19-37)	DXA Hologic	1.077	0.941*	-13%	
Cobb *et al.*, 2003	O/A=33 E=58	DXA Hologic	1.01	0.94	-7%	

Lumbar spine BMD in amenorrhoeic runners has been found to be significantly lower compared with eumenorrhoeic runners of the same age. One of the earliest cross-

sectional studies was conducted by Drinkwater *et al* (1984) who compared 14 amenorrhoeic and eumenorrhoeic runners, matched for age (mean age was 25 yr), body mass, percent body fat and age of menarche, and found that mean spine BMD was 14% lower in amenorrhoeic runners. Subsequent studies have made similar findings (Marcus *et al.,* 1985; Micklesfield *et al.*, 1995; Rencken *et al.,* 1996; Pettersson *et al.,* 1999; Gremion *et al.,* 2001).

Some studies report that amenorrheic runners have normal (Taaffe *et al.*, 1997; Gremion *et al.,* 2001) or above normal (Rutherford *et al.*, 1993; Okano *et al.,* 1995) BMD at weight-bearing sites, suggesting that running protects the lower body skeletal sites from bone loss. However several other studies have found that BMD is reduced below age-matched normal values, in female athletes participating in large amounts of running (Myerson *et al.,* 1992; Myburgh *et al.,* 1993; Rencken *et al.,* 1996; Pettersson *et al.,* 1999) which is regarded as a weight-bearing activity (Snow-Harter *et al.*, 1992). Conflicting results may have arisen due to variations in duration of amenorrhoea, the timing of amenorrhoea, genetic factors and factors other than amenorrhoea, such as energy and/or calcium deficiency, that may amplify the negative effects on bone.

iii) Sub-clinical menstrual disorders

Asymptomatic menstrual disorders such as luteal phase deficit (LPD), can only be identified using biochemistry, but may occur in apparently eumenorrhoeic athletes. An early study found reduced spine BMD in a group of 15 female marathon runners with LPD and it was concluded that their bone loss was associated with markedly decreased progesterone concentrations (Prior *et al.,* 1990). However, De Souza *et al* (1997) measured bone turnover markers in addition to BMD and menstrual status in

14 ovulatory runners and 10 runners with LPD. BMD was similar between groups and there were no differences in multiple assays of bone formation or bone resorption markers. It is also noteworthy that the runners in De Souza's group were matched for weight and training characteristics. Therefore it is possible that the low BMD in eumenorrhoeic compared to controls in Prior's study (Prior *et al.,* 1990), may not be due to LPD but to a different mechanism associated with body mass and training volume (Myerson *et al.,* 1992; Pettersson *et al.,* 1999; Cobb *et al.,* 2003).

2.4 Key inconsistencies in the literature

The literature seems to widely assume that reductions in sex hormones are the driving force behind the bone loss observed in distance runners. However, although oestrogen deficiency has an undisputed role in alterations in bone remodelling, the common surmise that this is the only cause of bone loss in athletes is not entirely substantiated. Indeed, a relationship between menstrual dysfunction and bone loss has been established, but several inconsistencies have emerged from work in both male and female runners to suggest that a reduction in sex hormones may not be the only factor involved in the genesis of their bone loss. This evidence will now be reviewed.

2.4.1 Bone Remodelling

There is evidence linking energy deficiency to a reduction in bone formation and bone turnover in women with anorexia (Grinspoon *et al.,* 1995; 1997; Heer *et al.,* 2002), amenorrhoeic distance runners (Okano *et al.*, 1995; Zanker and Swaine, 1998) in male runners under conditions of energy deficiency (Zanker and Swaine, 2000) and in

military female recruits with energy deficiency (Ihle and Loucks, 2004). As shown in table 2.6, this pattern of bone remodelling is different to that found in oestrogen deficiency (Manolagas and Jilka, 1995; Prestwood *et al.*, 2000) and energy deficiency may be contributing to the reported bone loss in runners. This has been systematically argued by Zanker (2004) in her recent review of the evidence that energy deficit causes metabolic and endocrine disruptions, altering bone turnover, with reductions in bone formation (Zanker, 2004).

Table 2.6: Bone remodelling as identified in postmenopausal women, amenorrhoeic runners and women with anorexia-nervosa

Study	Subjects	Bone turnover
Manolagas and Jilka, 1995 *Review*	Postmenopausal women	⇑ formation ⇑ resorption ⇑ turnover
Okano *et al.*, 1995	Adolescent amenorrhoeic distance runners	⇓ formation
Zanker and Swaine, 1998	Amenorrhoeic distance runners	⇓ formation ⇓ resorption ⇓ turnover
Grinspoon *et al.*, 1996	Amenorrhoeic women with anorexia nervosa	⇓ formation ⇓ turnover

Interestingly, from reviewing the literature a large number of studies was found reporting low energy intakes amongst amenorrhoeic running subjects but this received little attention in conclusions with no attempt by the authors to quantify energy balance (Drinkwater *et al.*, 1984; Marcus *et al.*, 1985; Nelson et al., 1986; Myerson *et al.*, 1992).

2.4.2 Treatment of bone Loss in female runners

Although results vary according to study design, duration and subject sample, it has been shown that the use of the oral contraceptive pill (excluding depot medroxyprogesterone acetate use) is associated with normal (Berenson *et al.,* 2004) and in women with primary ovarian failure, the oral contraceptive pill can maintain BMD (Castelo-Branco *et al.,* 2001). It has been argued by Zanker (2004), that if oestrogen deficiency is the major cause of bone loss in amenorrhoeic athletes, such oestrogen replacement therapy would be expected to prevent or even reverse this loss. However, as in women with anorexia nervosa (Kreipe *et al.,* 1993; Grinspoon *et al.,* 2002), amenorrhoeic runners appear to be less responsive to oestrogen treatment via the oral contraceptive pill (Warren and Holderness, 1992; Braam *et al.,* 2003; Hartard *et al.,* 2004).

Successful strategies for improving BMD in amenorrhoeic runners have shown to be increased nutrition, a reduction in training and a small degree of weight gain (Drinkwater *et al.,* 1990; Warren and Holderness, 1992; Keen and Drinkwater, 1997; Gibson *et al.,* 1999; Braam *et al.,* 2003). One study found that improved nutrition and slight weight gain in amenorrhoeic runners resulted in BMD increases of around 6% over 14 months (Drinkwater *et al.,* 1990) and a further follow-up study by the same team found no skeletal improvement despite several years of oral contraceptive pill use (Keen and Drinkwater, 1997). In addition to the independent beneficial skeletal effects of increased body mass, the reduction in training may have led to an improved energy balance and a subsequent increase in BMD.

Reversibility?

The reversibility of bone loss in previously amenorrhoeic women athletes who resume their menses, is not entirely clear as there are few studies examining this. In a follow-up study from Drinkwater's work (Drinkwater et al., 1990), 29 athletes were studied and relevant adjustments were made for the use of different BMD measurement techniques. It was found that despite the resumption of menses and apparently normal cycles, there were no significant improvements to BMD in previously amenorrhoeic runners (Keen and Drinkwater, 1997). Using a similar design, Micklesfield *et al*, (1995; 1998) support these findings with their observation of lower lumbar spine BMD in women marathon runners with a history of menstrual disturbance despite presently regular cycles (Micklesfield *et al.*, 1995). Longitudinally, the authors found that restoration of lumbar spine BMD was slow and did not reach the same level as controls (Micklesfield *et al.*, 1998).

2.4.3 Low BMD in eumenorrhoeic runners?

Although it is generally presumed that low BMD is primarily a problem for amenorrheic athletes only, there have been several indications of bone mineral deficits in eumenorrhoeic runners (Grimston *et al.*, 1993; Cobb *et al.*, 2003). Furthermore, a recent study described in chapter 1.3.5, found that regular menstruation can be maintained in women despite severe energy deficiency and significant bone mineral deficits (Miller *et al.*, 2004). Thus, bone loss may occur despite eumenorrhoea and due to energy deficiency. Table 2.7 summarises evidence from previous studies, for reduced lumbar spine bone mineral density in regularly menstruating runners. Grimston *et al* (1993) explored the calciotropic hormones and BMD in female distance runners and unexpectedly found that spinal osteopenia was present in 4 out

of 10 eumenorrhoeic athletes, whose menstrual status had been verified with biochemistry.

Table 2.7: Low lumbar spine bone mineral density in eumenorrhoeic runners as found in previous studies

Study	Subjects (age years)	Technique	Findings
Grimston *et al.*, 1993	14 runners (29-34)	DPA	4 ER with osteopenia at the spine (29%)
Burrows *et al.*, 2003	52 runners of various menstrual status (18-44)	DXA Hologic	Low LS BMD related to running mileage not menstrual status.
Cobb *et al.*, 2003	ER with high eating disorder scores(EDS)=8 ER with normal EDS=50 O/A=33 (18-26)	DXA Hologic *(no hormone tests)*	LS BMD (g/cm^2): ER=0.91 (-11% than C) C =1.02 O/A=0.94

O/A = oligo/amenorrheoic runners
ER = eumenorrhoeic runners
C = controls

Unfortunately, most of the studies cited earlier in section 1.6.4, table 2.5 did not include information on normal age-matched BMD, in order that the BMD of eumenorrhoeic runners could be compared to normal values. Of the three that did (Myerson *et al.*, 1992; Micklesfield *et al.*, 1998; Pettersson *et al.*, 1999) it appears that eumenorrhoeic runners had lower lumbar spine BMD than controls of -4%, -5% and -7% respectively[2]. These studies also clarified menstrual status by conducting biochemical tests. Oestradiol concentrations were significantly higher in the

[2] According to the mean BMD values for eumenorrheic runners and controls, provided by the authors

eumenorrhoeic runners and in Myerson's study, oestradiol levels were almost 5-fold higher than levels in the amenorrhoeic group (Myerson *et al.*, 1992). Thus, oestrogen deficiency could not explain the bone loss of the eumenorrhoeic runners.

Collectively, there is evidence to suggest that BMD may be reduced in athletes regardless of menstrual status, but that the severity is greatest in those who are amenorrhoeic. Thus, it appears that oestrogen deficiency may not be the only cause of compromised BMD in female runners.

2.4.4 Low BMD in male runners?

Although there is a lack of data regarding the bone health of male runners, several studies have revealed lumbar spine bone deficits of up to 19% in distance runners compared to controls (Bilanin *et al.*, 1989; Hetland *et al.*, 1993b). However, the mechanism by which this occurs is not yet known. Table 2.8 presents the results of the studies conducted in male runners and illustrates the need for further research to investigate the bone status of this population.

It is now recognised that both testosterone and oestradiol are significant for the bone health of men (Szule *et al.*, 2001; Leder *et al.*, 2003). However, in male runners, associations have not been found between BMD and levels of testosterone or oestrogen (Hetland *et al.*, 1993b; MacKelvie *et al.*, 2000; Maimoun *et al.*, 2003). Hetland *et al* (1993b) found significantly lower lumbar spine, total body and proximal femur BMC in elite runners compared to controls, but concentrations of testosterone were normal. MacKelvie *et al* (2000), found that running further than 40 miles per week coincided with significant reductions in testosterone ($p<0.005$) which were

unrelated to BMD. Although further research is required, results suggest that low bone density in male distance runners may be caused by factors other than reductions in sex hormones. This point has been argued elsewhere by Bennell (1996) and Zanker (2004).

Table 2.8: Reported bone status in male distance runners

Study	Male subjects (age years)	Technique	Findings
Bilanin *et al.*, 1989	runners=13 controls=11 (22-35)	Single photon absorptiometry	Runners had -9.7% spine BMD than controls ($p<0.05$)
MacDougall *et al.*, 1992	runners=53 controls=22 (20-45)	DXA	Runners had ⇑ leg BMD but similar BMD to controls at all other sites. No relation with testosterone levels.
Hetland *et al.*, 1993b	runners=120 (19-56)	DXA	⇓ LS, TB and PF BMC in elite high mileage runners. LS -19% than controls. No relation with testosterone.
MacKelvie *et al.*, 2000	runners=12 very high mileage runners=5 high mileage R=7 (40-55)	DXA	No benefits from running very high mileages *(+50miles)*, trend for ⇓ LS BMD. No relation with testosterone levels.

TB = total body *PF = proximal femur*

As in amenorrhoeic runners (Rencken *et al.*, 1996), BMD at weight-bearing sites such as the hip and femur in male runners, has been reported to be normal or lower than in controls (MacDougall *et al.*, 1992; Goodpaster *et al.*, 1996; Bennell *et al.*, 1997). Bennell *et al* (1997) compared the bone status of power athletes and endurance athletes (including distance runners). Male power athletes had higher BMD at all sites compared to the endurance group, even at the foot where it would be expected

distance runners would have an advantage due to the specific loading environment generated during running.

Summary

Despite over two decades of research, the paradox of low bone density in distance runners continues to present a challenge to researchers. On the one hand, the acquisition of maximum peak BMD is enhanced with regular weight-bearing exercise and the two are major strategies to prevent or offset the development of osteoporosis. However, although running is a vertical weight-bearing exercise, its mechanical characteristics appear to be insufficient to counteract bone loss at the spine and even the lower extremity cortical sites in certain distance runners.

Such skeletal deficits are most commonly observed in female distance runners with menstrual disorders, nevertheless this may reflect the fact that most research has only focussed upon this group. The predominant explanation for bone loss in these athletes has been a sex hormone deficiency. However, in the light of more recent evidence it appears that negative energy balance may have a separate and potentially more fundamental role. To date, factors found to be associated with low BMD in female runners are menstrual dysfunction, low BMI, high mileage training and disordered eating (elevated EDI scores).

Less research has been conducted in men, although several reports document low spine BMD in male runners, unrelated to reductions in sex hormones (Hetland *et al.*, 1993b; Bennell *et al.*, 1996). The only variable found to have correlated with reduced BMD in male runners has been high weekly running mileage, (above approximately

45 miles; MacDougall *et al.*, 1992; Hetland *et al.*, 1993b; MacKelvie *et al.*, 2000). Although the mechanism whereby this may have a negative effect on BMD is unclear, it may be associated with energy deficiency, arising from exceptionally high energy expenditures that are not compensated for in terms of dietary energy intake. To date, no study has investigated the relationship between energy balance and BMD. Hence, this is an aim of the present thesis.

Key Points

- Despite high levels of weight-bearing exercise and adequate calcium intake, there are frequent reports of low BMD in distance runners.
- Limited success of oestrogen for treating bone loss in amenorrhoeic athletes.
- Bone remodelling in amenorrhoeic runners is characteristic of bone loss due to under-nutrition rather than to oestrogen deficiency.
- Eumenorrhoeic female runners may also be at risk for bone loss.
- Reports of reduced BMD in male runners, unrelated to sex hormone suppression.
- Deliberate or involuntary energy deficiency may underlie low BMD in male and female distance runners, although research is required.

2.5 Aims and research questions

Distance running is a popular sport. However, serious bone mineral deficiencies are frequently reported in female distance runners with menstrual disorders (Marcus *et al.*, 1985; Gremion *et al.*, 2000). There is a serious prognosis for long-term skeletal health in female athletes who are diagnosed with osteopenia or osteoporosis in young adulthood, particularly as this bone loss may be irreversible (Drinkwater *et al.*, 1990; Keen and Drinkwater, 1995).

With regard to the consequences of persistent low BMD in athletes, low BMD and diminished bone quality has been associated with an increased incidence of stress fracture injury (Myburgh *et al.*, 1990; Bennell *et al.*, 1996). In the long-term, the failure to attain maximal peak bone density and the onset of early bone loss may predispose athletes to premature osteoporosis and future osteoporotic fracture which are just as severe in men as women (Ismali *et al.*, 2000; Blinkley *et al*,. 2002). However, as the main factor presumed to be responsible is oestrogen deficiency characterised by amenorrhoea (Drinkwater *et al.*, 1990; Tomten *et al.*, 1998), it is often assumed that male athletes are not at as high a risk for bone loss as females (Aulin, 1995). In support of this, research conducted in male runners has found that BMD is unrelated to levels of sex steroids (MacDougall *et al.*, 1992; Bennell *et al.*, 1993., Hetland *et al.*, 1993b; Maimoun *et al.*, 2003).

More recent, objective evidence indicates that energy deficit (the discrepancy between dietary energy intake and expenditure) independently leads to a reduction in bone formation (Grinspoon *et al.*, 1996; 2002; Zanker and Swaine, 1998; 2000; Ihle and Loucks, 2004; Miller *et al.*, 2004). Interestingly, short term induced energy restriction

in male runners has also led to reductions in P1CP, a bone (formation) marker of type 1 bone collagen synthesis (Zanker and Swaine, 2000). If this is the case, then male runners may be at a similar risk for bone loss due to exceptionally high energy requirements and the prevalence of low BMD in athletes may be more widespread than once thought.

Thus, there are two proposed theories for bone loss in athletes, oestrogen deficiency (Marcus *et al.,* 1985; De Souza and Williams, 2004) and energy deficiency (Zanker, 2004), with the latter only recently emerging as a potential independent factor. To date, the relationship between energy balance and BMD has not been studied in athletes and no study has previously investigated and compared the bone status of male and female runners. Therefore, it was envisioned that this work would contribute to the understanding of the prevalence and pathogenesis of low BMD in distance runners by fulfilment of two specific aims. Firstly, to determine whether male distance runners are at a comparable risk for bone loss compared to their female counterparts. Secondly, to determine if there is a relationship between reported energy balance and BMD in a large group of male and female distance runners.

With these aims in mind, the following research questions were developed.
The first three questions were designed to explore the incidence of low BMD in distance runners and to address factors other than energy balance that may influence BMD. This enabled the logical progression of the work conducted, to answer the main question concerning energy balance and BMD.

- What proportion of male and female distance runners have a low BMD?

(Chapter 4)

- Is BMD related to overt menstrual status in female runners?

(Chapter 5)

- Is BMD related to training characteristics and performance level in male and female runners?

(Chapter 6)

- Is there a relationship between energy balance and BMD?

(Chapter 7)

The next chapter comprises the thesis methodology and its rationale. Thereafter, chapters 4, 5, 6 and 7 are concerned with answering the above three questions respectively, commencing with a presentation of the results followed by their discussion. Chapter 8 is the overall discussion of the findings, evaluating any possible interactions between the measured variables and BMD of the runners. The concluding chapter (chapter 9) evaluates the key findings of this thesis and their implications.

Chapter 3

Methodology

"First have a goal, an objective. Second, have the necessary means; wisdom, money, materials and methods. Third, adjust all your means to that end".

Aristotle, Greek Philosopher. BC 384 - 322

The opening section of this chapter is the methodological review which provides the rationale for the methods used. Sections 3.2 to 3.8 describe these methods, detailing the tools and procedures used to gather the data.

3.1 Methodology review

There were two aims of this work: first, to investigate the incidence of low bone density in male and female distance runners and second, to determine if there is a relationship between reported energy balance and BMD. To achieve these aims two main outcome measures were required: bone mineral density (BMD) and energy balance. The rationale for the methods chosen to assess these parameters follows.

3.1.1 Measurement of bone

The technique used to measure BMD was bone densitometry by dual-energy X-ray absorptiometry (DXA). The principles of DXA will now be reviewed and a rationale

provided as to why DXA was considered the appropriate method for the research conducted in this thesis.

(i) Bone densitometry by dual-energy X-ray absorptiometry (DXA)

The measurement of BMD by DXA is currently the 'gold-standard' technique for the clinical diagnosis of osteoporosis (Melton, *et al.*, 2003; Watts, 2004) and is widely used in research (Pettersson *et al.*, 1999; MacKelvie *et al.*, 2000; Burrows *et al.*, 2003). BMD (g cm $^{-2}$) is the bone mineral content (BMC) per unit of projected area of a region measured using bone densitometry (Duan *et al.*, 1999; Seeman, 2003) and is a major predictor of osteoporotic fracture risk (Marshall *et al.*, 1996; Legrand *et al.*, 2000).

DXA has made valuable contributions to the understanding of bone development and factors affecting BMD (Soyka *et al.*, 2000), to the definition of 'osteoporosis' (Genant *et al.*, 1999; Lewiecki *et al.*, 2004) and to the identification of groups at risk of osteoporosis (Miller *et al.*, 2004). Furthermore, it has comprehensive reference databases for men and women of various ages (Maricic and Chen, 2000) and provides a calculation of diagnostic T-scores and Z-scores. The advantages and disadvantages of the technique are given in Table 3.1.

Table 3.1: Advantages and disadvantages of dual-energy X-ray absorptiometry (DXA)

Advantages	Disadvantages
Low radiation dose of 6.7-31 mSv (Genant *et al.*, 1991; Njeh *et al.*, 1999)	Unable to differentiate between cortical and trabecular bone tissue (Guglielmi *et al.*, 1994)
Accuracy[1] and precision[2] (Lilley *et al.*, 1991)	Overestimates BMD in the presence of osteoarthritis, scoliosis or a vertebral crush fracture (NOS, 2002)
Ability to measure BMD at axial and appendicular sites (Maricic and Chen, 2000)	Does not account for differences in bone size (Duan, 1999; Seeman, 2001)
Provision of additional accurate information regarding soft tissue composition (lean and fat mass) derived from the total body scan facility (Van Loan and Mayclin, 1992)	
Quality control procedures monitor instrument stability (Maricic and Chen, 2000)	
Short scan acquisition time with fan-beam DXA instruments	
Cost effective and widely available (Placide and Martens, 2003)	

(ii) Bone size

Low BMD is associated with increased fracture risk (Legrand *et al.*, 2000) although over the last decade it has emerged that the geometrical properties of bone may be equally as critical, in light that osteoporotic fracture occurs through both the structural and material failure of bone (Seeman, 2001; 2002; 2003; Henry *et al.*, 2004). Since DXA measures areal BMD (grams per square centimetre) and is a two-dimensional assessment, it fails to adjust for bone size (Duan *et al.*, 1999; Seeman, 2002),

[1] DXA value expressed as a % of the specified BMC. Approximately 3-8% (Lilley *et al.*, 1991)

[2] Degree of variability found in multiple measures of BMD. Usually expressed as co-efficient of variation. Approximately 1% at PA spine and 1-5% at femur (Lilley *et al.*, 1991)

"the densitometer is blind to bone size and shape, critical determinants of bone strength",

(Seeman, 2001, p4583)

This has become one of the major criticisms of DXA and is currently a topic of great interest amongst researchers and clinicians alike. However, DXA continues to be recognised as the most effective and efficient method for assessing BMD (IOF, 2004), accounting for around 60-70% of variation in bone strength (Ammann and Rizzoli, 2003).

Until recently, it was presumed that men have higher BMD than women, but it is now apparent that this discrepancy is due to the larger bone size in men (Henry and Eastell, 2000; Melton *et al.,* 2000; Seeman, 2001; 2002). It appears that previous studies of bone status in athletes have not compared BMD between the sexes. Although Bennell *et al* (1997) did include male and female athletes in their study and BMD comparisons were made between athletes from different sports, there were no comparisons between the sexes. In order to contribute to the literature, a main aim of this thesis was to evaluate and compare BMD status between male and female runners. To achieve this aim it was thus, considered relevant to adjust for the potentially confounding effects of bone size. DXA provides only a two-dimensional view of bone therefore, does not account for bone size.

(iii) Quantitative computed tomography (QCT)

Although quantitative computed tomography (QCT) accounts for bone size by measuring volumetric BMD (Henry and Eastell, 2000), compared to DXA (6.7-31 mSv), it transmits a higher dose of radiation (25-360 mSv) depending on the model and site measured (Njeh *et al.*, 1999). Consequently when using QCT, not only does it become more of a task to get ethical approval for the research, but it becomes more difficult when recruiting volunteers due to the associated risk from higher doses of radiation that might deter participation.

Unlike DXA, QCT cannot perform total body measurements, which were required in this work to quantify body composition. A further prerequisite of this work was to attain three BMD measurements (lumbar spine, dual femur and total body), in order to evaluate any site-to-site differences. QCT can measure forearm and lumbar spine BMD. However, measurements at the hip are less accurate due to the complex architecture of the femoral neck (Khan *et al.,* 2001; NOS, 2002). Moreover, additional scans would involve additional radiation. The cost of QCT is much greater than DXA and the machines are less widely available.

(iv) Bone mineral apparent density (BMAD)

Although DXA has been criticised for its failure to assess volumetric BMD (Duan *et al.,* 1999; Seeman, 2002), it has been suggested that this error may actually improve the effectiveness of areal BMD as a predictor of fracture, since bone size is also a determinant of bone strength (Kanis, 2002). Nevertheless, it was considered relevant to gain an insight into the potential differences in bone size between male and female runners, for this has previously not been investigated. It was also necessary to control

for the potential confounding effect of bone size on BMD in subjects of short stature (runners are often short and petite).

Although studies have attempted to correct for differences in body size by adjusting for body mass or BMI (Kelly *et al.,* 1990; Slosman *et al.,* 1994) this does not consider differences in skeletal size. However, the calculation of bone mineral apparent density (BMAD) provides an estimation of volumetric BMD and reduces the effect of bone size on DXA-derived BMD (Carter et al., 1992; Cvijetic and Korsic, 2004). BMAD ($mg.cm^{-3}$) was first defined by Carter *et al* (1992) and has since been used to determine gender and ethnic group differences in BMD whilst adjusting for bone size (Henry and Eastell, 2000; Melton *et al.,* 2000; Cvijetic and Korsic, 2004). The BMAD parameter has been used in the current study to adjust for differences in bone size between male and female runners at the lumbar spine and dual femur. Further details are provided in chapter 3.2.

(v) Bone biochemistry

Biochemical bone markers can provide information regarding the rate and nature of bone turnover and have previously been used to investigate bone turnover in women with anorexia (Grinspoon *et al.,* 1996) and in female and male runners (Zanker and Swaine, 1998; 2000).

In this study, bone biochemistry was not used as a relatively large number of runners were studied; also as multiple assays would have been required for an accurate assessment of bone turnover (Bennell *et al.,* 1997), it was financially not possible to do so. In addition, bone markers are susceptible to short and long-term fluctuations

depending on the time of day, year and in females, menstrual cycle (Watts, 1999), it would have been necessary to ensure that all subjects were measured at the same time (usually early in the morning following an overnight fast) and in females, at the same stage of the menstrual cycle. This would have required additional biochemical tests for sex hormone levels. Good quality storage and analysis facilities would have also been required, for biochemical bone markers are susceptible to assay imprecision and have a relatively high degree of biological variability (Compston, 1997).

Another consideration is that bone turnover markers reflect the total activity of bone remodelling throughout the skeleton (Eastell and Blumsohn, 1997). This is of particular importance when investigating bone turnover in runners. BMD is site-specific according to the distribution of loading in the given sport therefore distance runners may experience significant losses of bone mineral at the spine, but gains at the lower sites due to impact loading. In such cases total body BMD may be normal despite regional differences thus, it is possible that bone turnover may also appear normal, even though at certain sites significant loss of bone mineral may be occurring.

Studies using bone markers in young adults have had varying success. On the one hand, despite large differences in BMD, no differences have been found in bone markers between groups of athletes (Hetland *et al.,* 1993a; Bennell *et al.,* 1997). In a large group of athletes, Bennell *et al* (1997) hypothesised that athletes would have elevated bone formation and that changes in bone turnover markers would predict changes in BMD. However, over 12 months the bone markers were not associated with changes in BMD. The authors suggested that in order to fully characterise bone turnover, a combination of bone formation markers is required. In agreement, single

measurements of bone markers have been unable to identify differences in bone turnover between eumenorrheic and amenorrheic runners (Tomten *et al.,* 1998) and between runners and non-runners, despite differences in BMD (Hetland *et al.,* 1993a; Matsumoto *et al.,* 1997). On the other hand, several studies have found significant differences in athletes (Okano *et al.,* 1995; Zanker and Swaine, 1998; 2000; Creighton *et al.,* 2001).

Elevated levels of bone formation markers have been found in athletes of high impact sports compared to swimmers (Creigton *et al.,* 2001) and reductions of bone formation have been found in amenorrhoeic runners (Okano *et al.,* 1995; Zanker and Swaine, 1998). These studies used multiple markers of bone turnover. Zanker and Swaine (1998) used three assays for bone formation and two for bone resorption and determined that bone turnover and formation was significantly reduced in energy deficient, amenorrhoeic distance runners. The relationship between energy deficit and bone turnover has previously been identified (Grinspoon *et al.,* 1996; Zanker and Swaine, 1998; 2000; Zanker, 2004), although no study has investigated energy balance and BMD in athletes. Hence, this is the main aim of this thesis.

Summary

The chosen method of bone assessment was DXA-derived BMD and the calculation of BMAD to account for differences in bone size. As discussed, DXA is the universally accepted method for the diagnosis of osteoporosis, involves only low levels of radiation exposure and is non-invasive. The use of DXA was available for the duration of the study period and it was possible to obtain ethical consent for this. It was the aim of this work to assess BMD in runners, for bone markers have

previously been investigated in relation to energy balance in this athletic sub-population (Zanker and Swaine, 1998; 2000).

3.1.2 Assessment of energy expenditure

'Energy expenditure' may be defined as the cumulative amount of energy used by an individual after accounting for the energy cost of essential life processes, daily activities and exercise. It is usually expressed in kilocalories (kcal) or mega joules (MJ) of energy used per day. Throughout this thesis, energy expenditure is expressed as *kcal day* $^{-1}$. The assessment of energy expenditure requires determination of basal metabolic rate (BMR). BMR is the minimal rate of energy expenditure for an individual at rest (McArdle *et al.,* 1999) and constitutes 60-70% of total energy expenditure (Shetty *et al.,* 1994). It can be reliably measured using calorimetry (Battezzati and Vigano, 2001) although this can be time-consuming. For this reason, BMR was estimated using a predictive equation. Predictive equations are the most popular and practical method for estimating BMR although as inter-individual variation is large, the equations are continually being developed to account for factors known to influence energy expenditure, such as body mass and composition (Cunningham, 1980; Owen *et al.,* 1991).

(i) Lean Body Mass and BMR

Lean body mass is a major determinant of BMR because muscle tissue is more metabolically active than fat tissue and differences in lean body mass can explain 60-90% of the variation in BMR between individuals (Cunningham, 1980). As athletes

have a greater lean to fat ratio they will have a higher BMR than sedentary individuals (McArdle *et al.,* 1986, 1999). Numerous studies report higher BMR in athletes compared to non-athletes (Owen *et al.,* 1991; Thompson and Manore, 1996). In a study of male and female distance runners, BMR was measured at least 39 hours after their last run and it was concluded that the higher BMR in the athletes could be accounted for by differences in lean body mass (Thompson and Manore, 1996). Therefore, it is not surprising that De Lorenzo *et al.,* (1999) found that many equations, even those accounting for total body mass (Owen et al., 1987), largely underestimated BMR in athletes. For this reason, the 'Katch-McArdle' formula was chosen to estimate BMR in the runners, as this accounts for lean body mass and can be equally be applied to men and women (Katch *et al.,* 1996).

The Katch-McArdle Formula

BMR = 370 + (21.6 x lean body mass in kg)

(ii) Quantifying energy expenditure

The 'gold-standard' for quantifying energy expenditure in free-living conditions is the doubly-labelled water technique[3] (Livingstone and Black, 2003). Unfortunately, doubly labelled water requires sophisticated laboratory equipment and can be expensive. For the work in this thesis, energy expenditure from exercise data was gathered by 7-day prospective records. This method has been validated against calorimetry and doubly-labelled water, in contrast the 7-day recall method has not

[3] This involves the subject taking a dose of water enriched with deuterium isotopes and oxygen 18. Urine samples are collected at baseline, during and after the study period, then analysed by isotope ratio mass spectrometry. Based on biochemical principles, the rate of disappearance for each isotope determines energy expenditure (Livingstone and Black, 2003).

(Conway *et al.,* 2002). Prospective 7-day physical activity records have been recognised as effective for estimating energy expenditure in athletes (Irwin *et al.*, 2001) and groups (Kalkwarf *et al.*, 1989). Self-reported physical activity records are low cost and unobtrusive (Lamonte and Ainsworth, 2001), therefore were useful for this study.

(iii) Physical activity level

Average daily energy expenditure (EE) can be estimated from BMR multiplied by an appropriate activity factor (PAL) which is dependent on the nature and intensity of general physical activity (Scrimshaw *et al.,* 1994).

EE = BMR x PAL

The International Dietary Energy Consultation Group (IDECG) in the UK (1994) has provided gender-specific PAL values for individuals aged 19-50 years with 'very light', 'light', 'moderate', 'heavy' or 'very heavy' lifestyles (Scrimshaw *et al.,* 1994). These are shown in Table 3.2.

Table 3.2: Physical activity levels (PAL) from the IDECG report (1994)

	Male	**Female**
Very Light	1.3	1.3
Light	1.6	1.5
Moderate	1.7	1.6
Heavy	2.1	1.9
Exceptional	2.4	2.2

The PAL level of 1.3 was used for the runners in this study, unless their occupation reflected otherwise (a PAL level of 1.6/1.5 would be given to runner's who had a more taxing occupation). Although this level denotes a 'very light' lifestyle, it was unsuitable to choose 'moderate', 'heavy' or 'exceptional' levels, for each runner will vary according to their own level of training and exercise. Instead, average energy expenditure from training was determined from the 7-day prospective records, and added to the BMR X PAL equation.

EE = BMR x 1.3 + EE from Exercise

(iv) Energy expenditure from exercise

Energy cost of the runners' exercise training was estimated using Ainsworth's coding system for classifying physical activity by rate of EE (Ainsworth *et al.,* 1993). This system was designed to enhance the comparability of results across studies using self-reports of physical activity. The Compendium is organised by types or purpose of activity, each of which has a specific description in terms of form and intensity. All activities listed have an intensity unit based on their rate of EE and are expressed as METs. As BMR is fairly near to 1 kcal kgbody mass^{-1}.hour^{-1} (Ainsworth *et al.*, 1993), MET values can be multiplied by body mass (kg) to estimate the energy cost of exercise per hour.

EE (per hour) = MET x Body Mass (kg)

In order to accurately assess EE, it was essential that each subject recorded the intensity level and duration of their physical activity in the prospective record. Thus, the exercise record was designed to encourage this. The details of the procedures used

for the data collection have been described in chapter 3.4.2. The Compendium was regarded to be particularly useful for quantifying EE from exercise in the runners, as it provides a comprehensive list of MET values for running at different intensities (minutes per mile).

3.1.3 Assessment of energy intake

'Energy intake' may be defined as the total amount of dietary energy consumed from food or beverages. As with energy expenditure, energy intake is usually expressed in kcal or MJ per day. For this thesis, energy intake is expressed in *kcal day* $^{-1}$. There are several methods available for quantifying energy intake (EI), including food frequency questionnaires, 24 hour recall, diet history and prospective weighed dietary intake records. The technique chosen for the work conducted in this thesis was the 7-day diet record for several reasons. Firstly, validation studies have indicated that this provides a more accurate mean estimation of energy intake compared to diet history (Black *et al.*, 1995), 24 hour recall or food frequency questionnaires (Howat *et al.*, 1994). Secondly, it is low cost and unobtrusive. Prospective dietary records require each subject to weigh or accurately estimate all foods prior to consumption (Winters *et al.*, 1996) and although this is assumed to be the most accurate of the practically feasible methods, it relies greatly on the honesty and compliance of the subjects.

Under-reporting is a potential limitation of dietary records and occurs when the subject does not record all foods consumed, is selective in favour of 'healthy' foods and/or underestimates portion size (Schoeller *et al.*, 1995; Livingstone and Black, 2003). With regard to the potential for sex-differences in dietary reporting, a meta-

analysis of studies examining energy intake and expenditure using doubly-labelled water and report methods found no differences in the accuracy of reporting between female and male athletes (Sjoden *et al.,* 1994).

There appears to be an apparent prevalence of under-reporting amongst female athletes (Schoeller *et al.,* 1995; Livingstone and Black, 2003). This presumption is based on the observed discrepancies between energy intake and expenditure (Edwards *et al.,* 1993; Hill and Davies, 2002). However, biochemical indicators of chronic energy deficiency have been demonstrated in female athletes, who also have low body mass (Myerson *et al.,* 1991; Loucks *et al.,* 1992; Zanker and Swaine, 1998; De Souza *et al.,* 2003). It has also been claimed that female athletes underreport because they tend to remain weight stable throughout the study period, even though dietary records indicate that they should be losing approximately 0.5 kg week $^{-1}$ (Marcus *et al.,* 1985; Winters *et al.,* 1996). However, it is possible that such weight maintenance reflects energy conservation and the adaptation of BMR to low energy availability (chapter 2.2).

There is a degree of random error associated with day-to-day variation in reporting of nutritional intake and it has been suggested that certain nutrients require an increased number of food-recording days in order to gain greater precision[4] (Bingham, 1987). Bingham (1987) used results from several dietary surveys in adults to calculate the number of recording days necessary for various nutrients to be within +/- 10% of average. It was estimated that 5 days were required for energy intake, 6 days for carbohydrate, 7 days for protein, and less than 10 days for fat and calcium (Bingham,

[4] Precision is important to confidently detect the existence of differences between groups of people.

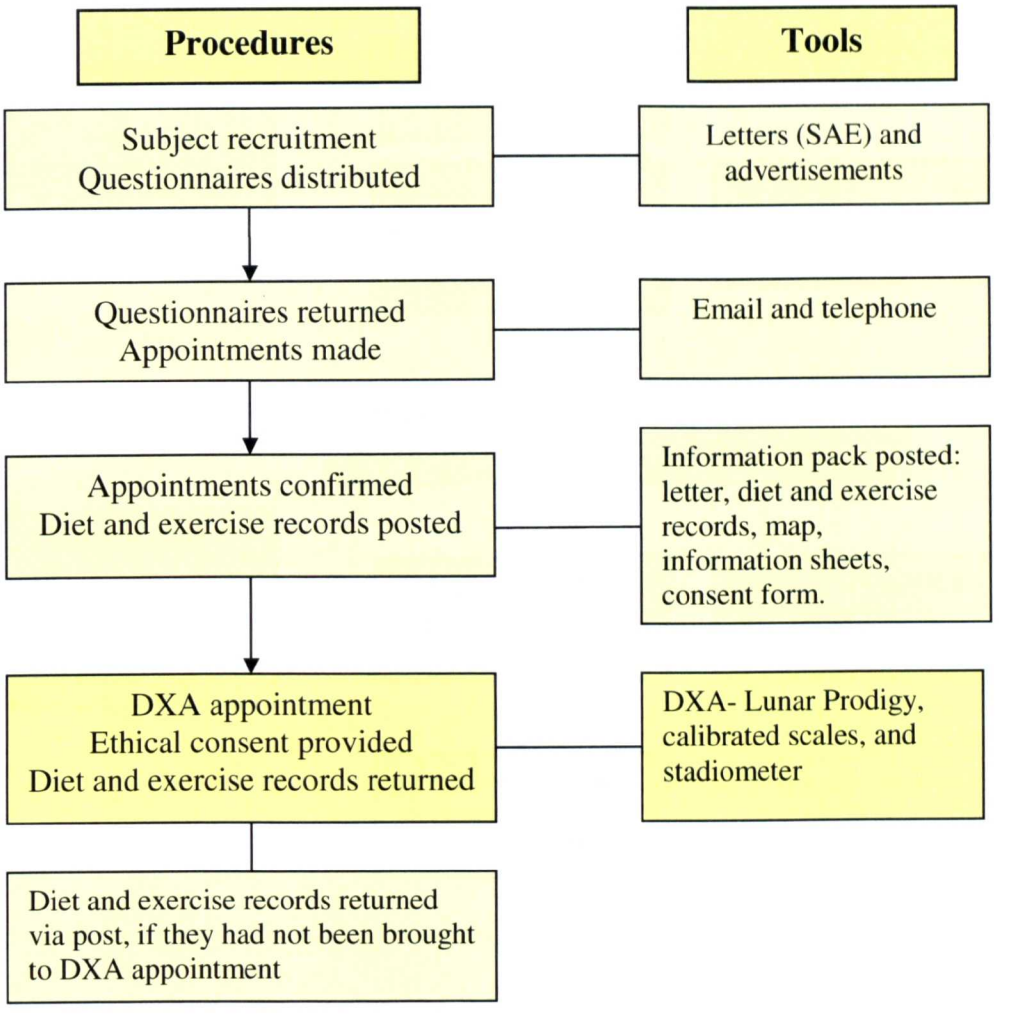
1987). Furthermore, a year-long study investigating variation in the daily energy intakes of men and women found reported energy intake was significantly greater on a Friday and Saturday compared to other days of the week (Basiotis *et al.,* 1989). Thus, studies using only a 3-day recording period may be less effective (Micklesfield *et al.,* 1995; Winters *et al.,* 1996, MacKelvie *et al.,* 2000) than those using 7 days (MacDougall *et al.,* 1992, Pettersson *et al.,* 1999; Burrows *et al.,* 2003). Prospective diet records were used for the current work as they appear to be the most practical and accurate of field methods and the duration of study was 7 days to reduce day-to-day variability. In addition to increasing the number of days in the recording period, precision can be improved by increasing the number of subjects studied (Bingham, 1991).

3.2 Study design and methods

3.2.1 Study design

A cross-sectional study design was used to achieve the aims of the investigation in accordance with the timescale permitted. The research was conducted between 2003 and 2004 at the Leeds Centre for Bone and Body Composition. Figure 3.1 provides an overview of the research procedures and the tools used.

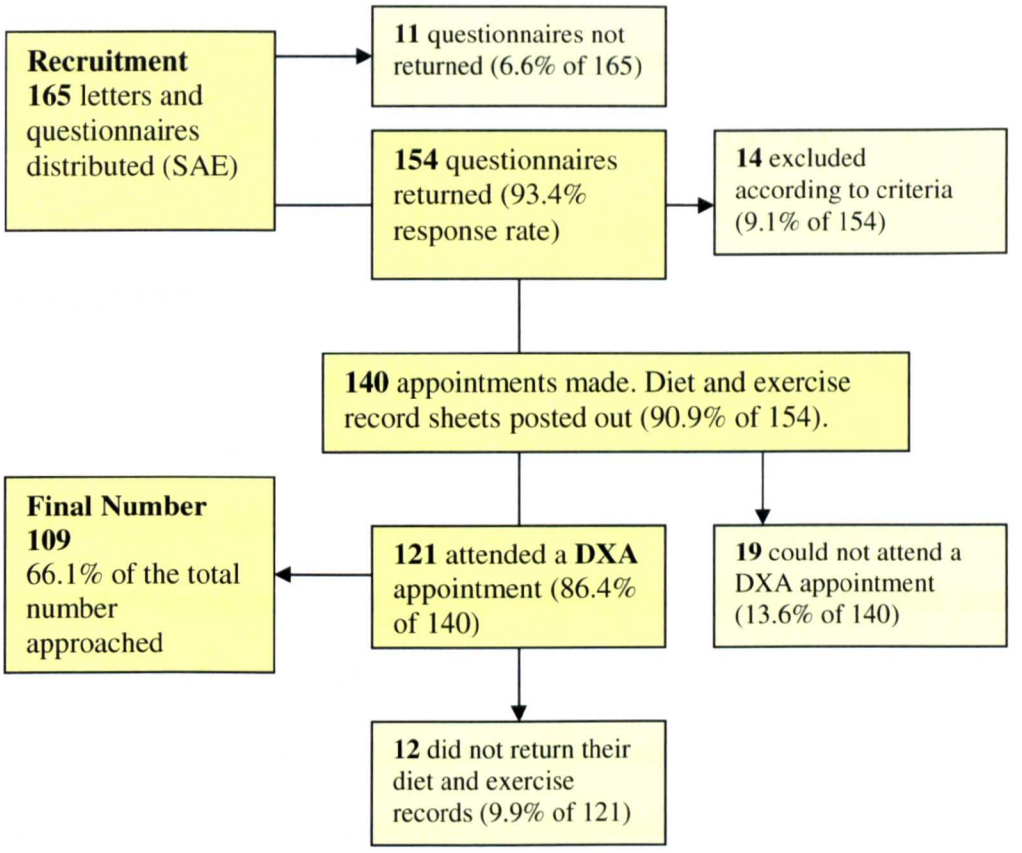
Figure 3.1: Research procedures and tools



3.2.2 Subject recruitment

Figure 3.2 is a flow chart illustrating the stages of the research undertaken, from subject recruitment to the final number of subjects used in the thesis. It also details the number of subjects who were eliminated or withdrew from the study at various stages of the research. As a heterogeneous subject sample was desired to reduce bias in subject selection, various recruitment strategies were adopted. It was anticipated that the final sample would consist of over 100 runners from across the UK.

Figure 3.2: Stages of research and determination of the final subject sample



165 runners were contacted and this number included around 80% of all UK distance runners who competed at elite level. The population of county and club level runners is far greater than that of elite runners, thus it is likely that our subject sample would be more representative of those competing at a higher level. Chapter 6 provides details of the proportion of runners from elite, county and club level. In order to reach elite distance runners, details of the study were posted to UK Athletics, who subsequently agreed to forward questionnaires and letters to Great Britain Team runners who were currently competitive and living in the UK. To reach runners at the 'county' and 'club' level, from across the United Kingdom (UK) rather than the local region, a letter was published in the national athletics magazine 'Athletics Weekly', providing a contact email address. Additionally, questionnaires were distributed to

runners at several organised races (Leeds, Birmingham, Newcastle and Loughborough) and contact was made with various coaches and secretaries from athletics clubs and universities. All questionnaires were posted in an A4 envelope and were accompanied by a letter and stamped addressed envelope (S.A.E).

3.2.3 Eligibility criteria

To be eligible to participate, male and female runners were aged between 18 and 50 years. The majority of bone development has occurred by the age of about 18 years in females and 20 years in males (Bonjour *et al.,* 1991; Nguyen *et al.*, 2001). Fifty years of age was set as the maximum limit as BMD declines after this age in both men and women (Warming *et al.,* 1997) and there is an elevated possibility that women over this age will be peri-menopausal or menopausal. Four runners were excluded for being outside the age range. It was also a requirement that the subjects ran at least 20 miles per week and that they had been running regularly at that level for at least 3 years. The exclusion criteria was a family history of osteoporosis as diagnosed in a blood relative aged under 70 years, (3 runners were excluded), ethnicity other than Caucasian (1 exclusion), pregnancy or lactation (3 exclusions), any disease, condition or medication known to influence bone density, including long-term regular use of corticosteroids (2 exclusions), but with exception of the oral contraceptive pill.
In line with IRMER (Ionising Radiation in Medical Exposure Regulations) 2000, pregnant women or women who were breast-feeding were excluded from the research due to the potential risks associated with radiation exposure. Only runners of Caucasian descent were included because bone structure, size and density are known to vary with ethnicity (Gilsanz *et al.,* 1998). Although bone size also differs according to gender, both male and female participants were included in order to address the

study aims. Oral contraceptive users were included in the sample in order to enable an insight into the BMD of this group and to see if any benefits were associated with oral contraceptive use. Only women who had used the oral contraceptive pill for at least 2 years were classed as oral contraceptive users. From the 159 who returned their completed questionnaires, 14 were excluded according to the above criteria.

3.2.4 Ethical approval and consent

Prior to the outset of the research conducted for this thesis, ethical approval was granted by the Leeds Research and Ethics Committee (LREC) (appendix 1). In accordance with the Declaration of Helsinki, each subject was provided with information regarding the reasons for the research, the procedures involved, the expected duration and the possible benefits and risks. After reading the information, subjects provided written consent to participate. These consent forms have been filed.

Ethical approval was also required for the use of DXA as it involves subject exposure to ionising radiation. The Medical Exposures Assessor and Radiation Protection Advisor for the Leeds Research Ethics Committee conducted a risk assessment and the total effective dose per person was quoted to be 0.1 mSv. This categorised the associated risk for participation in the study as '1', representative of a trivial risk. Certification for operator training in the Ionising Radiation (Medical Exposure) Regulations was completed in 2002 and certification for the National Training Scheme for Bone Densitometry (National Osteoporosis Society, 2002) was also attained in the same year. A copy of the certificate is provided in appendix 2.

3.2.5 Coding system

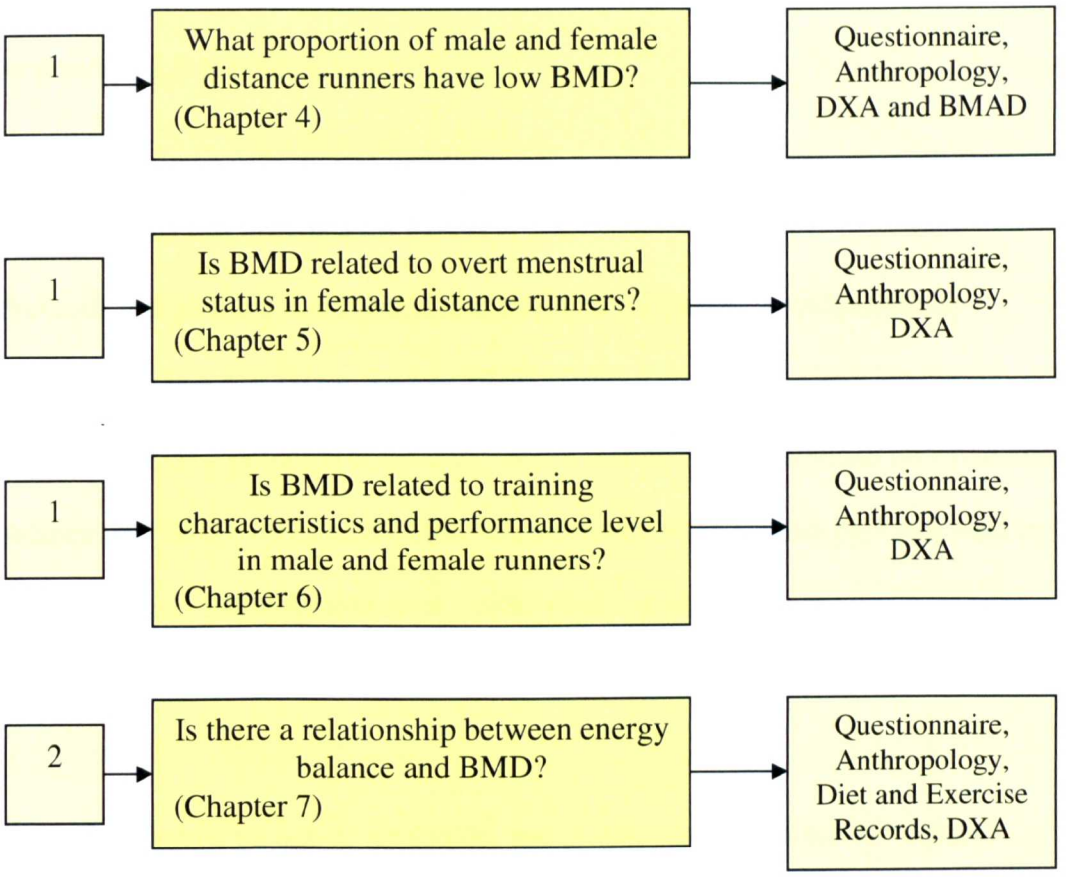
In accordance with the confidentiality requirement, each runner was allocated a code (numerical 1-109) which was used for the recording of the results and when conducting the analyses. The front page of the returned questionnaires included the subject's personal details, therefore these were removed and filed; the questionnaire was then coded. For the energy expenditure assessment and the dietary analyses, the coded system was also used.

3.2.6 Methods matrix

The matrix presented in figure 3.3 re-states the research questions for this thesis and the methods used to answer them.

Each question serves to achieve the thesis aims which were to determine whether male distance runners are at a comparable risk for bone loss compared to their female counterparts and to determine the relationship between reported energy balance and BMD in a large sample of male and female distance runners.

Figure 3.3: Methods matrix



The following sections detail the tools and procedures used for data collection. These include the questionnaire, diet and exercise records, anthropometry and DXA.

3.3 Questionnaire assessment

A self-administered questionnaire (provided in appendix 3) screened the subjects prior to selection and provided information on training, competition level, injuries, dietary habits, body image, menstrual status and menstrual history. The questionnaire was first piloted on a group of runners (6 male and 6 female) from an athletics club in

Gateshead, following which some minor changes were made. Questionnaires were returned to the Leeds Centre of Bone and Body Composition and this was encouraged by providing respondents with a SAE.

3.3.1 Menstrual status and history

Previous research has established that having a menstrual disorder such as amenorrhea, is a risk factor for low BMD (Drinkwater *et al.,* 1984; Pettersson *et al.,* 1999). Thus, as a potential confounder when testing for correlations between energy balance and BMD, menstrual status was assessed. As in several previous studies (Bennell *et al.*, 1997; Gibson *et al.,* 2000; Cobb *et al.,* 2003), the questionnaire assessed the menstrual status and history of the female runners.

As well as details on age of menarche, use of oral contraceptives or hormone replacement therapy, the runners were also asked to provide details of menstrual disorders (dates, duration and whether the onset of the disturbance coincided with any lifestyle alterations), past and previous menstrual disorders and whether the problem of menstrual disturbance had been addressed. When runners had not responded with sufficient detail, further information was obtained at their DXA appointment or via a telephone call.

(i) Menstrual status

Based on the data provided, the female runners were classified as eumenorrhoeic (10 or more menses per year), amenorrhoeic (0-3 menses per year) or oligomenorrhoeic (4-9 menses per year) or as users of the oral contraceptive pill (of which the required minimum duration was 2 years). The amenorrhoeic and oligomenorrhoeic runners

were combined to one group as there were no differences in BMD, BMI or other parameters. One woman runner who had been using the pill for less than 2 years was classed as eumenorrhoeic, in accordance to her menstrual status prior to using the pill. Fourteen runners were classed as oral contraceptive users and the mean duration of use was 3.8 (±1.4) years.

(ii) Menstrual history

The total years of oestrogen exposure (eumenorrhoea) was calculated by subtracting age of menarche from current age and then subtracting number of amenorrhoeic years from this. In oligomenorrhoeic subjects, when the sum of the number of menses missed reached 10, this was classed as 1 year, which was subsequently subtracted as shown below. This method was intended to provide an estimation of years of oestrogen exposure.

Oestrogen exposure (eumenorrhoeic years) = Present age - age of menarche – number of amenorrhoeic years

3.3.2 Competition, training and injury

A prerequisite of this study was to investigate differences in BMD between runners of different performance levels, therefore all subjects were asked to record details of their highest level of competition (and date this was achieved) and personal best times. From this information, the runners were classified as either *'elite'*, *'county'* or *'club'* depending on the highest level of competition they had achieved. Elite runners had represented Great Britain, England, Wales or Northern Ireland in International competition, home or abroad. The highest levels attained by several elite runners

included the Olympics and World Championships. The highest level attained by county runners was competing in the regional championships (North, South, Midlands etc) and for club runners, in harrier league meetings and fun runs. Competition distances ranged from 3000 km to the marathon. Runners were also asked to record the number of years they had been training regularly, any current or past injuries; the average number of miles ran in a typical week and a brief synopsis of the number of specific sessions undertaken each week.

3.3.3 Dietary habits and body image

Additional data regarding dietary habits and self-perception were obtained from the questionnaire. As distance runners have previously been shown to be at risk of eating disorders and to be concerned about their body weight, the questionnaire progressed to ask subjects: (i) whether they deliberately kept their body weight below a certain value, (ii) how they would best describe their build, (iii) whether they were happy with their present body weight and (iv) whether they followed a specific diet. It was anticipated that these questions would provide an insight into the runners' self-perception and to see if any had a distorted self-image, by viewing their responses in context of actual physical measurements. Although runners were asked whether they had a current or past eating disorder, the main aim of this thesis was to investigate energy balance and BMD therefore the Eating Disorder Inventory was not used. It was also possible that use of the EDI may have led to changes in the dietary behaviour of runners or influenced the reporting dietary intake in the prospective records that followed. Cobb *et al.,* (2003) have recently demonstrated an association between EDI scores and BMD in female runners regardless of menstrual status (Chapter 2.2.3).

3.4 Energy expenditure assessment

Daily energy expenditure (EE) without allowing for the energy cost of exercise, was estimated from the calculation of BMR (determined by the Katch-McArdle formula: 370 + 21.6 x lean body mass (kg)) multiplied by a PAL of 1.3 (or 1.5 depending on occupation) as described in Chapter 3.1.2. The energy cost of training was calculated using Ainsworth's compendium which allocates all activities an intensity unit based on their rate of EE, expressed in METs. As BMR is fairly near to 1 kcal kg bodymass^{-1} hour^{-1} (Ainsworth *et al.*, 1993), the MET values were multiplied by body mass (kg) to estimate the energy cost of exercise per hour.

EE (per hour) = MET x Body Mass (kg)

In order to accurately assess EE from exercise, it was essential that each subject recorded the intensity level and duration of their physical activity in the prospective record. Thus, the exercise record was designed to encourage this (appendix 4). The exercise record consisted of seven pages, with each page representing one day. Runners were provided with instructions on how to complete the record, which included detailing the time the exercise was performed, location, duration, measured intensity (heart rate or speed) and self-perception of intensity. In circumstances when a runner was injured, an excerpt from their training diary detailing a typical week was provided. This was not a problem as the majority of runners keep a detailed, up-to-date log of all training completed. In addition to training, information regarding extra exercise, including swimming, cycling and walking was provided.

The final equation used to calculate total EE was as follows:

EE = BMR x PAL + energy cost of exercise (kcal per day)

3.5 Energy intake assessment

3.5.1 7-day diet record

Nutritional intake was assessed from a 7-day prospective diet record (an example has been provided in appendix 4) of self-weighed (when possible) or self-estimated food and drink intake. This record was completed over the same week as the prospective 7-day exercise record. Runners were reminded of the importance of accuracy and honesty and were provided with instructions as to how to complete the record:

- weigh all food consumed when possible and when not, carefully estimate portion size;
- detail type of food and drinks, such as milk (full fat, semi-skimmed or skimmed) and bread (thick, medium or thin sliced; white, wholemeal, brown);
- detail all brands of food and drinks, and
- provide the time the food or drinks were consumed.

Many runners provided food labels and detailed nutritional information on the products they had consumed. The records were to be returned on the day of their DXA appointment, although many were unable to do so. Reasons included leaving short notice before arranging an appointment, losing their diet record booklet or forgetting to bring the diet record to their appointment. Several runners did not use the

allocated diet record booklets due to losing them, but recorded their intakes on paper. Others who had forgotten their records or who had not left enough time for their completion returned them via email or post. 12 runners did not return their records.

3.5.2 Nutritional analysis using CompEat Pro 5.8.0

Dietary intakes were analysed using the CompEat Professional Version 5.8.0 software programme (Nutrition Systems, Grantham, UK). This software is based on food composition data prepared by the Royal Society of Chemistry with agreement of the Ministry of Agriculture, Fisheries and Food. CompEat pro 5.8.0 contains 3587 types of food and drink, including numerous popular brands. It requires an input of weight or estimated portion size of the foods consumed. The mean daily intake of nutrients was obtained from the 7-day dietary analysis. Energy intake was the main parameter of interest, although as dietary calcium intake has an established role in bone health this was also assessed. In addition, fat and vitamin K intake were assessed for these may also confound correlation tests between energy intake and BMD (Craciun *et al.*, 1998; Kerstetter *et al.*, 2003).

3.5.3 Energy balance

Energy balance was assessed by deducting energy intake from energy expenditure. The methods used for the assessment of energy intake and expenditure were determined by the subject sample, available resources and time. It was predicted that runners in a negative energy balance would have a lower BMI and percentage body fat than those in a positive energy balance.

3.6 Anthropometry

All anthropological measurements were taken at the Centre for Bone and Body Composition, Leeds, prior to the DXA bone density tests.

3.6.1 Body mass, height and BMI

Body mass and height were measured with subjects wearing a hospital gown, no shoes, and no jewellery. Body mass was measured on electronic calibrated scales and recorded to the nearest 0.1 kg. Height was measured on a stadiometer and recorded to the nearest centimetre (cm). From these measurements, body mass index (BMI) was calculated:

$$\textbf{BMI (kg m}^{-2}\textbf{) = Body mass (kg) / Height (m)}^2$$

3.6.2 Body composition

The total body scan on the Lunar Prodigy (Lunar GE Systems) was used to estimate body composition, including percentage fat, fat mass and lean mass. The Lunar Prodigy software (version 5.00.211) also provides a body fat Z-score based on the UK total body reference population matched for age. Percentage body fat and lean body mass (g) has previously been associated with BMD in runners (Khosla *et al.*, 1996; Pettersson *et al.*, 1999; Burrows *et al.*, 2003) therefore these two parameters in addition to fat mass (g) and the body fat Z-score, were used in the analysis.

3.7 Bone measurements

Bone mineral density (BMD, g cm $^{-2}$) measurements were performed using fan-beam DXA (Lunar Prodigy, GE Systems, Madison, USA) and took place at the Leeds Centre for Bone and Body Composition Research. Figure 3.4 shows the DXA machine used to conduct the bone densitometry. As the sole investigator and following certification in bone densitometry, I conducted all tests which eliminated intra-operator error. Advice was sought on two occasions from a fellow bone densitometrist when abnormal images required further visual analysis. Lumbar spine (anterorposterior projection, L2-L4), dual femur (total hip) and total body BMD was measured. The software version 5.00.211 was used for all measurements.

3.7.1 Quality assurance and control

The calibration of the DXA machine was employed by the Centre's quality assurance and quality control protocols. This enabled the stability of the machine to be monitored throughout the study period.

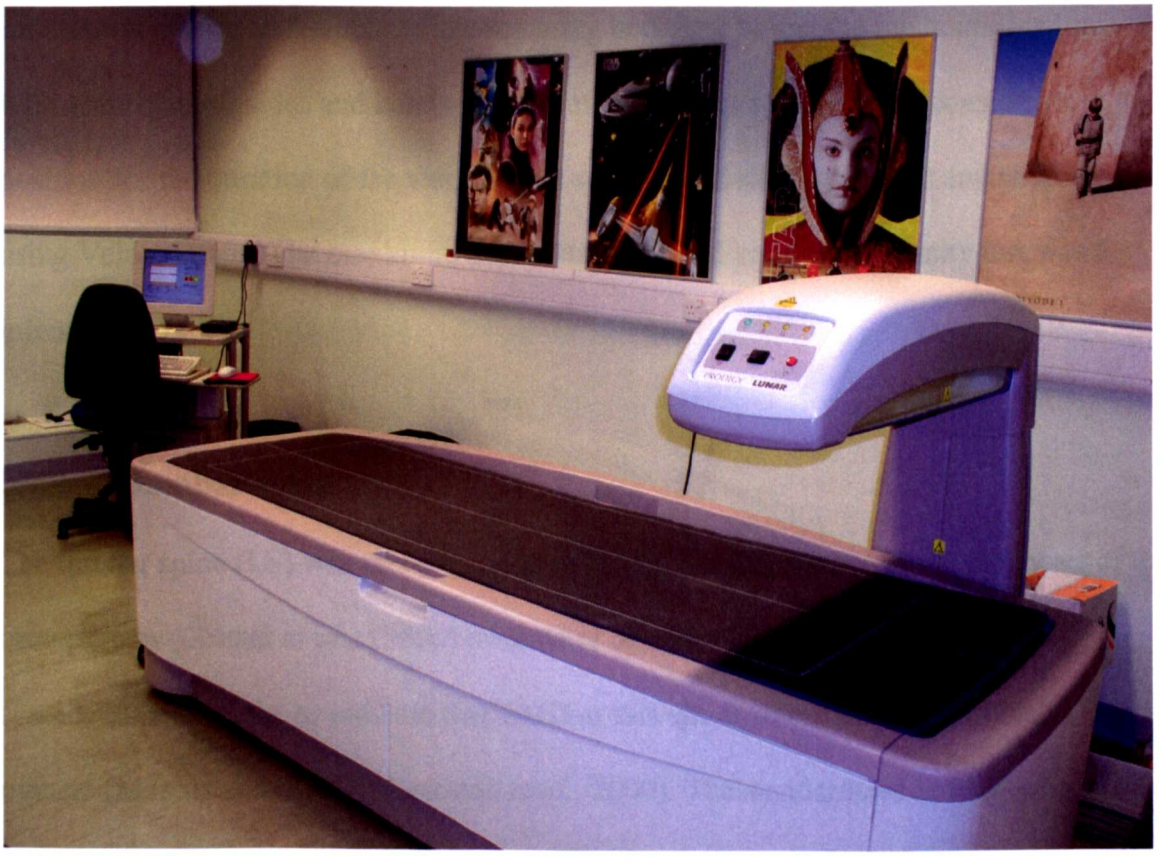
(i) Quality assurance

Quality assurance (QA) refers to the internal calibration of the instrument and involves daily instrument calibration checks. On the morning of each runner's DXA appointment, the QA protocol was performed using the Lunar calibration block. This block was scanned on a predefined position on the scanning bed and the system software provided a 'pass-fail' evaluation.

(ii) Quality control

Quality control (QC) is the evaluation of the instrument's calibration over time and this was performed across the duration of the scanning period for this research. The QC procedure involves scanning the Lunar gold spine phantom 3 to 4 times per week. On most occasions the scanning of this phantom was performed by a clinical bone densitometrist in the Centre for Bone and Body Composition. The results for each QC test were saved and archived to the software database. This enabled analysis of the QC results over the period of bone density testing for this research. The results showed no significant machine drift according to the adapted Shewhart Rules.

Figure 3.4: The Lunar Prodigy DXA (GE Systems)



3.7.2 Subject preparation

Prior to the DXA tests, each subject changed into a hospital gown, removed their shoes and all items that may attenuate the X-ray beam, including clothing with zips, buckles and snaps and jewellery. The subject details (height, body mass and date of birth) were then entered into the software database.

3.7.3 Total body

Although the WHO criteria for diagnosing osteoporosis cannot be applied to total body BMD results, this measurement provided useful information on overall BMD and body composition. Precision at this site (% coefficient of variation) is 0.8%. The subject was positioned supine on the scanning bed, 3cm below the top boundary line with arms by their sides and hands facing down. In order to maintain a consistent position for the duration of the test, the feet were turned slightly inward and supported using Velcro straps. The maximum duration of the total body bone density test was 5 minutes.

3.7.4 AP lumbar spine

The lumbar spine (LS) was measured due to its clinical importance in the diagnosis of osteoporosis (Genant *et al.*, 1999; Lewiecki *et al.*, 2004) and because there is considerable evidence to indicate that BMD at this site is often reduced in female runners (Marcus *et al.*, 1985; Gremion *et al.*, 2000). The standardised anteroposterior (AP) lumbar spine scan was conducted and BMD results at the L2 to L4 vertebrae were used. Precision at this site (% coefficient of variation) is 0.6%. Subjects lay supine on the scanning bed with legs elevated to rest on a foam pillow. Raising the

legs opens the intervertebral disc spaces, which helps with the analysis. Their arms were placed at either side and palms faced down. The X-ray beam was then positioned above the L5 vertebrae and each scan took 2 to 3 minutes.

3.7.5 Dual femur

The measurement of the dual femur is that of the total hip, and throughout this thesis the term dual femur is used since this is the term used by the Lunar system. Again, this measurement was chosen for its clinical relevance but also to enable a comparison with LS BMD. Several studies have previously found elevated BMD at weight-bearing sites such as the hip and legs compared to the spine (MacDougall *et al.,* 1992; Gremion *et al.,* 2000) although some have not (Myerson *et al.,* 1992; Rencken *et al.,* 1996). Precision at this site (% coefficient of variation) is 1.5%. Subjects lay supine on the scanning table with legs abducted by 15 degrees and this was assisted with use of the Dualfemur device (GE Lunar Systems). This position separates the ischium from the lesser trochanter, enabling the Lunar Smart fan feature to detect the contra-lateral femur without the need to reposition the subject. The legs are then internally rotated by 25 degrees. The total duration of this scan is approximately 4 minutes.

3.7.6 Scan analysis and reference data

The analysis of each measurement is performed automatically by the Lunar software system (Lunar Prodigy, GE Systems). Following visual analysis of each image, it was occasionally necessary to move the intra-vertebral markers to accurately separate the vertebrae (L2, L3 and L4) and to ensure that sufficient soft tissue is on either side of

the bone image in accordance with the NOS densitometry guidelines (NOS, 2002). The results were then archived to the database, printed and filed.

The results obtained from each measurement were BMD (g cm^{-2}), bone area (cm^2), T-scores and Z-scores.

(i) Adjusting for bone size

To adjust for bone size, bone mineral apparent density (BMAD) was calculated using DXA-derived measurements of bone area and BMD. This provides an approximation of volumetric BMD and aims to normalise BMD measurements for bone size. BMAD was estimated for the lumbar spine and dual femur (Carter *et al.*,1992; Cvvijetic and Korsic, 2004) and expressed in milligrams per cubic centimetre (mg cm^3). As in recent work comparing BMD in healthy men and women (Cvvijetic and Korsic, 2004), BMD at the lumbar spine or dual femur was divided by the square root of the lumbar spine (L2-L4) or total femur area to give BMAD. The square root of the bone area estimates the depth.

$$\textbf{BMAD} = \textbf{BMD} / \sqrt{\textbf{Bone Area}}$$

(ii) T-scores and reference data

In order to standardise BMD data to provide an indication of BMD relative to age- and sex-matched normal values, T-scores and Z-scores were used. As the majority of runners were aged 20 to 40 years, their Z-scores were similar to their T-scores therefore for the majority of the analyses, T-scores were used. As described in chapter 1.3.2, a T-score result indicates the difference between an individual's BMD and the

ideal BMD of healthy young adults (aged 20 to 40 years) and the WHO definitions of osteopenia and osteoporosis are based on these values.

$$\text{T-score} = \frac{\text{Measured BMD} - \text{Young Adult Mean BMD}}{\text{Young Adult SD}}$$

Evidence indicates that the WHO criteria can be applied to men if based on gender-specific T-scores (Blinkley *et al.*, 2002; Lewieksi *et al.*, 2004). The UK male reference data provided by the Lunar manufacturer (Lunar, GE Systems), enabled BMD in the male runners to be expressed as T-scores. Although T-scores were originally intended for postmenopausal women, recent guidelines indicate that they may be used in young adults (Lewieksi *et al.*, 2004) and for this study the use of T-scores were adopted to provide an insight into the current and potential future bone status of male and female distance runners.

The reference data for the determination of the T-scores at each measured site were obtained from the Lunar manufacturer (Lunar, GE Systems). The data sets were based on BMD measured at the lumbar spine, dual femur and total body in healthy men and women from the UK, Northern Europe and Australia. There were greater normal subject numbers for scans conducted at the lumbar spine and dual femur. The numbers for each data set are shown in table 3.3.

Table 3.3: Numbers of healthy male and female subjects comprising the reference data sets for the bone measurements

	20 -29 years		30 – 39 years		40 – 49 years	
	Female	Male	Female	Male	Female	Male
Lumbar Spine (L2-L4)	707	186	1006	217	2302	240
Dual Femur (total)	696	167	994	254	2154	307
Total Body	179	91	192	112	259	135

3.8 Statistical procedures

Statistical analyses were computed using SPSS for Windows, version 11.5.0 (LEAD Technologies Inc. ©) and results were presented as means and standard error of the means (SEM). Following the Kolmogorov-Smirnov test for normality, differences between two groups (male *v* female; osteopenic *v* normal; stress fracture *v* non-stress fracture; BMI <18 *v* >18 kg m^{-2}) were tested using either one-way analysis of variance (ANOVA) for parametric data or the Mann-Whitney U test for non-parametric data. When there were three or more groups (menstrual status, performance level, energy status), differences between groups were tested using ANOVA and Games-Howell post-hoc analysis. The Games-Howell post-hoc analysis was used as this test is specifically designed for situations when sample sizes are unequal.

'Observed' and 'adjusted' (by ANCOVA) results were presented. Analysis of covariance (ANCOVA) removed the potential bias of variables such as age, body mass, BMI and body fat when computing differences between groups. The adjusted means were based on the Sidak confidence interval adjustment. Post-hoc analyses were also conducted for ANCOVA using the Games-Howell technique.

To test for relationships between two or more variables, scatter charts were plotted and two-tailed bivariate correlation tests computed for male and female data separately. For correlations between parametric data, Pearson's product moment correlation coefficients were computed and to test for correlations between non-parametric data, Kendall's tau-b correlation tests were conducted. The Kendall's tau-b test was used rather than the Spearman's correlation test as it draws more accurate generalisations about the population (Field, 2000). Two-tailed partial correlations were also computed between certain factors and BMD to control (hold in constant) variables such as age, BMI, eumenorrhoeic years, energy intake and dietary fat intake between groups.

Multiple regression analyses were used to determine which factors were most predictive of BMD in all runners and of menstrual status in female runners. To determine which factors were most predictive of menstrual status, binary logistic regression (backward) was computed and all potential determinants were included in the model. The Hosmer and Lemeshaw's measure was used to estimate the percentage of variance to be accounted for by the final model (R^2 = model chi square / original 2 log-likelihood). This method was chosen for it does not assume linearity and accepts categorical predictors such as performance level. To determine which variables were

most predictive of BMD and T-scores at the various sites in runners, stepwise multiple linear regression analyses were performed. All potential covariates were entered into the equation and were removed by order of least significance. For all statistical tests, probability values of less than 0.05 were considered to be significant.

Chapter 4

Bone status of male and female distance runners

One aim of the work conducted for this study was to determine whether male distance runners are at a similar risk for low BMD compared to their female counterparts and with respect to this aim it was asked,

"What proportion of male and female distance runners have a low BMD?"

This first research question was addressed by analysing BMD values and T-scores, BMD expressed as percentage of expected value for age and sex, and also BMD adjusted for a potential gender difference in bone size (BMAD).

4.1 Results

4.1.1 Subject characteristics

The characteristics of the runners are shown in Table 4.1. Male and female runners were similar in age, body fat Z-score and number of years in training. The age range for female runners was 18.4 to 50.9 years and 19 to 50.1 years for the male runners. two female runners were aged 18.4 and 18.5 years and 2 others were aged 19.6 years. Two male runners were aged 19 and 19.6 years. The range of self-reported weekly running mileage was 20 to 95 miles for females and 20 to 117 miles for males.

Groups differed in height, body mass, BMI, percentage body fat lean body mass and average weekly running mileage and these differences are shown in Table 4.1. All differences were significant after using the appropriate parametric (ANOVA) or non-parametric (Mann-Whitney U) statistical test depending on the distribution of the data.

Table 4.1: Subject characteristics (mean and SEM)

	Female (n=65)	Male (n=44)	Distribution Normal?	ANOVA	Mann Whitney U test
Age (years)	26.8 (0.9)	27.0 (1.1)	n		ns
Height (cm)	165.0 (0.8)	178.2 (1.1)	y	$p<0.001$	
Body mass (kg)	52.6 (0.7)	68.1 (1.0)	y	$p<0.001$	
BMI (kg m $^{-2}$)	19.2 (0.2)	21.1 (0.2)	y	$p<0.001$	
Body fat (%)	16.4 (0.6)	11.2 (0.7)	n		$p<0.001$
Body fat Z-score	-1.5 (0.9)	-1.2 (0.1)	n		ns
Lean body mass (kg)	42.4 (0.5)	59.6 (0.8)	y	$p<0.001$	
Weekly mileage	51.4 (1.7)	60.5 (2.8)	n		$p<0.05$
Years in training	9.6 (0.7)	11.2 (1.0)	n		ns

4.1.2 BMD and T-scores

4.1.2.1 Differences between groups

Table 4.2 presents the BMD (g cm $^{-2}$) values and T-scores for the male and female runners.

(i) Observed BMD and T-scores

T-scores were provided by the Lunar software and were also used for runners aged 18 to 19 years for they were not found to be significantly different from Z-scores. Observed T-scores at the lumbar spine, dual femur and total body were similar in male and female runners. BMD T-scores were reduced in males as in females (-0.8 *v* -0.8 respectively) whereas observed BMD at the dual femur (0.5 *v* 0.6) and total body (0.3 *v* 0.2) were slightly above normal. Observed lumbar spine T-scores ranged from -2.6 to 0.9 in females and -3.3 to 0.6 in the males. At the dual femur, the ranges were -1.4 to 2.8 in the females and -1.8 to 2.4 in the males. Observed BMD values were greater in male compared to female runners at the lumbar spine ($p<0.05$), dual femur ($p<0.001$) and total body ($p<0.001$).

Table 4.2: Observed and adjusted lumbar spine, dual femur and total body bone status in distance runners, mean (SEM)

	Female (n=65)	Male (n=44)	Difference
Lumbar spine T-score			
Observed	-0.8 (0.1)	-0.8 (0.1)	ns
Adjusted*	-0.7 (0.2)	-1.1 (0.1)	$p<0.05$
Lumbar spine BMD (g cm^{-2})			
Observed	1.10 (0.01)	1.14 (0.01)	$p<0.05$
Adjusted*	1.12 (0.01)	1.12 (0.01)	ns
Dual femur T-score			
Observed	0.5 (0.1)	0.6 (0.1)	ns
Adjusted*	0.8 (0.1)	0.1 (0.2)	$p<0.01$
Dual femur BMD (g cm^{-2})			
Observed	1.10 (0.0)	1.15 (0.0)	$p<0.001$
Adjusted*	1.09 (0.01)	1.11 (0.02)	ns
Total body T-score			
Observed	0.3 (0.1)	0.2 (0.1)	ns
Adjusted*	0.6 (0.1)	-0.2 (0.1)	$p<0.001$
Total Body BMD (g cm^{-2})			
Observed	1.15 (0.001)	1.24 (0.009)	$p<0.001$
Adjusted*	1.17 (0.008)	1.20 (0.01)	ns

* *Adjusted for age, body fat and BMI by ANCOVA*

(ii) Adjusted BMD and T-scores

There were significant differences in BMI and percentage body fat between the male and the female runners, therefore, adjustments to the BMD results were made for these differences using analysis of covariance (ANCOVA). Although age did not differ between the male and female groups, the bone data were also adjusted for age because of the relatively large range between subjects (18 to 50 years). There were no adjustments for lean body mass or height, since these variables were likely to be accounted for when adjusting for body fat and BMI. The contribution of menstrual status to bone density in the female runners is addressed in chapter 5.

Adjusted lumbar spine T-scores were lower in male compared to female runners (-1.1 *v* -0.7; $p<0.05$) and adjusted mean BMD was identical between groups. Adjusted dual femur T-scores were also lower in males (0.1 *v* 0.8; $p<0.01$), and adjusted BMD were similar. Again, adjusted total body T-scores were lower in male than female runners (-0.2 *v* 0.6; $p<0.001$). Thus, after adjustment for age, BMI and body fat, BMD in male runners was lower than in female runners.

4.1.2.2 Osteopenia and osteoporosis

(i) Prevalence

Table 4.3 gives the numbers and percentages of runners meeting the WHO criteria for the diagnosis of osteoporosis and osteopenia at the lumbar spine. Over one third (36.4%) of the male runners were classified as osteopenic (T-score less than -1.0) and 41.6% of female runners were classified as osteopenic. One male runner and 1 female runner had lumbar spine osteoporosis. Surprisingly, at the dual femur, 3 male and 2 female runners were classified as osteopenic. One male runner with osteopenia in the

dual femur (hip) had suffered a stress fracture in the left hip which developed into a more serious hip fracture. This runner also had lumbar spine osteoporosis. Another male runner had osteopenia in the hip but not in the lumbar spine which was particularly surprising considering his regular weight-bearing exercise. The majority of runners had a negative T-score at the lumbar spine and approximately one third had a negative total body T-score[1].

Table 4.3: Numbers and percentages of runners meeting WHO diagnostic criteria for osteoporosis and osteopenia at the lumbar spine

	Osteoporosis (T-score < -2.5)	Osteopenia (T-score <-1.0, >-2.5)	Total *
Female	1 (1.5%)	27 (41.6%)	28 (43.1%)
Male	1 (2.3%)	16 (36.4%)	17 (38.6%)
Total group	2 (1.8%)	43 (39.5%)	45 (41.3%)

* *number of runners meeting diagnostic criteria for either osteoporosis or osteopenia*

(ii) Differences between runners with and without osteopenia

Runners with ('1') and without ('0') lumbar spine osteopenia or osteoporosis were compared using ANOVA, as indicated in Table 4.4. As shown, female runners with lumbar spine osteopenia were lighter and had a lower percentage body fat compared to female runners with T-scores above -1.0. This may in part, reflect their higher weekly running mileage which may lead to or maintain a low body mass with low levels of body fat. Fewer years of eumenorrhoea and hence oestrogen exposure were found in runners with osteopenia and these women also had a significantly later age of menarche.

[1] Total Body T-scores cannot be used for diagnosis and have been used purely for the research purpose

Table 4.4: Characteristics (mean and SEM) of runners with (< -1.0) and without (> -1.0) osteopenia at the lumbar spine

	Female		Male	
	T-score > -1.0 (n=37)	T-score < -1.0 (n=28)	T-score > -1.0 (n=27)	T-score < 1.0 (n=17)
Body mass (kg)	53.6 (0.8)	51.2 (1.2)*	69.2 (1.4)	66.3 (1.5)
BMI (kg m $^{-2}$)	19.8 (0.2)	18.6 (0.3)*	21.4 (0.2)	20.6 (0.3)
Body fat %	17.6 (0.8)	14.9 (0.9)*	12 (0.9)	10 (0.9)
Body fat Z-score	-1.5 (0.1)	-1.6 (0.8)	-0.9 (0.2)	-1.6 (0.1)*
Lean body mass (kg)	42.8 (0.6)	41.8 (0.7)	60.3 (1.0)	58.4 (1.3)
Weekly running mileage	46.8 (2.0)	57.1 (2.4)*	51.5 (2.5)	74.9 (4.2)*
Menarche (years)	13.8 (0.2)	14.9 (1.6)*	n/a	n/a
Years of eumenorrhoea	13 (1.5)	6.8 (1.1)*	n/a	n/a
Years in training	9.9 (1.0)	9.0 (0.9)	10.1 (1.3)	12.9 (1.5)

* *p<0.05*

Male runners with osteopenia also ran more miles per week than runners with a T-score greater than -1.0 ($p<0.05$). Although runners with osteopenia weighed less and had a lower BMI, the wide range of scores within this sample may have prevented the differences reaching statistical significance. Body fat Z-scores were significantly lower in male runners with osteopenia.

Differences between runners with and without osteopenia at the dual femur were not computed due to the extreme inequalities of subject sizes. It was found that the two female runners with osteopenia in the dual femur had a low BMI and were amenorrhoeic. However, no other risk factors were found. No risk factors were found in the male runners who had dual femur osteopenia.

4.1.2.2 Correlations with BMD

Relationships were investigated using Pearson's product moment or Kendall's-tau correlation procedure depending on the data distribution.

(i) Lumbar spine

In female runners, there was a positive effect of age ($r = 0.242$; 0.245; $p<0.05$), total number of eumenorrhoeic years ($r = 0.292$; 288; $p<0.05$), body mass ($r = 0.371$; 0.392; $p<0.01$), BMI ($r = 0.440$; 0.444; $p<0.005$) and percentage body fat ($r = 0.232$; 0.224; $p<0.05$) on lumbar spine T-score and BMD respectively, whereas weekly running mileage had a negative effect ($r = -0.435$; -0.467; $p<0.05$). In male runners, lumbar spine T-score and BMD were positively associated with BMI ($r = 0.258$; 0.238; $p<0.05$) and body fat Z-score ($r = 0.292$; 0.285; $p<0.05$) and as in females, were negatively associated with weekly running mileage ($r = -0.488$; -0.483; $p<0.01$).

(ii) Dual femur

In female runners, body mass and BMI correlated positively with dual femur T-score ($r = 0.270$; 0.366; $p<0.05$) and BMD ($r = 0.278$; 0.362; $p<0.05$). There was no relationship between the number of eumenorrhoeic years or weekly running mileage and dual femur T-score or BMD in the female runners. In male runners, only an inverse correlation with age was observed ($r = -0.346$; -0.357; $p<0.01$).

(iii) Total body

Total body BMD for female runners correlated positively with age ($r = 0.263$; $p<0.01$), number of eumenorrhoeic years ($r = 0.313$; $p<0.05$), body mass ($r = 0.536$; $p<0.01$), BMI ($r = 0.576$; $p<0.01$), percentage body fat ($r = 0.189$; $p<0.05$) and lean body mass

(r =0.274; p<0.05). In male runners, total body BMD was positively associated with BMI (r =0.261; p<0.05) and lean body mass (r =0.214; p<0.05). An inverse correlation between weekly running mileage and total body BMD (r = -0.222; p<0.05) was demonstrated for the group of male runners. Thus, in male and female runners, the consistent correlates with BMD were BMI, body mass, body fat and weekly running mileage (-).

Additional analyses were conducted to investigate relationships between regional BMD and body composition (with the exclusion of the head and trunk due to large variability and interference during scanning), as the effect of both leptin and muscle tissue on bone may be localised. However, with the exception of the arms in female runners, where a weak relationship between regional BMD and body fat was observed (r=0.373; p-0.002), there were no other correlations between regional BMD and body composition in male or female runners.

4.1.3 Comparisons to reference data

In order to compare the runners' BMD to expected values for age and sex, BMD was expressed as percentages of age and sex-matched reference data. The results are shown in Table 4.5 and all data was normally distributed. The leg and arm BMD values were obtained from the total body DXA measurement using the regional analysis option. Mean lumbar spine BMD percentages for male and female group were lower than normal values and ranged from 74 to 108% in the females and 73 to 106% in the males. Mean arm percentages were also lower and ranged from 84 to 112% in the females and 74 to 111% in the males. Dual femur and leg BMD were above normal. Dual femur percentages ranged from 81 to 130% in the females and 85

to 125% in the males. Leg percentages ranged from 90 to 129% in the females and 88 to 121% in the males. Overall, total body BMD was normal, ranging from 90 to 115% in the females and 80 to 110% in the males.

Table 4.5: BMD of runners measured by DXA and expressed as percentages of age and sex-matched normal reference data (mean and SEM)

	Female (n=65)	**Male (n=44)**
Legs %	113.2 (0.9)	107.2 (0.9)*
Arms %	96 (0.7)	94.6 (0.9)
Lumbar spine %	91.6 (1.1)	91.6 (1.0)
Dual femur %	104.9 (1.2)	104.7 (1.5)
Total body %	100.4 (1.7)	101 (0.7)

** Males < females, p<0.001*

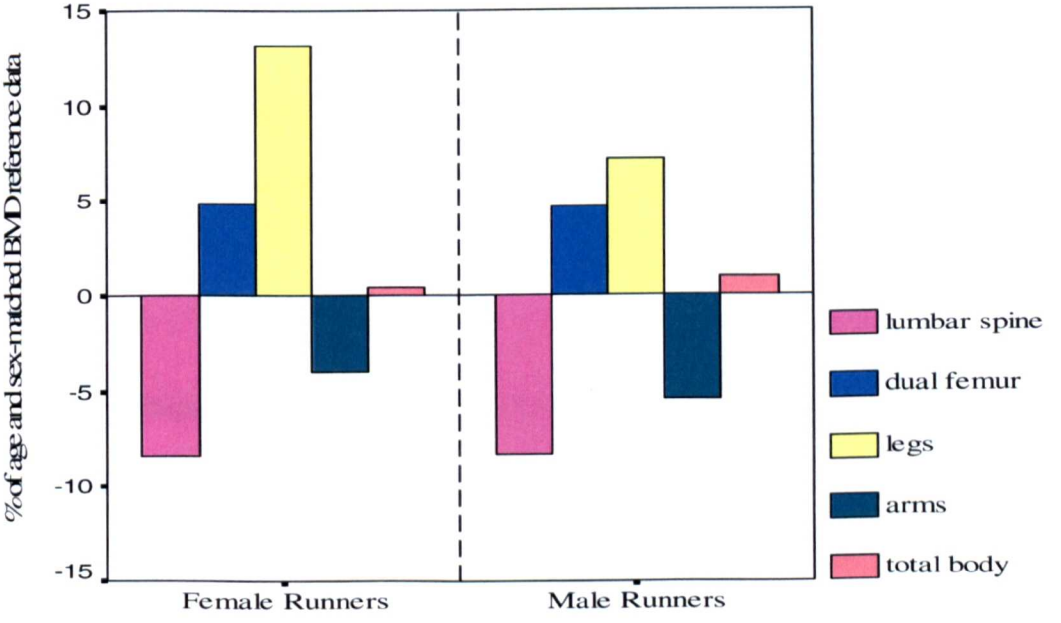


Figure 4.1: Site-specific bone status of runners expressed as percentages of age and sex-matched normal reference data

Bivariate correlations revealed positive correlations between number of eumenorrhoeic years with, leg ($r = 0.272$; $p<0.005$) and arm ($r = 0.275$; $p<0.005$) values in female runners. There were also positive correlations with BMI and body mass at the legs, total body, dual femur and arms ($r = 0.347$ to 0.474; $p<0.005$) and with percentage body fat at the total body and arms ($r = 0.189$; 0.182; $p<0.05$). In the male runners, BMI and body mass was associated with values for the total body, legs and arms ($r = 0.236$ to 0.347; $p<0.005$). There was a negative correlation between years ran, dual femur, legs and total body values ($r = -0.291$ to -0.407; $p<0.005$). A negative correlation was observed between age and dual femur values ($r = -0.332$; $p<0.01$) in male runners.

4.1.4 Bone mineral apparent density

As discussed in chapter 1, men have larger bones than women and differences in bone size can confound DXA-derived areal BMD results between men and women (Henry and Eastell, 2000; Seeman, 2001). BMAD was calculated to give an approximation of volumetric bone density in male and female runners in order to reduce the confounding effect of bone size when comparing the BMD of male and female runners. Table 4.6 presents the lumbar spine and dual femur bone area and BMAD results.

There were significant gender differences in bone area. Male runners had larger bone area than female runners at the lumbar spine which coincided with lower BMAD ($p<0.001$). Bone area was also greater at the dual femur in male runners, but mean BMAD did not differ significantly from the female runners. Bone area at the lumbar spine and dual femur correlated positively with body mass in male ($r = 0.538$; $r =$

0.640; p<0.001) and the female runners (r = 0.481; r = 0.561; p<0.001) and with height in male (r= 0.583; r = 0.491; p<0.001) and female runners (r = 0.585; r = 0.687; p<0.001).

Table 4.6: DXA-derived bone area and bone mineral apparent density (BMAD) in runners (mean and SEM)

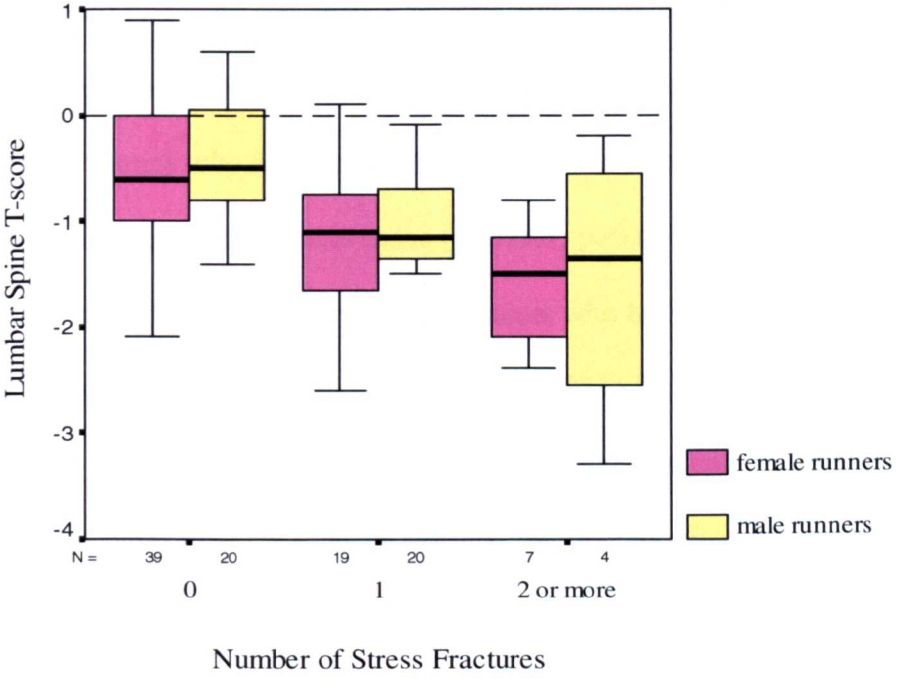
	Female (n=65)	Male (n=44)	Distribution normal?	ANOVA	Mann Whitney U Test
Lumbar spine bone area (cm^2)	44.1 (0.5)	53.0 (0.7)	n		$p<0.001$
Lumbar spine BMAD ($mg\ cm^{-3}$)	167.3 (2.0)	157.6 (2.0)	y	$p<0.001$	
Dual femur bone area (cm^2)	30.9 (0.3)	37.7 (0.6)	n		$p<0.001$
Dual femur BMAD ($mg\ cm^{-3}$)	191.6 (2.3)	189.8 (2.9)	n		ns

Dual femur BMAD correlated negatively with age in male runners (r = -0.246; p<0.05) but not in female runners. BMI positively correlated with lumbar spine and dual femur BMAD in the female runners (r = 0.335; r = 0.292; p<0.05) and with lumbar spine BMAD in male runners (r =0.335; p<0.05). In female runners, lumbar spine BMAD was positively associated with number of eumenorrhoeic years (r =0.280; p<0.05). In male and female runners, there was an inverse relationship between weekly running mileage and lumbar spine BMAD (r = -0.448; -0.401; p<0.005).

4.1.5 BMD and stress fractures

Information gathered from the questionnaire revealed that 25 (38.5%) female runners had suffered a stress fracture with 7 of these runners having suffered 2 or more stress fractures. Twenty four (54.6%) male runners had suffered a stress fracture, and 4 (9.1%) of these runners had suffered more than 2 stress fractures. Figure 4.2 illustrates the relationship between lumbar spine T-scores and stress fracture incidence in the male and female runners.

Figure 4.2: Lumbar spine T-score and stress fracture incidence in runners



There were negative associations between stress fracture incidence and T-scores at the lumbar spine (r= -0.453) and total body (r= -0.330, $p<0.005$). Table 4.7 presents the mean lumbar spine T-scores for the runners when allocated to groups depending on stress fracture history.

Table 4.7: Mean (SEM) lumbar spine T-score for runners grouped according to stress fracture history

Number of stress fractures	Female (n=65)	Male (n=44)
0	-0.6 (0.1)	-0.3 (0.1)
1	-1.2 (0.2)†	-1.1 (0.1)*
2 or more	-1.4 (0.4)†	-1.6 (0.7) *

* *T-score lower than those who have never had a stress fracture, p<0.005*

ANCOVA revealed that male and female runners with stress fractures had significantly lower lumbar spine T-scores than those who had never sustained a stress fracture. Female runners who had experienced at least one stress fracture also had lower BMD and BMAD at the lumbar spine ($p<0.001$; $p=0.01$ respectively) and dual femur ($p=0.006$; $p=0.003$ respectively). Male runners who had experienced a stress fracture had lower lumbar spine BMD ($p<0.001$) and BMAD ($p<0.001$), but not lower dual femur BMD or BMAD. There were no significant correlations between age, body mass, height, BMI, weekly running mileage, years in training or bone area and stress fracture incidence in male and female runners.

4.2 Discussion

This chapter investigated and compared the bone status of male and female distance runners. In doing so, the results confirm previous reports of reduced lumbar spine BMD in female runners (Marcus *et al.*, 1985; Pettersson *et al.*, 1999a; Burrows *et al.*,

2003), but the major new finding from this study is that there was a similar incidence and severity of bone mineral deficiencies in male as in female runners. Although overall total body bone status was normal, bone status varied between the sites measured, with lower values in the lumber spine and arms, and greater values in the dual femur and legs.

How does this data compare to that from previous studies? In agreement with earlier research (Winters *et al.*, 1996; Gremion *et al.*, 2001; Burrows *et al.*, 2003) reduced lumbar spine BMD was observed in female runners. Although less research has been conducted in men, some studies have found normal lumbar spine BMD in male runners (MacDougall *et al.*, 1992; Goodpaster *et al.*, 1996). However, in agreement with others (Bilanin *et al.*, 1989; Hetland *et al.*, 1993b), the results from this study indicate that lumbar spine BMD is reduced in male runners.

No previous investigation has compared bone status between male and female runners and in doing so, the observed mean lumbar spine T-scores for male and female runners were comparably low at -0.8 (the highest scores were 0.6 and 0.9 and the lowest were -3.3 and -2.6 respectively). Previous studies have reported mean lumbar spine T-scores of -1.2 (Myburgh *et al.*, 1993), -1.6 (Drinkwater *et al.*, 1984) and -2.1 (Micklesfield *et al.*, 1995), but these have been in small samples of female amenorrhoeic runners (n=9 to 13). In contrast, the present study investigated BMD using a larger subject sample of 109, comprising of male runners and of female runners of varying menstrual status and thus, it is more representative of the population of runners compared to previous work using smaller samples.

How does the incidence of osteopenia at the lumbar spine compare to that expected in the general population? The prevalence of lumbar spine osteopenia was higher in this sample of runners compared to that expected for the general population of young adults. It has been estimated that approximately 15% of the young adult female population are osteopenic and that 0.5% are osteoporotic (Kanis *et al.,* 1994). In this study, 41.6% of female runners were osteopenic and one runner (1.5%) was osteoporotic. These figures indicate at least a 2.5-fold higher prevalence than that expected in the general population. Of interest, a similar percent of male runners had a lumbar spine T-score in the osteopenic range (38.6%) and one runner was osteoporotic (2.3%). Although the prevalence of osteopenia in young male adults is unclear, it would be expected to be lower than that observed in females therefore these results would seem to be of particular significance. The prevalence of osteopenia in the male runners in this study, was similar to that previously recorded for amenorrhoeic and oligomenorrhoeic runners (Rencken *et al.,* 1996; Pettersson *et al.,* 1999; Gremion *et al.,* 2000). This is of special relevance as the primary cause of low BMD in endurance athletes has almost invariably been attributed to be sex steroid deficiencies, manifested by menstrual disorders in female athletes. This is discussed in chapter 5, which focuses on the results as analysed in terms of the menstrual status of female runners, but which also includes comparative work with the male runners.

The observation that 41.3% of the young adult runners in this study had a lumbar spine T-score indicative of osteopenia (< -1.0) is of clinical relevance for these athletes may become osteoporotic at an earlier age and have an increased risk of fracture later in life. Admittedly, caution should be applied when interpreting T-scores in young male and female adults, for this parameter of bone status was originally

intended for use in postmenopausal women. Nonetheless, the use of T-scores in this study provided an insight into the extent of low BMD in young adult distance runners. It is possible that some of the runners had not reached skeletal maturity for 4 female runners were aged 18 to 19 years and 2 male runners were aged 19 years. This may account for their low BMD, due to the possibility that they are still accumulating bone. This should be considered when interpreting the results. However, the majority were aged in their mid-twenties as indicated by the mean age of 27 and 26.8 years for the male and female runners respectively. Thus, it would seem that skeletal deficits at this age are of particular concern for these runners may not have attained an optimal peak bone density, which will put them at risk of developing future osteoporosis. Low BMD is a useful predictor of future fracture risk in both sexes and vertebral fractures occur with a higher incidence earlier in life than other types of osteoporotic fractures (Blinkley *et al.,* 2002; Fogelman *et al.,* 2002). Thus, it is possible that continuing exposure to the cause of their bone mineral deficiencies may predispose runners to low BMD and future vertebral fracture in the foreseeable future. However, prospective, longitudinal research is required to test this hypothesis.

In addition to BMD, bone size is a major determinant of fracture risk (Seeman *et al.,* 2001). It is known that men have larger bones than women and that this confers a biomechanical advantage against fracture (Henry and Eastell, 2000; Seeman *et al.,* 2001). As expected, male runners had a significantly greater lumbar spine bone area than female runners. This coincided with a significantly lower bone mineral apparent density (BMAD) and is consistent with reports from studies in healthy young men and women (Henry and Eastell, 2000; Cvijetic and Korsic, 2004). Thus, the net amount of bone mineral in the lumbar spine of male runners may be less than in female runners,

but because of a greater bone area, they may be have a biomechanical advantage over female runners against vertebral fracture. However, it should be considered that other indices of bone strength, notably cortical thickness, were not measured in this study for this required separate specialist equipment.

Smaller bones increase the risk of fracture (Seeman, 2001). However, although male runners had greater bone area at the lumbar spine and dual femur (and thus, larger bones), a higher percentage of male (54.6%) than female (38.5%) runners had suffered a stress fracture of the lower extremities (hip/sacrum to foot). Furthermore, there were no differences in bone area between runners who had or had never had a stress fracture. It appears that BMD may be more important than bone size for although bone area was similar, lumbar spine BMAD was significantly lower in male and female runners who had previously had stress fractures and dual femur BMAD was lower in female runners who had experienced a stress fracture. A similar pattern was revealed with dual femur T-scores. Low BMD has been shown to be associated with stress fracture occurrence in runners in several previous investigations (Myburgh *et al.*, 1990; Johnson *et al.*, 2001). In this study, runners who had suffered 2 or more stress fractures had the lowest T-scores, which were mid-range osteopenia (-1.6 and -1.4). Indeed factors other than BMD are likely to contribute to stress fracture incidence including bone geometry, running terrain and foot strike. These were not assessed by the current study. Weekly running mileage, body mass and BMI were unrelated to stress fracture incidence to suggest that the magnitude and duration of running training may not be the most permissive determinants of stress fracture risk.

Repetitive loading increases microdamage in bone (Frost, 1997). One elite male runner had experienced a stress fracture to the hip but continued to attempt to train on this injury. Approximately one year later, this runner had a more serious hip fracture requiring surgery and is unable to train competitively. Despite previously running high weekly mileages, this runner had osteopenia in the dual femur which indicates diminished bone integrity. However, for the total group, no correlations were observed between training volume and stress fracture incidence. Additionally, there were no differences in number of years in training between runners who had, or had not suffered a stress fracture. These results suggest that BMD is more predictive of stress fractures in runners, regardless of training volume and history.

It is unclear whether running *per se* is a beneficial stimulus at more loaded skeletal sites. Several studies have found reduced BMD even at sites recipient of greater magnitude of loading during running exercise, such as the proximal femur in male (Hetland *et al.,* 1993b) and female (Myburgh *et al.,* 1993; Rencken *et al.,* 1996) runners. In this study it was surprising that several runners were osteopenic in the dual femur despite participating in a regular running regime. Low BMD at this site elevates the risk for hip fracture, the most serious of osteoporotic fractures with nearly one third of patients dying within one year (Keene *et al.,* 1993). Indeed, one male runner with osteopenia in the dual femur had recently suffered a serious fracture to his left hip. However, the mean results of the current study are in agreement with those which have reported increased or normal BMD at the lower extremities in runners (Bennell et al., 1997; Gremion et al., 2001). Despite normal mean total body bone status, there were site-specific differences in BMD, with reduced BMD in the lumbar spine and arms, and greater BMD in the legs and dual femur. This is likely to reflect a site-

specific effect from loading during running exercise and was present in both male and female runners.

Mechanical loading of the skeleton by ground reaction forces or muscular contraction leads to an increase in bone density (Lanyon, 1996; Frost, 1997). As running is a vertical, weight-bearing exercise, the ground reaction forces are attenuated as they transmit upward. Although running does not generate as high a magnitude compared to sports such as gymnastics, forces at the lower limbs can be 2 to 5 times greater than body mass (Subotnick, 1985). In contrast, those at the lumbar spine are 1.75 greater than body mass (Cappozzo, 1983). Thus, the observation in this study that BMD at the dual femur and lower limbs was normal or above normal, is likely to be resultant of a weight-bearing effect. In contrast, the spine and arms receive minimal bone strain during running and may, as a consequence, be less dense (Frost, 1997).

The findings also suggest that the repetitive, low magnitude strains associated with running are sufficient to maintain BMD at lower, but not upper extremity sites. These results are consistent with the majority of studies, which suggest that bone gain in distance runners only occurs in lower extremity skeletal sites (Heinonen *et al.,* 1993; Bennell *et al.,* 1997) or that low BMD in runners is limited to upper body sites, in particular, the spine (Bilanin *et al.,* 1989; Pettersson *et al.*, 1999; Gremion *et al.,* 2001).

There are site-specific differences in the composition of bone throughout the skeleton which influence the rate of bone turnover and hence, the skeletal response to stimuli. Trabecular bone is more sensitive to metabolic and hormonal alterations than cortical

bone because of its rapid turnover rate (Compston, 1997; Heaney, 1999). The lumbar spine is predominantly comprised of trabecular bone, which may partly explain the severity of bone mineral deficits in many runners. In contrast, the dual femur and long bones have a greater cortical component (up to 50%), which has a lower turnover rate (Myerson *et al.,* 1992; Heaney, 1999). Thus, it is possible that adverse changes in BMD at these sites may not become apparent until the negative environment has been sufficiently prolonged to enable a response through an alteration in remodelling. Reflecting this, the present study found that in female runners, number of eumenorrhoeic years correlated positively with leg and arm BMD values. In male runners, there was a significant negative association between years in training and age with bone values at the legs and dual femur, suggesting that BMD may decrease as years in training increase. The relationship with age is unlikely to reflect significant natural loss of bone with age, for this usually occurs after the age of 50. It may simply reflect the finding that older men (above the age of 30 years) had been training for a longer period of time ($p<0.005$).

Although many athletes ran high mileages (up to 117 miles per week), there were no positive correlations with bone status at weight-bearing sites, suggesting that more miles of running will not necessarily increase BMD at the lower extremities. In fact, there was a trend for lower dual femur BMD as training volume increased. The 'more is less' concept is most clearly applicable for lumbar spine BMD. Weekly running mileage was the strongest predictor of lumbar spine BMD in all runners. This negative relationship indicates that large volumes of running training may be harmful to bone. Bone status is examined with respect to the training characteristics and performance level of the runners in chapter 6.

High strain magnitude is a prerequisite for optimal skeletal loading (Chilibeck *et al.,* 1995). A higher body mass or BMI imposes greater strain magnitude in bone, than a lower body mass or BMI (Chilibeck *et al.*, 1995; Frost, 1997). Thus, the positive correlations observed between BMI and bone status at all measured sites, was expected and corroborate results from previous studies (Drinkwater *et al.,* 1984; 1990; Rencken *et al.,* 1996; Pettersson *et al.,* 1999; Burrows *et al.,* 2003). It is also possible that low body mass, BMI and body fat amongst the runners, indicates a chronic energy deficit, arising from high energy expenditures with insufficient energy intake. Indeed, as discussed in the introductory chapters, independent from other factors, an energy deficit may have negative implications for bone (Zanker and Swaine, 1998; Heer *et al.,* 2002). Energy balance and BMD results are presented and discussed in chapters 7 and 8.

This study was cross-sectional and therefore limited in the sense that no conclusions can be drawn about when and how rapidly these runners started to lose BMD. A second consideration is that of self-selection. Subjects of lower body mass and BMI may have self-selected to the sport of distance running in suiting with their body type. Thus, as low BMI is a risk factor for osteoporosis, this may predispose runners to low BMD. However, if this was the case, then total body BMD would have been expected to be reduced and this was not the case. Furthermore the problem of self-selection can only be controlled in intervention studies and this design would not have been suitable to address the study aims. The strengths of this study were that it adopted a different stance in the research of BMD in athletes by comparing male and female runners using T-scores and the subject sample was relatively large in comparison to the

majority of previous work in this area. Moreover, this study provided information regarding differences in bone size between male and female runners.

The results of this study show that the problem of low lumbar spine bone density in runners may be more widespread than previously thought, affecting both male and female runners. Thus, in conclusion to the first research question, reduced lumbar spine BMD in distance runners does not sex discriminate, since over one third of male and female runners were osteopenic at this site and the mean T-scores were identical. It is possible that female runners are at a greater risk for osteoporotic fracture in later life because of their smaller bone size. However, as this study was cross-sectional this could not be determined. Stress fractures were associated with reduced BMD and not reduced bone size. High weekly running mileage and low BMI were particularly predictive of low lumbar spine, dual femur and total body BMD. In addition, total number of eumenorrhoeic years correlated positively with lumbar spine BMD in the female runners. As menstrual disorders are known to be a major risk factor for low BMD in female athletes, this has been addressed in the following chapter.

Chapter 5

Bone mineral density and overt menstrual status in female distance runners

The results have shown that lumbar spine BMD is reduced in both male and female distance runners. Although this may suggest that a similar aetiology of low BMD in male and female distance runners, previous literature has established a role for menstrual dysfunction in female athletes, arising from the associated oestrogen deficiency. In this chapter, the second research question was investigated:

"Is BMD related to overt menstrual status[1] in female runners?"

5.1 Results

5.1.1 Menstrual history and bone status

The mean age of menarche for the female runners was 14.3 (±1.6) years and ranged from 11 to 19 years. Three highly-trained runners reported one menstrual cycle at the age of 15/16 years, but no menstrual cycles thereafter and were now aged 20 and 21 years. The mean total number eumenorrhoeic years was 10.2 (± 8.4), and ranged from 0 to 36 years. As expected, total number of eumenorrhoeic years correlated positively with age ($r = 0.590$; $p < 0.005$) and negatively with age at menarche ($r = -0.322$;

[1] 'Overt menstrual disorder' is defined as a menstrual disorder that can be symptomatically identified by the absence or irregularity of menses.

$p<0.001$). Age of menarche was unrelated to BMD or T-scores for the lumbar spine, dual femur or total body. Total number of eumenorrhoeic years correlated positively with lumbar spine BMD and T-scores (both $r = 0.29$; $p<0.01$), total body T-scores ($r = 0.31$; $p<0.001$), percentage of normal leg BMD ($r = 0.373$; $p<0.005$) and percentage of normal arm BMD ($r = 0.404$; $p<0.001$). There were no relations between BMD measures and number of eumenorrhoeic years during adolescence (18 years *minus* age at onset of menarche).

5.1.2 Menstrual status

More than half (52.3%) of the female runners were eumenorrhoeic, 15 (23.1%) were currently amenorrhoeic, 2 (3.1%) were oligomenorrhoeic and 14 (21.5%) were using the oral contraceptive pill. The mean duration for use of the oral contraceptive pill was 3.8 years (±1.4), ranging from 2 to 7 years. Three runners had primary amenorrhoea, 1 was aged 18 years and 2 were aged 19 years. As only 2 runners were oligomenorrhoeic and as there were no differences between these runners and the amenorrhoeic group, oligomenorrhoeic and amenorrhoeic runners were grouped together for the main analyses and have been referred to as 'amen/oligomenorrhoeic'. Table 5.1 presents the characteristics of the female runners, grouped by menstrual status.

There were no differences in age, lean body mass, age of menarche and years in training. Amen/oligomenorrhoeic runners had fewer years of eumenorrhoea than the eumenorrhoeic runners ($p<0.05$). Amen/oligomenorrhoeic runners were lighter and had lower levels of body fat compared to eumenorrhoeic runners.

Amen/oligomenorrhoeic runners and runners using the oral contraceptive pill ran more miles per week than did eumenorrhoeic runners ($p<0.05$).

Table 5.1: Characteristics (mean and SEM) of the female runners by menstrual status

	Eumenorrhoeic (n=34)	Amen/oligomenorrhoeic (n=17)	Oral contraceptive users (n=14)
Age (years)	27.8 (1.5)	25.5 (1.8)	26 (1.3)
Body mass (kg)	54.1 (0.8)	48.8 (1.4) *	53.2 (1.6)
BMI (kg m $^{-2}$)	19.8 (0.5)	18.2 (0.3) *	19.2 (0.3)
% Body fat	18.3 (0.7)	13.6 (1.3) *	15.3 (1.5)
Body fat Z-score	-1.3 (0.3)	-1.9 (0.8) †	-1.7 (0.2)
Lean mass (kg)	42.6 (0.6)	40.9 (1.0)	43.7 (1.0)
Menarche (years)	14.1 (0.3)	14.2 (0.4)	14.8 (0.5)
Years of eumenorrhoea	12.7 (1.6)	5.6 (1.6) †	9.8 (1.6)
Weekly mileage	46.5 (2.4)	55.1 (2.9) ‡	58.8 (2.5) ‡
Years in training	9.9 (1.0)	8.0 (1.0)	10.5 (1.6)

* *< eumenorrhoeic runners, $p<0.005$*
† *< eumenorrhoeic runners, $p<0.05$*
‡ *> eumenorrhoeic runners, $p<0.05$*

5.1.3 Menstrual and bone status

(i) Differences between menstrual groups

The bone measurement results (observed and adjusted for BMI) for the female runners grouped by menstrual status are presented in Table 5.2. Results for measurements of bone mineral apparent density (BMAD) have been included to control for potential differences in bone size between the groups.

Table 5.2: Bone status of female runners by menstrual status

	Eumenorrhoeic (n=34)	Amen/oligomenorrhoeic (n=17)	Oral Contraceptive users (n=14)
Lumbar spine T-score			
Observed	-0.4 (0.3)	-1.4 (0.2) *	-1.1 (0.1) *
Adjusted*	-0.5 (0.2)	-1.2 (0.2) *	-1.1 (1.5) *
BMD (g cm^{-2})			
Observed	1.15 (0.02)	1.03 (0.02) *	1.07 (0.02) †
Adjusted*	1.14 (0.01)	1.05 (0.02) *	1.05 (0.02) †
Bone area (cm^2)	44.6 (0.6)	42.7 (1.0)	44.9 (1.3)
BMAD (mg cm^{-3})	174.2 (2.8)	159.8 (3.0) *	160 (3.1) †
Dual femur T-score			
Observed	0.7 (0.1)	0.0 (0.2) *	0.5 (0.2)
Adjusted*	0.8 (0.1)	0.1 (0.1) *	0.5 (0.2)
BMD (g cm^{-2})			
Observed	1.09 (0.02)	0.99 (0.2) *	1.06 (0.02)
Adjusted*	1.09 (0.02)	1.01 (0.02) *	1.06 (0.02)
Bone area (cm^2)	31.2 (0.4)	29.9 (0.4)	31.6 (0.6)
BMAD (mg cm^{-3})	196.6 (3.2)	183.2 (4.6) †	189.8 (3.8)
Total body T-score			
Observed	0.5 (0.1)	-0.2 (0.2) ‡	0.5 (0.1)
Adjusted*	0.3 (0.1)	0.0 (0.2)	0.5 (0.2)
Legs % normal	+15.6 (1.2)	+7.2 (2.0) * ‡	+14.6 (1.7)
Arms % normal	-1.8 (1.1)	-8.2 (1.0) *	-3.7 (1.5)

* < *eumenorrhoeic runners, p<0.005*
† < *eumenorrhoeic runners, p<0.05*
‡ < *oral contraceptive pill users, p<0.05*

Mean lumbar spine BMD and T-scores were lowest in the amenorrhoeic and oral contraceptive groups ($p<0.005$) and these differences remained significant when adjusted for BMI ($p<0.05$). Lumbar spine BMD was 10.4% lower in amen/oligomenorrhoeic runners and 7% lower in oral contraceptive users, compared to eumenorrhoeic runners.

Despite a trend for smaller lumbar spine bone area, amen/oligomenorrhoeic runners had the lowest BMAD at this site, although BMAD was also lower in the oral contraceptive users compare to eumenorrhoeic runners. Dual femur BMD and BMAD and total body BMD were also lowest in amen/oligomenorrhoeic compared to eumenorrhoeic runners ($p<0.005$) and differences at the dual femur were independent of BMI and percentage body fat ($p<0.05$). Only the amenorrhoeic group had a negative mean total body T-score, which was significantly lower than the T-score for the oral contraceptive group ($p<0.05$). Despite having lower lumbar spine BMD and BMAD, oral contraceptive users had normal BMD at the more weight-bearing sites of the dual femur and legs, and total body. Percentages of age-matched normal data at the legs and arms were lower in amenorrhoeic compared to eumenorrhoeic runners ($p<0.005$).

(ii) Osteopenia and Osteoporosis

Ten (58.8%) amen/oligomenorrhoeic runners were osteopenic at the lumbar spine. One amenorrhoeic runner had a T-score of less than -2.5, indicating osteoporosis. At the dual femur, 2 amenorrhoeic runners had a T-score of less than -1.0 to indicate osteopenia.

Surprisingly, 10 (71.4%) oral contraceptive users and 7 (23.3%) eumenorrhoeic runners had a lumbar spine T-score indicative of osteopenia. The lowest T-score in the eumenorrhoeic group was -2.3 and this individual had never experienced amenorrhoea. The lowest T-score in the oral contraceptive users group was -2.4, nearing the osteoporotic threshold and all oral contraceptive users had been taking the pill for at least 3 years. ANOVA was performed to look for any differences in

eumenorrhoeic women and oral contraceptive users with and without lumbar spine osteopenia. The results of the analysis found that the 7 eumenorrhoeic runners who had osteopenia ran significantly more miles per week than those without osteopenia (55.7 *v* 44.1; $p<0.005$). There were no differences in bone area, BMI, body fat or number of eumenorhoeic years. The 10 oral contraceptive users found to have osteopenia had significantly lower body fat (12.9 *v* 19.5%; $p<0.001$) and BMI (18.7 *v* 20.1 $kg.m^{-2}$; $p<0.05$). There were no differences in bone area, weekly running mileage, years ran, eumenorrhoeic years or duration of oral contraceptive use.

(iii) Within-group correlations

Pearson's product moment and Kendall's-tau correlation tests were performed to investigate potential relationships between the measured variables and bone status of each menstrual group. The majority of correlations were only found for the eumenorrhoeic group which may reflect the greater number of subjects in this group. Weekly running mileage correlated negatively with lumbar spine BMD in the eumenorrhoeic group ($r = -0.428$, $p<0.001$) but no relationships were found for runners in the other menstrual groups and the 7 eumenorrhoeic runners with lumbar spine osteopenia ran significantly more miles per week than their eumenorhoeic counterparts. Body mass was only associated with total body BMD in the eumenorrhoeic group ($r = 0.461$ $p<0.05$). In the amen/oligomenorrhoeic group, the number of eumenorrhoeic years correlated positively with lumbar spine BMD ($r = 0.650$; $p<0.005$) and total body BMD ($r = 0.551$; $p<0.05$). There were no correlations between bone status and body fat, lean body mass, BMI or number of years in training within the three menstrual groups.

5.1.4 Comparisons to male runners

ANOVA and the Games-Howell post-hoc tests were used to compare bone status of male and female runners when accounting for menstrual status in the female runners. Table 5.3 summarises the mean data for the three female sub-groups and the male runners.

Table 5.3: Bone status (mean and SEM) of male runners and of female runners grouped by menstrual status

	Eumenorrhoeic (n=34)	Amen-oligomenorrhoeic (n=17)	Oral contraceptive users (n=14)	Male (n=44)
Lumbar spine				
T-score	-0.4 (0.3)	-1.4 (0.2)	-1.1 (0.1)	-0.81 (0.1) *
Adjusted*	-0.4 (0.1)	-1.1 (0.2)	-1.0 (0.2)	-1.0 (0.1) *
BMD (g cm^{-2})	1.15 (0.02)	1.03 (0.02)	1.07 (0.02)	1.14 (0.01) †
Adjusted*	1.16 (0.02)	1.06 (0.02)	1.08 (0.02)	1.13 (0.02)
Bone area (cm^2)	44.6 (0.6)	42.7 (1.0)	44.9 (1.3)	53.0 (0.7) †‡¥
BMAD (mg cm^{-3})	174.2 (2.8)	159.8 (3.0)	160 (3.1)	157.6 (2.0) *
Dual femur T-score	0.7 (0.1)	0.0 (0.2)	0.5 (0.2)	0.6 (0.1)
Adjusted*	0.8 (0.1)	0.1 (0.2)	0.6 (0.2)	0.4 (0.1)
BMD (g cm^{-2})	1.09 (0.02)	0.99 (0.2)	1.06 (0.02)	1.15 (0.0) †‡¥
Adjusted*	1.09 (0.02)	1.02 (0.03)	1.07 (0.03)	1.14 (0.02)
Bone area (cm^2)	31.2 (0.4)	29.9 (0.4)	31.6 (0.6)	37.7 (0.6) †‡¥
BMAD (mg cm^{-3})	196.6 (3.2)	183.2 (4.6)	189.8 (3.8)	189.8 (2.9)
Total body T-score	0.5 (0.1)	-0.2 (0.2)	0.5 (0.1)	0.19 (0.1)
Adjusted*	0.5 (0.1)	0.2 (0.2)	0.7 (0.2)	-0.1 (0.1)*¶
Legs % normal	+15.6 (1.2)	+7.2 (2.0)	+14.6 (1.7)	+7.2 (0.9) *¥
Arms % normal	-1.8 (1.1)	-8.2 (1.0)	-3.7 (1.5)	-5.4 (0.9) *

**Scores adjusted for age, BMI and body fat*

* *< eumenorrhoeic runners, p<0.01*
‡ *> amenorrhoeic runners, p<0.05*
‡ *> eumenorrhoeic runners, p<0.05*
¥ *> oral contraceptive users, p<0.05*
¶ *< oral contraceptive users, p<0.05*

Male runners had a lower observed lumbar spine T-score compared to female eumenorrhoeic runners (-0.8 *v* -0.4; $p<0.005$). Lumbar spine bone area was greater in the male group compared to the female groups, indicating that men have larger bones than women. The larger bone size in men explained the higher lumbar spine BMD in this group compared to female amen/oligomenorrhoeic runners (1.14 *v* 1.03; $p<0.05$) and oral contraceptive users (1.14 *v* 1.07 g cm^{-2}, $p<0.05$), for BMAD was lower in the male runners and lower in the male than in the female eumenorrhoeic runners (157.6 *v* 174.2 mg cm^{-3}, $p<0.005$).

Although dual femur BMD was greater in male than female runners, again this could be attributed to the greater bone area at this site in men. Dual femur BMAD was similar for all groups of runners with a non significant trend for lower BMAD in male compared to female eumenorrhoeic runners.

When adjusted for differences in age, BMI and body fat, the mean lumbar spine T-score for male runners was similar to that for amen/oligomenorrhoeic runners and oral contraceptive pill users. Adjusted total body T-scores were lower in male runners than in female eumenorrhoeic runners and those using the oral contraceptive pill.

Percentage of age and sex-matched normative data for BMD of the legs and arms, as determined using regional analysis from the total body DXA measurement, were lower in male compared to the female eumenorrhoeic runners (legs: +7.2 *v* +15.6%, $p<0.001$; arms: -5.4 *v* -1.8%, $p<0.005$). The mean percentage of age and sex-matched leg BMD was identical in male as in female amen/oligomenorrhoeic runners (+7.2%).

5.1.5 Stress fractures

Seven (20.6%) eumenorrhoeic runners had suffered 1 stress fracture and 2 (5.9%) had suffered 2 or more stress fractures. In the oral contraceptive group, 6 (42.9%) runners had suffered 1 stress fracture and 2 (14.3%) had 2 or more. Five (29.4%) amen/oligomenorrhoeic had suffered 1 stress fracture and 3 (17.6%) had suffered 2 or more stress fractures. There were no differences in number of eumenorrhoeic years, BMI, body fat or weekly running mileage in those who had suffered a stress fracture compared to those who had never had a stress fracture.

5.2 Discussion

The results presented confirm previous findings that female runners with amenorrhoea have lower BMD than their eumenorrhoeic counterparts (Drinkwater *et al.*, 1984; Marcus *et al.*, 1985; Rencken *et al.*, 1996; Pettersson *et al.*, 1999). These runners also had significantly lower BMI compared to eumenorrhoeic runners and this is consistent with previous findings (Zanker and Swaine, 1998). Significant differences were found for all measured sites and in agreement with previous studies, the largest deficit was observed at the lumbar spine (Marcus *et al.*, 1985; Cobb *et al.*, 2003).

Numerous cross-sectional studies comparing bone status between eumenorrhoeic and amenorrhoeic runners have expressed reduced BMD in amenorrhoeic runners as a percentage of BMD in eumenorrhoeic runners. In this study, the mean difference at the lumbar spine was -10.4%. The highest deficits reported in the literature are from Pettersson *et al* (1999), who found an overall deficit of 16% and Drinkwater *et al* (1984) who recorded a 14% deficit. However, in such studies, ascertainment bias may be present because subjects were specifically recruited according to their menstrual

status and subject sample sizes were small (10 to 14 runners per group). A recent study conducted by Cobb *et al* (2003), used a greater number of subjects (overall n=91; oligoamenorrhoeic n=33) and found that the mean difference in lumbar spine BMD was 7%. As in the Cobb *et al* study (2003), the difference in lumbar spine BMD between amen/oligomenorrhoeic and eumenorrhoeic runners was independent of BMI and body fat, suggesting additional influential variables are associated with reduced BMD in runners with amenorrhoea.

There was a trend for reduced bone size in amenorrhoeic runners. Although this may reflect disrupted bone development during puberty, for bone size is usually complete by the end of puberty (Seeman, 2001); there were no associations between bone size and age at menarche. The differences observed in lumbar spine BMD between amenorrhoeic and eumenorrhoeic runners were not due to differences in bone size. A number of previous studies have reported that amenorrhoeic runners are at increased risk for stress fractures in the lower extremities (Marcus *et al.,* 1985; Myburgh *et al.,* 1990). Although in chapter 4, low lumbar spine BMD was shown to be a major predictor of stress fracture occurrence, there was no difference in the incidence of stress fractures between female runners with varying menstrual status.

Amenorrhoea and the underlying oestrogen deficiency are associated with low BMD in female athletes (Tomten *et al.,* 1998; Gibson *et al.,* 2000). In this respect, the use of the oral contraceptive pill as a form of oestrogen therapy would be expected to maintain BMD. However, lumbar spine BMD was reduced in runners who had been using the oral contraceptive pill for at least 2 years (mean duration, 3.8 years), to a similar degree as observed in amenorrhoeic runners. It is unlikely that these results

were confounded by differences in group size as between-group differences were tested using the Games-Howell statistical method which accounts for such inconsistencies. Furthermore, the similarities in BMD between the oral contraceptive users and amenorrhoeic runners, were independent of BMI, body fat and bone size.

To date, whereas the oral contraceptive pill (excluding depot medroxyprogesterone acetate use) is associated with maintenance or improvement of BMD in normal women (non-runners; Sultana *et al.,* 2002; Berenson *et al.,* 2004) and women with primary ovarian failure (Castelo-Branco *et al.,* 2001), the suitability of the oral contraceptive pill in terms of promoting BMD in female athletes is unclear. Studies have reported conflicting results (Warren and Holderness, 1992; Hergenroeder *et al.,* 1995; Gibson *et al.,* 1999) and there has been no randomised intervention trial of a suitable design or duration, in this area. If oestrogen deficiency is the major cause of low BMD in amenorrhoeic athletes, oestrogen replacement therapy such as the oral contraceptive pill, would be expected to prevent or even reverse this. In this study there were no positive associations between oral contraceptive use and lumbar spine BMD in female runners. Rather, oral contraceptive pill users had low BMD that was comparable to the amenorrhoeic group. However, there are several potential explanations as to why this may be. Firstly, it is possible that the BMD of these runners was low due to previous amenorrhoea and the use of the oral contraceptive pill for 2.5 to 7 years (mean duration 3.8 years) may have been insufficient. Secondly, it is possible that the form of synthetic oestrogen used in the oral contraceptive pill does not have the same effect as the natural oestradiol produced by the ovaries or provided by HRT. Unfortunately there has been no randomised intervention trial to

determine the efficacy of the oral contraceptive pill, even though it is currently offered to amenorrhoeic athletes as a preventative treatment against osteoporosis.

Although the oral contraceptive pill is currently offered to amenorrhoeic athletes to help maintain BMD (Shangold, 1990; Cumming, 1996), its efficacy is unclear for studies have reported conflicting results. The majority of studies have described little or no effect and there have been problems with compliance (Warren and Holderness, 1992; Keen and Drinkwater, 1997; Gibson *et al.,* 1999; Braam *et al.,* 2003). Similarly, the oral contraceptive pill has not been able to prevent bone loss or improve BMD in women with anorexia nervosa (Kreipe *et al.,* 1993; Grinspoon *et al.,* 2002). Thus, it is possible that female runners and women with anorexia lose bone due to a similar mechanism.

Ten (71.4%) oral contraceptive users had a lumbar spine T-score indicative of osteopenia. On closer analysis of the data, these runners had a significantly lower BMI ($p<0.05$) and in particular, a significantly lower percentage body fat compared to oral contraceptive users who had a normal lumbar spine T-score ($p<0.005$). There were no differences in eumenorrhoeic years. Low body fat levels have previously been associated with reduced BMD (Khosla *et al.,* 1996; Pettersson *et al.,* 1999; Burrows *et al.,* 2003). Body fat may have a contributory role in bone health for it is associated with the secretion of oestrogen, IGF-1 and leptin (Warren, 1999; Bollag *et al.*, 2001; Ong *et al.,* 2002; Thong *et al.,* 2000). Leptin has a particularly strong relationship with body fat and has recently been found to be a physiological mediator of bone metabolism and in particular, bone formation (Thomas *et al.,* 1999; Cornish *et al.*, 2002). Thus, low body fat may signify a reduction in these bone active hormones

and as a consequence, an alteration in bone remodelling. Furthermore, in contrast to the uncertainty regarding the effectiveness of the oral contraceptive pill, there is a consensus in the literature that improvements in BMD, regardless of whether or not there is a resumption of menses, are attained by female amenorrhoeic runners who gain weight and body fat (Drinkwater *et al.,* 1990; Warren and Holderness, 1992; Keen and Drinkwater, 1997). This indicates a particularly fundamental role for normal body mass and body fat, for the maintenance and improvement of BMD in runners.

In addition to the observation of osteopenia in women runners using the oral contraceptive pill, lumbar spine osteopenia was also found in 7 eumenorrhoeic runners (23.3%). It is possible that these eumenorrhoeic athletes may have had sub-clinical menstrual disorders, such as anovulatory cycles or luteal phase deficits. However, the evidence linking such disorders to low BMD is conflicting. Prior *et al* (1990) reported lower spine BMD in women with luteal phase deficiency whereas De Souza *et al* (1997) did not. Information provided by another study (Pettersson *et al.,* 1999) indicated that despite normal oestradiol levels, eumenorrhoeic runners still had 7% lower lumbar spine BMD than controls. Furthermore, Grimston *et al* (1993) verified menstrual status with hormone tests and found that 4 out of the 10 eumenorrhoeic runners studied, had spinal osteopenia. In contrast, Gibson *et al* (2004) reported the mean lumbar spine T-score for their group of eumenorrhoeic runners (n=17) to be +0.4, whereas in the current study, the mean lumbar spine T-score was found to be -0.4. The 7 eumenorrhoeic runners from the sample studied in the current research who were found to be osteopenic ran significantly more miles per week than those with normal BMD ($p<0.001$) and weekly running mileage was negatively

associated with lumbar spine BMD in all runners. Regarding correlations for the female runners as one group, higher weekly running mileages, low BMI and low body fat were associated with lumbar spine osteopenia.

It is unclear whether running *per se*, can maintain or improve BMD at loaded sites in runners with menstrual disorders. On the one hand, there are some studies which have found significantly reduced BMD at loaded sites such as the proximal femur, trochanter and total femur in amenorrhoeic runners (Myburgh *et al.,* 1993; Rencken *et al.,* 1996; Pettersson *et al.,* 1999). On the other hand, the present study is consistent with results from several other studies (Taaffe *et al.,* 1997; Gremion *et al.,* 2002) which indicate that the repeated impact strains produced during running, may offer a benefit at loaded skeletal sites. In this study, although T-scores and BMD at the dual femur, legs and total body were significantly lower in amenorrhoeic than eumenorrhoeic runners, T-score values were within the normal range. It is interesting to note that although oral contraceptive users had significantly reduced lumbar spine BMD, to a level comparable to amenorrhoeic runners, they were found to have significantly greater total body and leg BMD values. Furthermore, BMD at the dual femur was also greater than in amenorrhoeic runners (although narrowly missed statistical significance). These results suggest that the oral contraceptive pill users may obtain a skeletal benefit at cortical sites recipient of greater impact loading, but not at the predominantly trabecular spine, which receives less impact loading during running.

How does the bone status of female runners grouped by menstrual status compare to that of the male runners? It was unexpected that male runners had significantly lower

observed lumbar spine T-scores and BMAD compared to eumenorrhoeic runners. Once adjusted for BMI and percentage body fat, the mean lumbar spine T-score for male runners was similar to those found for amen/oligomenorrhoeic runners and runners using the oral contraceptive pill, with all at -1.0 or less. Actual BMD values for male runners showed a trend to be lower than for eumenorrhoeic runners, despite their larger bone size. This suggests that bone mineral may be jeopardised to a greater extent than bone size in male runners although further research of bone size, structure and quality is required to confirm this observation.

There are several considerations to make when interpreting the data from the current research. One is the absence of biochemistry. Unfortunately, this was beyond the allocated means available for the study and would not have been feasible in light of the fact that the majority of participants had to travel at various times in the day and evening for their appointments. Thus, levels of sex hormones in both male and female runners and their bone turnover were not determined.

It is recognised that the self-report method of categorisation of menstrual status may not have been sufficiently sensitive to separate women with differences in basal oestrogen secretion. Whilst prolonged amenorrhoea (> 1 year) and a low BMI (<18 $kg.m^{-2}$) has been shown to coincide with oestrogen levels that appear inadequate, oligomenorrhoea often coincides with adequate oestrogen levels (Zanker and Swaine, 1998). Evidence linking oligomenorrhoea (attributable to low levels of progesterone or anovulation), to reduced BMD, is insufficient (Prior *et al.,* 1990; De Souza *et al.,* 1997). In women, ovarian suppression is also accompanied by testosterone deficiency, which may disturb the balance of bone turnover (Pacifici *et al.,* 1996). The

contribution of such potentialities could not be determined from the current study but would be useful to evaluate in future research.

Another consideration is that the oral contraceptive pill may not adequately replace ovarian hormones in women runners with endogenous oestrogen deficiency due to hypothalamic amenorrhoea. The main form of oestrogen used in the oral contraceptive pill is synthetic ethinyloestradiol, which may be less effective than endogenous oestradiol and its metabolites for the maintenance of normal bone turnover. Thus, it is possible that runners using the oral contraceptive pill may be deficient of the required form of oestrogen.

In male runners, although previous work indicates that the cause of skeletal deficiencies in male runners does not involve the sex steroids, this could not be assumed in the current study. Indeed, endurance training may suppress testosterone levels (Wheeler *et al.,* 1991), but this has not been related to low BMD or alterations in bone turnover of male runners elsewhere (Hetland *et al.*, 1993b; MacKelvie *et al.,* 2000; Maimoun *et al.,* 2003). Although oestrogen may mediate the skeletal response to testosterone, a recent study found normal levels of oestrogen in male runners with low BMD (Maimoun *et al.,* 2003).

In conclusion to the original research question, BMD in female runners was found to be related to menstrual status to a certain extent. On the one hand, amen/oligomenorrhoeic runners had lower BMD at all measured sites compared to eumenorrhoeic runners, although the most severe deficits were at the lumbar spine. On the other hand, low BMD in oral contraceptive using runners and in 7

eumenorrhoeic runners suggests that oestrogen deficiency may not be the only cause of reduced BMD. The finding that T-scores in male runners were comparable to those in female runners with amenorrhoea, were of particular interest and questions the 'female' athlete triad. The results of this study indicate that the problem of low lumbar spine BMD in runners may be more widespread than once previously thought and more widespread than concluded in chapter 4.

Chapter 6

Bone mineral density, training characteristics and performance level

Chapters 4 and 5 have shown that lumbar spine BMD was reduced to a similar extent in male and female distance runners. Skeletal sites exposed to greater loading during running, such as the dual femur and legs, appeared to be relatively protected against bone loss. Low lumbar spine BMD was more severe in female runners with menstrual disorders. However, osteopenia was also particularly prevalent in those using the oral contraceptive pill and in male runners. Therefore, factors other than amenorrhoea could be implicated in the aetiology of bone loss in the running group. In this chapter, training characteristics and differences in performance level were examined to investigate any potential associations with low BMD in runners. The third research question of this study was,

> *"Is BMD related to training characteristics and performance level in male and female distance runners?"*

6.1 Results

6.1.1 Training characteristics and BMD

The training characteristics for the male and female runners are presented in Table 6.1.

Table 6.1: Training characteristics of the runners

	Female (n=65)	Male (n=44)
Weekly running mileage (mean and SEM)	51.4 (1.7)	60.5 (2.8)
Twice-a-day training at least twice per week*	47 (72.3)	36 (81.8)
Rest day less frequent than once per week*	19 (29.2)	21 (47.7)
2 or more sessions of strength training per week*	27 (41.5)	15 (34.1)

** Number of runners and % of group*

(i) Twice-a-day training

There were no differences in BMD or T-scores between male runners who did or did not train twice-a-day. The inability to detect a difference in the male group is likely to be because the large majority of male runners trained twice a day. In females, those who ran twice-a-day had lower lumbar spine BMD (1.08 *v* 1.16; $p<0.01$) and T-scores (-1.0 *v* -0.4; $p<0.005$) than those who did not run twice-a-day. There were no differences at the dual femur or total body.

(ii) No regular rest day

Male runners who did not take a complete rest day from training at least once a week, had lower lumbar spine BMD (1.09 *v* 1.20; $p<0.001$) and T-scores (-1.3 *v* -0.4: $p<0.001$) and total body BMD (1.22 *v* 1.26; $p<0.05$) and T-scores (-0.1 *v* 0.5; $p<0.005$), than those who did take a rest day. The results were similar in female runners who did not take a weekly rest day compared to those who did, at the lumbar spine (BMD: 1.03 *v* 1.14; T-score: -1.5 *v* -0.6; $p<0.001$) and total body (BMD: 1.12 *v* 1.16; T-score: -0.1 *v* 0.4; $p<0.05$). All differences were significant after adjustment for BMI using ANCOVA.

(iii) Strength training

Male and female runners who participated in organised strength training, such as weight training, at least twice a week had greater lumbar spine BMD than those who did not. These runners did not differ in body mass or BMI. Mean lumbar spine BMD in female runners who participated in regular strength training, was greater than those who did not (1.14 *v* 1.08; $p<0.001$) as was lumbar spine T-score (-0.5 *v* -1.1; $p<0.01$). The results were similar at the lumbar spine in male runners (BMD: 1.20 *v* 1.12; $p<0.01$; T-score: -0.4 *v* -1.1; $p<0.05$). Male and female runners who participated in regular strength training had greater total body BMD and T-scores compared those who did not ($p<0.05$). All differences remained significant after controlling for BMI using ANCOVA.

(iv) Weekly running mileage

Mean weekly running mileage for male and female runners was 60.5 and 51.4 respectively. Weekly running mileage correlated negatively with lumbar spine BMD and T-scores in male ($r = -0.488$; -0.483; $p<0.005$) and female ($r = -0.328$; -0.354; $p<0.005$) runners, even after controlling for BMI. The inverse relationship between weekly running mileage and lumbar spine T-score is shown in Figure 6.1.

No relationships were found for weekly running mileage, leg and dual femur bone status in male or female runners. Similarly, there were no associations between running mileage and total body BMD in female runners, but there was an inverse correlation between weekly running mileage and total body T-score ($r = -0.291$; $p<0.01$) in male runners.

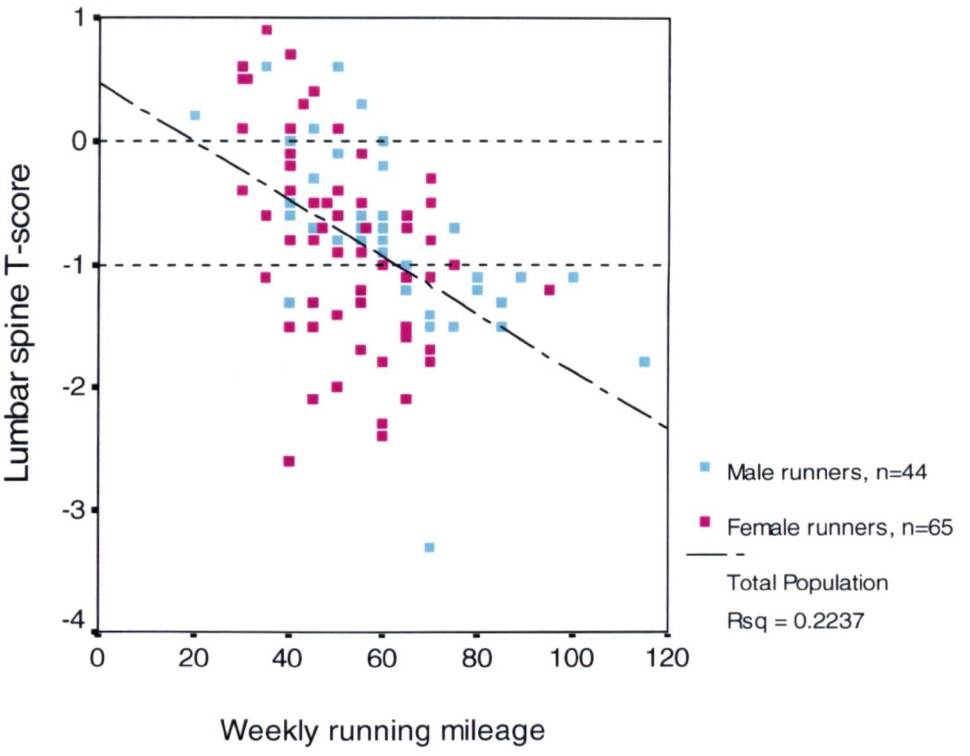


Figure 6.1: Scatter chart showing an inverse relationship between lumbar spine T-score and weekly running mileage in runners

To investigate the relationship between training volume and lumbar spine T-scores further, runners were divided into two groups: there were those who ran less than 50 miles per week ('0' n=40) and those who ran 50 miles or more per week ('1' n=69). ANOVA was used to test for differences in T-scores between the two groups. T-scores were used for this analysis as male and female runners were combined as one group. Mean lumbar spine T-scores in those running 50 miles or more per week were lower than in athletes running less (-1.0 *v* -0.4; $p<0.001$). There were no differences in dual femur or total body T-scores or in percentages of age- and sex-matched normal leg BMD.

6.1.2 Performance level and BMD

Of the total group of runners, 55 (50.5%) were elite, 30 (27.5%) were county and 24 (22%) performed at club level. Table 6.2 shows the number of the male and female runners who performed at elite, county or club level. As runners were not selected for participation in the study by performance level, the subject groups were unequal in size. The Games-Howell post-hoc multiple comparisons procedure was used to test for correlations as this accounts for differences in sample size.

Table 6.2: Number of runners (%) grouped by performance level

	Elite	County	Club	Total
Female	31 (47.7)	20 (30.8)	14 (21.5)	65
Male	24 (54.5)	10 (22.7)	10 (22.7)	44
Total	55 (50.5)	30 (27.5)	24 (22)	109

6.1.2.1 Subject characteristics

Tables 6.3 and 6.4 present the general characteristics of the female and male runners respectively, when grouped by performance level.

Female elite runners were younger than club runners. Club runners had experienced more years of eumenorrhoea than their elite and county level counterparts and had an earlier age of menarche compared to their county counterparts. Approximately one third of elite (32.3%) and county (30%) runners were classed as amenorrhoeic or oligomenorrhoeic. Approximately one quarter (25.8%) of elite runners were using the oral contraceptive pill and 41.9% were eumenorrhoeic. Of the county group, 15%

used the oral contraceptive pill and 55% were eumenorrhoeic. A greater proportion of eumenorrhoeic runners were club level (71.4%), 21% used the oral contraceptive pill and one club runner was amenorrhoeic.

Table 6.3: Characteristics (mean and SEM) of female runners by performance level

	Elite (n=31)	County (n=20)	Club (n=14)	Differences
Age (years)	24.5 (1.1)	26.1 (1.4)	32.6 (2.7)	elite< club, $p<0.05$
BMI (m kg $^{-2}$)	18.8 (0.2)	19.5 (0.3)	19.8 (0.4)	ns
Body fat %	14.0 (0.8)	18.2 (0.9)	19.2 (1.2)	elite< county, $p<0.01$ elite< club, $p<0.005$
Body Fat Z- score	-1.8 (0.1)	-1.3 (0.2)	-1.2 (0.3)	elite< county, $p<0.05$
Lean body mass (kg)	42.9 (0.5)	41.1 (0.9)	43.0 (1.3)	ns
Weekly running mileage	58.2 (2.5)	47.5 (2.5)	42.0 (1.9)	elite< county, $p<0.05$ elite< club, $p<0.001$
Years of training	9.0 (0.9)	10.9 (1.3)	8.6 (1.6)	ns
Menarche (years)	14.2 (0.2)	15.3 (0.4)	13.2 (0.4)	county> club, $p<0.05$
Years of eumenorrhoea	8.8 (1.2)	8.0 (1.8)	16.6 (2.7)	elite and county> club, $p<0.05$

There were no differences in lean body mass or years of training between the performance groups. Male and female elite runners ran significantly more miles per week than county ($p<0.05$) and club runners ($p<0.001$). They also had a lower body fat percentage than county ($p<0.05$) and club runners ($p<0.01$). Male elite runners had a lower BMI than county runners ($p<0.005$).

Table 6.4: Characteristics (mean and SEM) of male runners by performance level

	Elite (n=24)	County (n=10)	Club (n=10)	Differences
Age (years)	25.8 (1.2)	29.9 (3.0)	26.9 (2.3)	ns
BMI (m kg $^{-2}$)	20.6 (0.2)	22.1 (0.4)	21.5 (0.4)	elite< county, p<0.005
Body fat %	9.1 (0.7)	13.6 (1.2)	13.9 (1.8)	elite< county, p<0.05 elite< club, p<0.01
Body Fat Z- score	-1.5 (0.1)	-0.7 (0.3)	-0.9 (0.3)	elite< county, p<0.05
Lean body mass (kg)	59.8 (1.0)	61.0 (1.7)	57.7 (2.0)	ns
Weekly running mileage	71.6 (3.6)	61.0 (1.7)	47.0 (4.4)	elite> county, p<0.05 elite> county, p<0.01
Years of training	10.9 (1.1)	12.9 (3.0)	10.0 (2.0)	ns

6.1.2.2 Bone measurement data

Tables 6.5 and 6.6 present the bone measurement data for the female and male runners respectively, grouped according to performance level. BMD and T-scores for the female runners were adjusted for total number of eumenorrhoeic years to reduce the effect of varying menstrual status between the performance groups.

(i) Female runners

Elite female runners had lower lumbar spine T-scores and BMD than club runners ($p<0.05$). These differences existed after adjusting data for age and total number of eumenorrhoeic years, to suggest that factors other than oestrogen exposure may be predictive of the low bone density in elite runners. Lumbar spine T-scores indicative of osteopenia were found in 61.3% (n=19) of elite female runners, 35% (n=7) of county runners and 7% (n=1) of club runners. Dual femur T-scores indicative of osteopenia were found in one elite and one county female runner.

(ii) Male runners

Lumbar spine T-scores and BMD were also lower in elite male runners compared to county and club runners ($p<0.05$). The mean lumbar spine T-score for the elite group was -1.1. Lumbar spine T-scores indicative of osteopenia were found in 62.5% (n=15) of elite male runners, 20% (n=2) of county and 10% (n=1) of club runners. One male runner from each performance group had a dual femur T-score indicative of osteopenia. Overall, bone status at the dual femur and the total body was normal and did not differ between the groups.

Table 6.6: Bone status (mean and SEM) of male runners by performance level

	Elite (n=24)	County (n=10)	Club (n=10)	Difference ($p<0.05$)
Lumbar spine T-score	-1.1 (0.1)	-0.5 (0.3)	-0.4 (0.2)	*elite< county elite< club*
BMD (g cm^{-2})	1.11 (0.02)	1.19 (0.03)	1.2 (0.02)	*elite< county elite< club*
Bone area (cm^2)	52.5 (1.0)	55.7 (0.1)	51.5 (1.4)	*ns*
BMAD (mg cm^{-3})	152.8 (2.5)	159.3 (4.2)	167.8 (3.6)	*elite< club*
Dual femur T-score	0.6 (0.2)	0.4 (0.3)	0.7 (0.4)	*ns*
BMD (g cm^{-2})	1.16 (0.02)	1.14 (0.03)	1.18 (0.05)	*ns*
Bone area (cm^2)	37.5 (0.7)	38.6 (0.9)	37.4 (1.9)	*ns*
BMAD (mg cm^{-3})	191.1 (3.5)	183.6 (6.2)	193.1 (7.2)	*ns*
Total Body T-score	0.0 (0.2)	0.3 (0.1)	0.4 (0.2)	*ns*
BMD (g cm^{-2})	1.23 (0.01)	1.25 (0.01)	1.25 (0.02)	*ns*

There was a trend for the elite male runners to have smaller lumbar spine bone area compared to county runners, although this did not reach significance ($p=0.07$). Male club runners had higher BMAD than elite runners at this site ($p<0.05$).

6.1.2.3 Within-group correlations

Elite runners ran more miles per week than county and club runners and the following error bar charts (Figure 6.2 and Figure 6.3) collectively illustrate an inverse relationship.

Figure 6.2: Error bar chart of mean (95% confidence interval) lumbar spine T-scores for runners by performance level

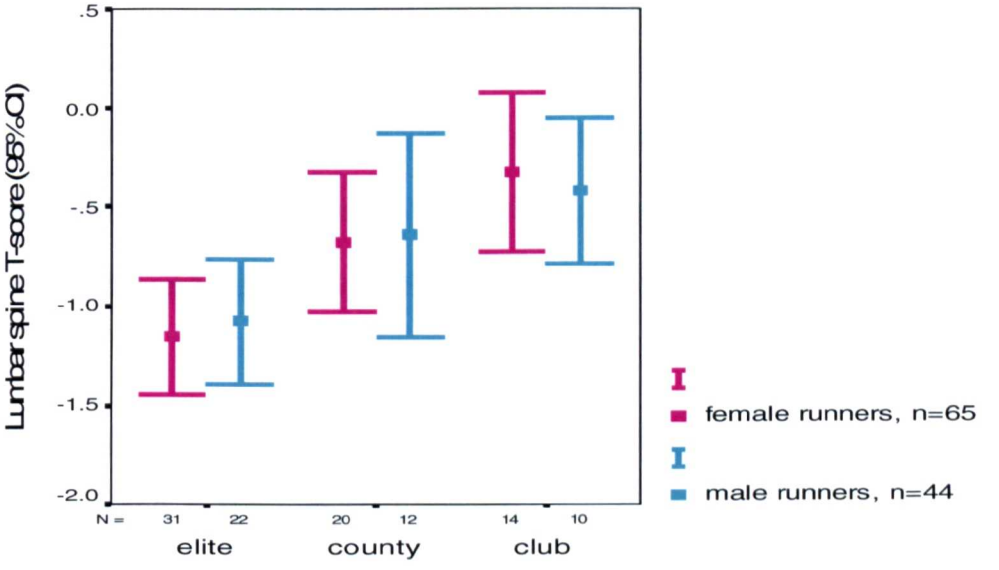
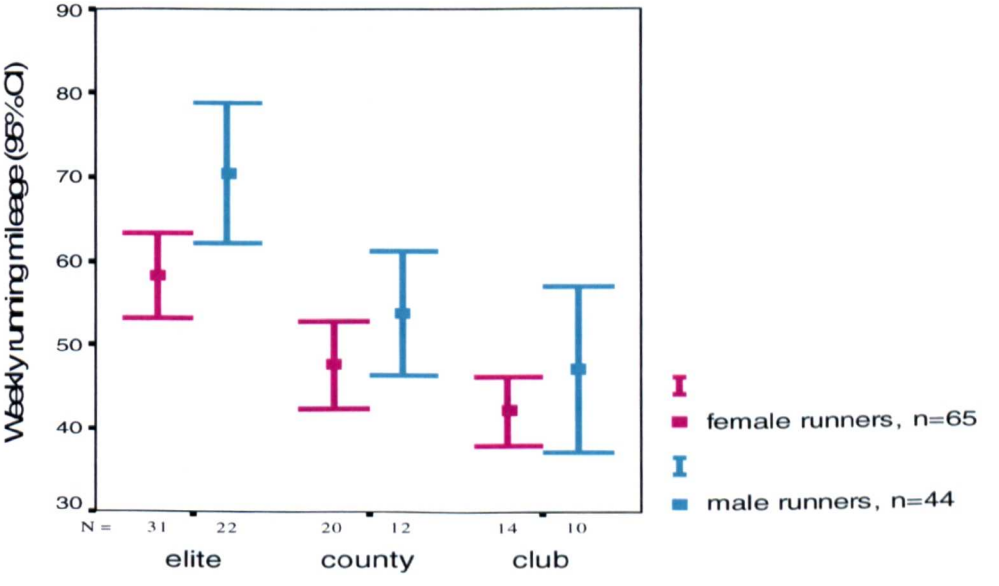


Figure 6.3: Error bar chart of mean (95% confidence interval) weekly mileage of runners by performance level



Correlations between variables and bone status were investigated for each of the three performance groups.

(i) Female runners

In elite runners, BMI positively correlated with lumbar spine BMD ($r = 0.401$; $p<0.05$) and T-score ($r = 0.465$; $p<0.01$) and total body BMD ($r = 0.560$; $p<0.001$) and T-score ($r = 0.660$; $p<0.001$). In county runners, BMI was positively associated with lumbar spine BMD ($r = 0.487$; $p<0.05$) and T-score ($r = 0.479$; $p<0.05$); dual femur BMD ($r = 0.538$; $p<0.001$) and T-score ($r = 0.547$; $p<0.001$) and total body BMD ($r = 0.615$; $p<0.001$) and T-score ($r = 0.605$; $p<0.001$). BMI was associated with total body BMD ($r = 0.549$; $p<0.05$) and T-score ($r = 0.543$; $p<0.05$) in female club runners.

Number of eumenorrhoeic years was positively associated with lumbar spine BMD and T-score ($r = 0.439$; 0.425; $p<0.05$) only in elite runners. Lean body mass correlated positively with total body BMD and T-score ($r = 0.668$; 0.697; $p<0.005$) in elite runners whereas percentage body fat correlated positively with dual femur BMD and T-score ($r = 0.490$; 0.466; $p<0.01$) in county runners. Weekly running mileage (ranging from 30 to 70 miles) negatively correlated with lumbar spine BMD and T-score ($r = -0.525$; 0.551; $p<0.05$) in county runners.

(ii) Male runners

In elite male runners, the number of years training negatively correlated with lumbar spine BMD and T-score ($r = -0.638$; -0.629), dual femur BMD and T-score ($r = -0.674$; -0.612) and total body BMD ($r = -0.811$; -0.703) and T-score (all $p<0.01$). As in elite female runners, there was a positive association between lean body mass and total

body BMD in elite male runners ($r = 0.411$; $p<0.05$). As in female county runners, there was an inverse relationship between weekly running mileage (ranging 35 and 70 miles per week) and lumbar spine T-score in the male county runners, ($r = -0.684$; $p<0.01$). Dual femur BMD and T-score correlated positively with BMI in county runners ($r = 0.784$; 0.791; $p<0.01$). In male club runners, BMI correlated positively with total body BMD and T-score ($r = 0.750$; 0.732; $p<0.01$) and lean body mass correlated positively with total body BMD ($r = 0.714$; $p<0.01$).

In summary, male and female elite runners ran more miles per week and had lower lumbar spine BMD and BMAD than club and county runners. In county runners, weekly running mileage was negatively associated with reduced lumbar spine BMD. BMD and BMAD at the dual femur and total body were similar between groups suggesting that these sites are relatively protected from whatever is causing the bone loss at the lumbar spine, at least in the short term.

6.2 Discussion

In this chapter, the potential associations between bone status and training characteristics were investigated and comparisons were made between runners grouped according to their level of performance. In doing so, it was found that those training characteristics likely to result in high energy expenditures were associated with low lumbar spine and in some cases, total body BMD. This study also found that elite runners had significantly lower lumbar spine BMD than runners who competed at lower levels.

In agreement with previous reports (Etherington *et al.*, 1996; Bennell *et al.*, 1997; MacDougall *et al.*, 1992; Dook *et al.*, 1997) and as discussed in chapters 4 and 5, male and female distance runners had normal or above normal BMD in the dual femur and legs, but reduced BMD in the lumbar spine.

A significant inverse relationship was found between weekly running mileage and lumbar spine BMD in all runners, confirming previous reports in female (Winters *et al.*, 1996; Burrows *et al.*, 2003) and male runners (Bilanin *et al.*, 1989; Hetland *et al.*, 1993). To date, there has been no identification of a threshold of running mileage, above which running may have negative implications for lumbar spine BMD. In this study, runners with a weekly mileage above 50 had significantly reduced lumbar spine BMD compared to those with a mileage below 50. However, as this was a cross-sectional study, these results do not imply causation and it is likely that individuals vary in their skeletal response to running training, mediated by confounding variables such as genetics, diet, hormonal status, training surfaces and bone size.

In contrast, mechanical loading generated by running may protect weight-bearing sites from bone loss. However, as no differences in dual femur and leg bone status were detected between those who ran above, and those who ran below 50 miles per week, it is suggested that higher running mileages do not result in a dose-dependent increase in BMD at weight-bearing sites. These results are consistent with those from previous studies in distance runners (MacDougall *et al.*, 1992; MacKelvie *et al.*, 2000) and are supportive of Frost's 'Mechanostat Theory' (Frost, 1992), that the magnitude of strain required to 'switch on' the 'mechanostat' is dependent on the

initial status of bone prior to loading. Bone can become accustomed to constant loading of a similar magnitude and will not increase its strength until a higher magnitude load is applied (Frost, 1992; 1997). In line with this concept, distance running generates highly repetitive strain but because this strain is of a relatively low and consistent magnitude, bone density would reach a plateau rather than continue to increase. This may explain why there was no positive association between weekly running mileage and BMD at the dual femur and legs. Furthermore, in chapter 4 it was found that in male runners dual femur and total body BMD negatively correlated with number of years in training to suggest that over a longer period of time, running may become of little stimulus to bone at weight-bearing sites.

Weekly running distances and training years (in males) were negatively associated with lumbar spine BMD and the absence of a positive correlation between dual femur and total body BMD, indicates that the number of loading cycles is not of major importance in stimulating bone adaptation to loading. This agrees with previous intervention studies conducted in animals (Rubin and Lanyon, 1984; Raab-Cullen *et al.,* 1994). As discussed in chapter 4, there were positive correlations between body mass and BMI and BMD in runners as a group and in this chapter it was shown that there were similar correlations for the runners when grouped by performance level. Thus, it could be argued that strain magnitude (as that derived from a higher body mass) is of more significance than the number of loading cycles (from running distances).

Evidence suggests that the insertion of recovery periods in between loading sessions enhances bone adaptation to mechanical stress by restoring the mechano-sensitivity of

the bone cells (Robling *et al.,* 2001; Srinivasan *et al.*, 2002). In support of this concept, this study found that female runners who ran twice a day had lower lumbar spine BMD than those who trained daily. Twice-daily training would be expected to increase the total volume of running as well as reducing recovery in between loading sessions. Thus, whereas running three times per week has resulted in an increase in lumbar spine BMD (Snow-Harter *et al.,* 1992), twice-a-day training in this study was associated with lower lumbar spine BMD. The inability to detect a difference in the male group is likely to be because the majority of male runners trained twice a day.

Similarly, male and female runners who did not include one rest day per week had significantly lower lumbar spine and total body BMD and T-scores than those who did. Female runners who did not have one rest day per week had significantly lower dual femur BMD. Bone has been shown to respond more to alternate day loading rather than consecutive loading (Raab-Cullen *et al.,* 1994) and continuous training without sufficient recovery does not appear to be optimal for generating a positive skeletal response (Raab-Cullen *et al.,* 1994; Robling *et al.*, 2001).

From the evidence presented the question follows why may high volume endurance training predispose athletes to compromised bone density? The mechanism is unclear, but it appears to affect both male and female runners. As shown, distance runners and in particular elite runners, engage in large amounts of training which can be twice-a-day and deficient of adequate recovery. From a mechanical perspective, the training regimes of these runners do not appear to be optimal for promoting bone gain. This is most likely to apply to the lower skeletal sites which are in closer proximity to the mechanical forces generated during running. However, as the spine receives

considerably less impact, it is likely that the mechanical loading regime would have little effect. Instead bone loss at this site may be due at least in part, to metabolic factors.

High volume training regimes are likely to result in exceptionally high energy expenditures, which if not matched with sufficient dietary energy intake, could result in several metabolic alterations that may have a negative effect on bone. A recent study conducted in 462 healthy men and women investigated the mechanical and metabolic components of exercise and suggested that there may be a metabolic threshold above which, extra exercise becomes deleterious to BMD at the lumbar spine and total body (Bakker *et al.,* 2003). Indeed the spine is more susceptible to such influences due to its high trabecular content. As discussed in Chapter 5, amenorrhoea is also likely to contribute to reduced BMD in female runners.

One aspect of training that was associated with increased BMD in male and female runners was strength training and this association remained after adjustment for BMI to suggest that the relationship was not confounded by differences in body size. Runners who participated in an organised form of strength training (weight training, plyometrics and circuit training) at least twice per week, were found to have significantly greater lumbar spine and total body BMD than those who did not. This supports the concept that high magnitude strains, in unusual directions and with sufficient recovery, encourage bone gain (Lanyon, 1996; Frost, 1997; Jiang *et al.*, 1999). Strains generated during strength training are likely to be in the 'physiological loading zone' or the 'overload zone' (Forwood and Turner, 1995; Frost, 1997) which

will result in either the maintenance of BMD or an increase in BMD. These results need confirmation by future prospective intervention studies.

Lumbar spine BMD and T-scores were lower in elite female runners compared to club runners, even after adjustment for total number of eumenorrhoeic years. This suggests that factors other than oestrogen exposure may be predictive of low BMD in elite runners. Male and female elite runners had significantly lower lumbar spine BMD and T-scores than county or club level runners. As elite runners ran significantly more miles per week than club or county runners, these results may represent a negative effect of the higher self-reported running volumes amongst this group. Furthermore, elite runners had significantly lower BMI and levels of body fat, which may be indicative of a catabolic state. This has been addressed in chapter 7. In contrast to bone status at the lumbar spine, there were no differences in bone status at the dual femur and legs between elite, county and club runners. This was despite the higher weekly running mileage of the elite group and as discussed earlier, this may reflect the protective effect of mechanical loading.

A smaller bone area would be expected to reflect an overall higher BMAD (Melton *et al.*, 2000; Cvijetic and Korsic, 2004). Elite female runners had significantly smaller bones than club runners. The smaller bone size in elite runners may reflect the fact that smaller, lighter bones will confer a performance advantage, as success in distance running often comes together with lightness. It was of particular relevance that despite smaller bone area at the lumbar spine, female elite runners had significantly lower BMAD than club runners. Reduced bone size and BMD are risk factors for future osteoporotic fracture, thus female elite runners may be more prone to future fracture

at the lumbar spine. In contrast, at the dual femur, although elite female runners had a smaller bone area they had a greater BMAD compared to club runners. This suggests that whatever was causing their reduced bone mineral at the lumbar spine was counteracted at the dual femur, a site recipient of greater impact loading during running. Although lumbar spine bone area was similar between male runners, elite runners had lower BMAD than club runners. These results suggest that lumbar spine BMD in elite male runners was lower than in club runners and that this difference was not due to smaller bone size. In this study, BMAD was based on a predictive equation rather than actual volumetric bone measurements, therefore future studies would benefit from use of techniques such as QCT which could also assess cortical thickness and bone composition. Never-the-less the evidence presented suggests that in the highly-trained elite runners, bone at the lumbar spine may be under-mineralised to a greater degree than expected from their BMD results. Future studies are required to investigate the bone structure and volumetric BMD of distance runners to gain a greater insight into the potential bone fragility of this population. Self-selection however, cannot be discounted.

In conclusion, lumbar spine BMD was lower in runners who participated in higher volumes of training and who had lower levels of body fat and BMI. Elite runners were engaged in high volume training and had lower lumbar spine BMD. As discussed in chapter 2, high volume training results in high energy expenditures which if not matched by energy intake, will result in an overall energy deficit with reductions in body mass and body fat over a longer period of time. The following chapter evaluates the potential contribution of energy deficit to BMD in runners.

Chapter 7

Energy balance and bone mineral density in male and female distance runners

"Is there a relationship between energy balance and BMD?"

With reference to the first aim of this research, chapters 4 to 6 demonstrate that low BMD at the lumbar spine is prevalent amongst the distance runners studied, regardless of gender. The second aim of the research was to determine whether there was a relationship between energy balance and BMD in male and female distance runners. There are two parts to this area of the research: the current chapter, which evaluates the contribution of reported energy deficit to reduced BMD in these runners as a group and chapter 8, which brings together the evidence from chapters 4 to 7, by evaluating energy balance and BMD according to the menstrual status of the female runners and to the performance level of all runners.

7.1 Results

This section presents the energy balance and BMD results.

7.1.1 Energy status of male and female runners

Table 7.1 presents the energy status data derived from the 7-day nutritional analysis.

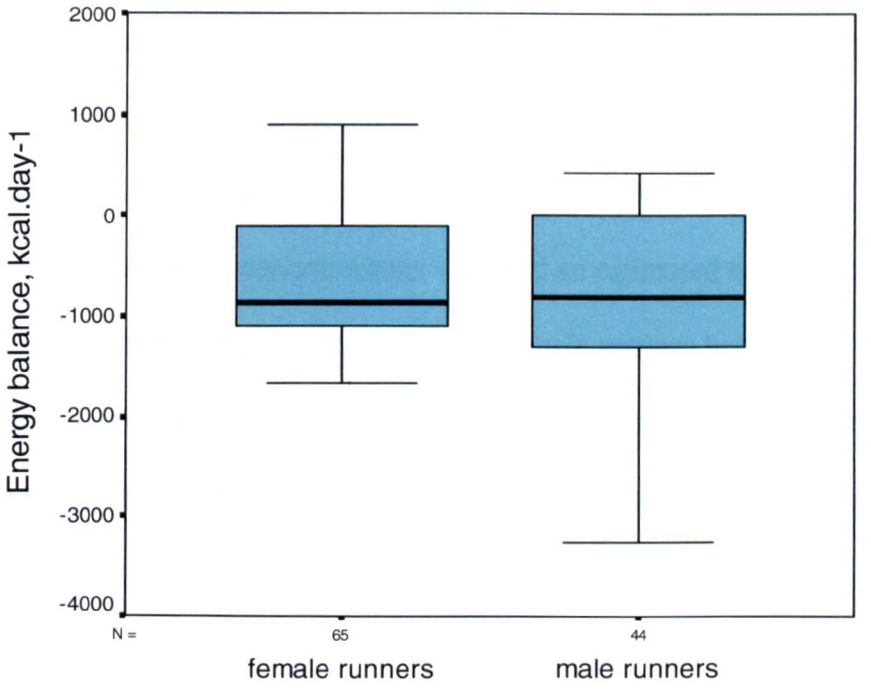
Table 7.1 Energy status (mean and SEM) in distance runners

	Female (n=65)	Male (n=44)	ANOVA	Mann Whitney U Test
Energy intake (kcal day^{-1})	1798.3 (67.5)	2513.5 (88.3)	p<0.001	
Range	*638 - 3081.8*	*1366.5 - 3954.3*		
Energy intake per kg body mass (kcal kg^{-1})	34.1 (1.1)	37.2 (1.3)	ns	
Range	*14.5 - 59*	*19.1 - 57.8*		
Energy expenditure (kcal day^{-1})	2434.9 (34.3)	3288.4 (61.2)		p<0.001
Range	*1828.5 - 3220*	*2286.6 - 4633.6*		
Energy balance (kcal day^{-1})	-638.0 (76.6)	-779.4 (115.6)		ns
Range	*-1660.9 – 895.7*	*-3267- 429.1*		
BMI (kg m^{-2})	19.2 (0.2)	21.1 (0.2)	p<0.001	
Range	*15.3 - 24*	*19 - 24*		
Body fat %	16.4 (0.6)	11.2 (0.7)		p<0.001
Range	*4.4 - 24*	*5.4 - 24.9*		
Body fat Z-score	-1.5 (0.9)	-1.2 (0.1)		ns
Range	*-3.1 - 1*	*-2.7 - 1*		

(i) Energy status

Male runners reported consuming more calories per day than female runners ($p<0.001$). However, when energy intake was expressed per kg body mass, intakes were similar ($p<0.08$) and well below recommendations for endurance athletes, estimated to be approximately 50 kcal kg^{-1} (Economos et al., 1993). As expected from their higher weekly running mileage ($p<0.05$), male runners expended more energy per day than females ($p<0.001$). Figure 7.1 is a box plot illustrating the subsequent energy balance for the male and female runners.

Figure 7.1: Box-plot illustrating mean (SEM) energy balance in distance runners



Male and female runners had a similar mean energy deficit (p=0.08). Almost three quarters of male (72.7%) and female (73.8%) runners were energy deficient to various extents. As shown in Figure 7.1, there was a wide range of energy balance in the male group, with one runner having a daily energy deficit as high as -3267 kcal. The largest energy deficit recorded in the female group was -1660.9 kcal. The highest energy excess in the female group was +895.7 kcal day^{-1}, whereas the highest energy excess in the male group was less than half of this value (+429.1 kcal day^{-1}). The highest expenditure recorded from the male group was 4633.6 kcal day^{-1} and the highest from the female group was 3220 kcal day^{-1}.

(ii) Body fat, BMI and energy balance

Mean body fat levels in male and female runners were lower than expected for normal healthy men and women. Mean body fat Z-scores were -1.2 and -1.5 in the male and female runners respectively, with the minimum score -3.3 in the female and -2.7 in the male group. Percent body fat was higher in female runners. In the female group, the lowest body fat was 4.4% for one runner who had an estimated energy deficit of 1296.8 kcal day^{-1}. A further four energy deficient female runners had a body fat less than 7%. The lowest body fat in the male group was 5.4% and ten male runners had a body fat percentage lower than 7%. All but one of these runners, were in negative energy balance.

BMI was greater in male compared to female runners, with the lowest BMI 15.3 kg m^{-2} in the female group and 19.0 kg m^{-2} in the male group ($p<0.001$). Thirteen female runners had a BMI less than 18 kg m^{-2}. After categorising female runners as '0' (BMI >18) or '1' (BMI< 18), ANOVA showed that runners with the lowest BMI had higher energy deficits compared to those with a BMI > 18 kg m^{-2} ($p<0.01$). Results were computed similarly for male runners who had a BMI > or < 19 kg m^{-2}. There were no significant differences for male runners when grouped by BMI.

It was predicted that if the runners were chronically energy deficient, this would correlate with body mass and body composition measurements. A positive correlation was found between energy balance and percentage body fat in female runners ($r =0.182$; $p<0.01$), and energy balance and body fat Z-score in male runners ($r =0.235$; $p<0.005$). Energy expenditure was negatively related to percent body fat in the female ($r = -0.239$; $p<0.005$) and the male group ($r = -0.224$; $p<0.005$).

In the female runners, there was a positive correlation between BMI and energy balance ($r = 0.285$; $p < 0.001$) and between body mass and energy balance ($r = 0.291$; $p < 0.05$). Energy balance also correlated positively with body mass ($r = 0.707$; $p < 0.001$), BMI ($r = 0.147$; $p < 0.05$) and percentage body fat ($r = 0.483$; $p < 0.483$) for the group as a whole.

(ii) Self-reported body image and dietary habits

In addition to the quantitative data presented, the questionnaire gathered qualitative information concerning the beliefs, attitudes and dietary practices of the athletes studied. The responses are quantified in Table 7.2.

Table 7.2: Self-reported body image and dietary behaviours by distance runners

	Female (n=65)	Male (n=44)
Unhappy with body mass	25 (38.5%)	5 (11.4%)
Self-imposed weight limit	29 (44.6%)	19 (43.2%)
Dietary fat restriction	22 (33.8%)	14 (31.8%)
High carbohydrate diet	3 (4.6%)	4 (9.1%)

Approximately one third of male and female runners voluntarily admitted to consciously restricting fat in their diet, in many cases, "as much as possible". Almost half of the sample had a self-imposed weight limit which they stayed below for competition. Many remarked that they believed their body mass was normal for an average individual but too high for a 'runner', despite having a normal or below normal BMI.

Runners were divided into groups ('0' and '1') by their questionnaire responses and ANOVA was computed. There were no differences in energy balance, BMI, body fat percentage or Z-score between runners who reported that they were unhappy with their body mass and those who weren't. Similarly, runners with a self-imposed weight limit did not have significantly lower energy intakes compared to those without a weight limit. In contrast, those runners who stated that they purposefully restricted dietary fat had lower fat intakes (34.6 *v* 62.3 g day^{-1}; $p<0.001$) and body fat percentage (14.7 *v* 17.3%; $p<0.05$) and were in more severe energy deficit (- 937.8 *v* - 484.7 kcal day^{-1}; $p<0.01$). These results suggest that those who stated that they purposefully restricted fat intake, did, and that these runners may have also been particularly restrictive with their overall calorific intake.

(iii) Intakes of nutrients other than energy

In addition to energy status, several nutrients that may affect bone were also assessed. This enabled relationships between energy balance and bone status to be investigated whilst considering potential confounding effects from other nutrients. Table 7.3 presents the data derived from the 7-day nutritional analysis, with regard to nutrient intakes other than energy.

Table 7.3: Intakes of nutrients other than energy (mean and SEM) in runners

	Female (n=65)	Male (n=44)	ANOVA	Mann Whitney U Test
Calcium (mg day^{-1}) *Range*	884.0 (40.8) *291 – 1864.1*	1269.3 (56.3) *481.6 – 2057.8*	p<0.001	
Vitamin D (ug day^{-1}) *Range*	3.4 (0.3) *0.02 – 12.3*	3.9 (1.1) *0.14 – 47.9*		0.436
Fat (g day^{-1}) *Range*	52.9 (3.2) *4.6 – 132.3*	73.8 (3.6) *34.9 – 128.7*		p<0.001†
Protein (g day^{-1}) *Range*	73.0 (2.8) *21.1 – 134.7*	99.6 (3.3) *54 - 178*	p<0.001†	
Carbohydrate (g day^{-1}) *Range*	269.2 (9.7) *137 - 525*	360.8 (15.4) *134 - 563*		p<0.001†

Mean calcium intake was higher in males than females (p<0.001). Both groups had a mean intake that was well above the current UK RDA (700mg day^{-1}) but the females had a mean intake below the 1000mg day^{-1} recommended for athletes. Vitamin D intakes were low, although it was assumed that further vitamin D would be obtained from sunlight due to the large amounts of time the runners spent training outside. Although the male runners consumed more fat, protein and carbohydrate than the female runners, this was expected due to their larger energy intakes. Although there was a wide range, dietary fat intakes for most runners were well below recommended allowances for average men (95g) and women (70g). In the female group, 73.8% had a fat intake below 70g day^{-1} and in the male group, 81.8% consumed less than 95g day^{-1} dietary fat. The minimum fat intake in the female group was 4.6 g day^{-1} and in the male group, 34.9 g day^{-1}. Protein intakes were also calculated relative to body mass. Female runners had a mean intake of 1.37 (0.4) g kg day^{-1}, ranging from 0.5-2.5 g kg day^{-1}. Similarly, male runners had a mean intake of 1.47 (0.3) g kg day^{-1},

ranging from 0.7- 2.6 g kg day^{-1}. Twenty two female runners and 5 male runners had a protein intake below the 1.2 g kg day^{-1} recommended for endurance athletes.

7.1.2 Energy balance and bone status

Potential relationships between energy status and bone status were investigated and the results are shown in Table 7.4. As the bone score data were normally distributed Pearson's correlation coefficients were computed for all tests.

(i) Female runners

There was a significant relationship between energy balance, intake and bone status at all three measured sites in the female runners, with the strongest coefficient at the lumbar spine. When coefficients were computed controlling for calcium, fat and protein, a positive relationship was observed at the lumbar spine but not at the dual femur and total body. This suggests that such factors may have a more significant relationship with BMD at these sites. When controlling for energy intake, there were positive correlations between protein intake and dual femur T-score ($r=0.367$, $p<0.005$), and between fat intake and total body T-score ($r=0.443$; $p<0.005$). When controlling for energy, fat and protein, partial correlation coefficients between calcium intake and total body BMD were significant ($r=0.322$; $p<0.005$). Thus, in female runners, energy balance and energy intake were significantly associated with lumbar spine bone status, whereas protein intake appeared to be more predictive of dual femur T-score and calcium and fat intake, predictive of total body T-score.

Table 7.4: Pearson's correlation coefficients between energy and bone status in runners

	Female (n=65)			Male (n=44)		
	Energy balance	Energy intake	Energy expenditure	Energy balance	Energy intake	Energy expenditure
Lumbar spine T-score	0.861†	0.790†	-0.385†	0.715†	0.672†	-0.381†
Adjusted*	0.576†	0.359†	ns	0.607†	0.634†	ns
Lumbar spine BMD	0.866†	0.800†	-0.380†	0.603†	0.690†	-0.408†
Adjusted*	0.579†	0.413†	ns	0.622†	0.602†	ns
Dual femur T-score	0.450†	0.464†	ns	0.330‡	0.370‡	ns
Adjusted*	ns	ns		ns	ns	
Dual femur BMD	0.438†	0.452†	ns	0.348‡	0.364‡	ns
Adjusted*	ns	ns		ns	ns	
Total body T-score	0.547‡	0.611†	ns	0.340†	0.432‡	ns
Adjusted*	ns	ns		ns	ns	
Total body BMD	0.550‡	0.618†	ns	ns	0.419‡	ns
Adjusted*	ns	ns		ns	ns	

**Partial correlation coefficients: controlling for calcium, fat and protein intake*

† $p<0.001$; ‡ $p<0.05$

As described earlier, when grouped as having a BMI above 18 or less than 18 kg m^{-2}, female runners with a BMI less than 18 had significantly greater energy deficits. Subsequent analyses revealed that these runners also had lower BMD at all sites, fewer eumenorrhoeic years and higher weekly running mileages ($p<0.01$).

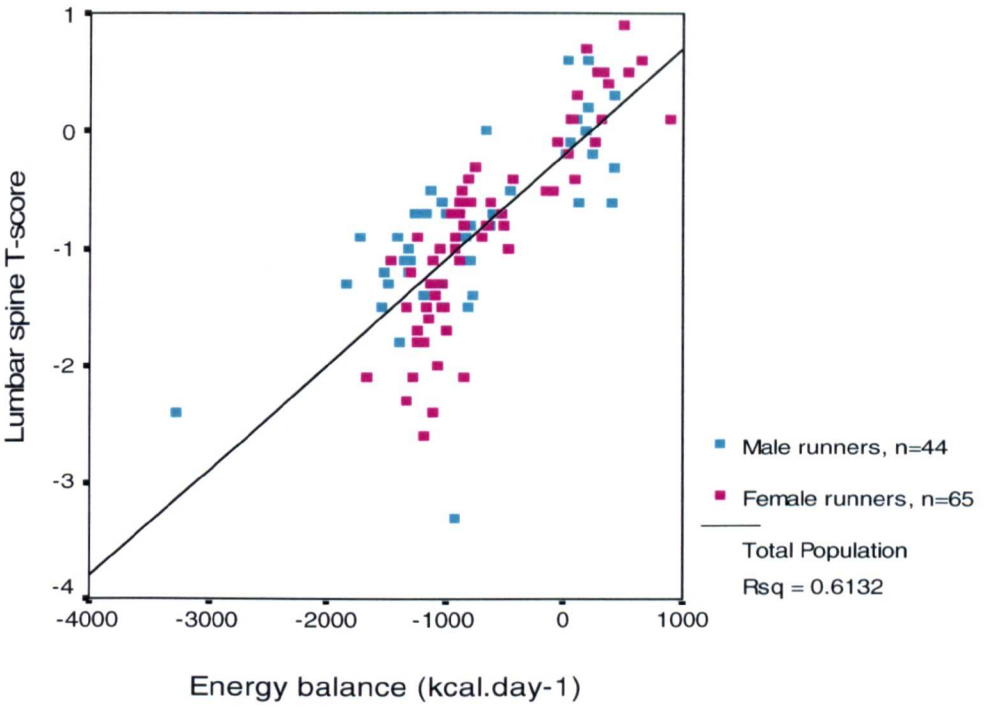
(ii) Male runners

As in the female runners, energy intake was positively associated with bone status at all three measured sites in male runners ($p<0.001$). Energy balance positively correlated with lumbar spine T-score and BMD and with total body T-score ($p<0.001$; $p<0.05$). Partial correlation coefficients were computed between bone data and energy

intake and balance, controlling for calcium, fat, protein and carbohydrate intake. Significant relationships remained between energy intake and lumbar spine bone status and between energy balance and lumbar spine bone status ($p<0.001$). Correlations at the dual femur and total body were no longer significant after controlling for energy intake. However, unlike in female runners there were no correlations with calcium intake. This suggests that factors other than those nutritionally mediated may be associated with dual femur and total body BMD in male runners.

There was a significant inverse relationship between energy expenditure and lumbar spine T-score and BMD, suggesting that higher metabolic requirements from exercise may contribute to low BMD at this site in runners. When this high expenditure is not matched with sufficient energy intake, negative energy balance occurs and as previously shown, energy balance was significantly and positively related to lumbar spine bone status in male and female runners. This relationship is further illustrated by the line of best fit and the large r^2 value in Figure 7.2.

Figure 7.2: Scatter plot showing a positive relationship between energy balance and lumbar spine T-score in runners



(iii) Energy deficiency and BMD

In order to investigate the relationship between energy deficiency and BMD, runners were grouped by the severity of their energy deficit. Using ANOVA and Games-Howell post-hoc analysis, between-group comparisons were made between runners who were in positive energy balance (entered as '0'), those who were energy deficient by up to 1000 kcal day^{-1} ('1') and runners who were energy deficient by over 1000 kcal day^{-1} ('2'). The results are shown in Table 7.5 and 7.6 for the female and male groups respectively.

Table 7.5: Characteristics of female runners by energy balance status

	Positive energy balance (n=16)	Up to -1000 kcal day^{-1} energy deficit (n=36)	Over 1000 kcal day^{-1} energy deficit (n=13)
Weekly mileage	38.7 (2.0)	54.1 (1.9)†	59.6 (3.8)†
BMI (kg m^{-2})	20.1 (0.8)	19.2 (0.3)	18.1 (0.3)‡
Body fat Z-score	-1.2 (0.2)	-1.6 (0.1)	-1.6 (0.2)
Years of eumenorrhoea	14.5 (2.7)	9.6 (1.2)‡	6.8 (1.6)‡
Lumbar spine T-score			
Observed	0.3 (0.1)	-1.0 (0.1)‡	-1.6 (0.1)‡
Adjusted*	0.1 (0.1)	-1.0 (0.1)‡	-1.5 (0.1)‡
Dual femur T-score			
Observed	1.0 (0.2)	0.5 (0.1)	-0.1 (0.2)‡
Adjusted*	0.9 (0.2)	0.5 (0.1)	-0.1 (0.2)‡
Total body T-score			
Observed	0.9 (0.1)	0.3 (0.1)‡	-0.4 (0.2)‡
Adjusted*	0.6 (0.1)	0.3 (0.1)	-0.1 (0.2)₸

* *T-scores adjusted for BMI, years of eumenorrhoea and calcium intake using ANCOVA*
† *greater than P group, p< 0.001*
‡ *less than P group, p< 0.005*
₸ *less than P group, p< 0.05*

Energy deficient male and female runners ran more miles per week than those who were in a positive energy balance ($p<0.001$). Energy deficient female runners had lower BMI and body fat Z-scores ($p<0.005$) and energy deficient male runners had lower body fat Z-scores, than runners in positive energy balance ($p<0.005$).

Tables 7.5 and 7.6 indicate a lower lumbar spine T-score in runners as the severity of energy deficit increases. Mean lumbar spine T-score of male and female runners with an energy deficit of less than 1000 kcal day^{-1} was indicative of osteopenia (borderline at -1.0), and male and female runners with a more severe energy deficit had a mean T-score of -1.2 and -1.6 respectively. This pattern was similar for female runners when T-scores were adjusted for differences in total number of eumenorrhoeic years,

calcium intake and BMI, and in male runners when the T-scores were adjusted for calcium intake and BMI.

Table 7.6: Characteristics of male runners by energy balance status

	Positive energy balance (n=13)	Up to -1000 kcal day^{-1} energy deficit (n=12)	Over 1000 kcal day^{-1} energy deficit (n=19)
Weekly mileage	44.2 (2.9)	64.1 (4.1)†	69.5 (4.3)†
BMI (kg m^{-2})	21.6 (0.3)	21.0 (0.4)	20.9 (0.3)
Body fat Z-score	-0.7 (0.3)	-1.4 (0.1)‡	-1.5 (0.2)‡
Lumbar spine T-score			
Observed	0.0 (0.1)	-1.0 (0.2)‡	-1.2 (0.1)‡
Adjusted*	0.0 (0.1)	-1.0 (0.2)‡	-1.3 (0.1)‡
Dual femur T-score			
Observed	0.9 (0.3)	0.5 (0.3)	0.4 (0.2)
Adjusted*	1.0 (0.3)	0.6 (0.3)	0.3 (0.2)
Total body T-score			
Observed	0.6 (0.1)	0.0 (0.3)	0.0 (0.1)‡
Adjusted*	0.6 (0.1)	-0.1 (0.3)	0.1 (0.3)‡

** T-scores for BMI and calcium intake using ANCOVA*
† greater than P group, p< 0.001
‡ less than P group, p< 0.005

Dual femur and total body T-scores, showed a trend to be lower in the energy deficient female runners, with both T-scores reduced in those who were energy deficient by over 1000 kcal day^{-1} ($p<0.005$). A comparable trend was observed in male runners, although statistical significance was only attained from comparisons in total body T-scores. Results were similar when T-scores were adjusted for the previously listed covariates.

(iv) Energy balance and bone mineral apparent density (BMAD)

Analysis of the covariates found a significant correlation between energy balance and lumbar spine BMAD in female runners, when controlling for calcium intake ($r = 0.771$; $p < 0.001$). There were no correlations with calcium intake when controlling for energy intake. The coefficients were larger than those found for the relationship between total number of eumenorrhoeic years and lumbar spine BMAD ($r = 0.280$; $p < 0.05$). Furthermore, energy balance was positively associated with dual femur BMAD in female runners after controlling for calcium intake, BMI and eumenorrhoeic years ($r = 0.324$; $p < 0.01$).

In the male runners, correlation coefficients also indicated a positive relationship between energy balance and lumbar spine BMAD when controlling for calcium intake ($r = 0.774$; $p < 0.001$). Energy balance was also positively correlated with dual femur BMAD after controlling for calcium intake and BMI ($r = 0.38$; $p < 0.01$). In both male and female runners, there were no associations between energy balance and bone size.

7.2 Discussion

The results demonstrate a positive association between energy balance and lumbar spine BMD in male and female distance runners. What follows is a discussion of the energy balance results and relationships with BMD.

i) Energy balance

As expected, distance runners had high energy requirements arising from prolonged endurance training that was often twice-a-day. In agreement with previous reports of sub-optimum energy intakes amongst endurance athletes (Marcus *et al.,* 1985; Niemen *et al.,* 1989; Pettersson *et al.,* 1999; Burke *et al.,* 2003), it was found that the majority of runners failed to ingest sufficient calories to match energy expenditure. The mean energy intakes for male and female runners were 37.2 and 34.1 kcal per kg body mass respectively, both of which were well below recommendations for male and female endurance athletes (50 and 45-50 kcal kg^{-1} day^{-1} respectively) (Economos *et al.,* 1993). The positive association between energy expenditure and intake, suggests that most runners increased their energy intake in response to greater energy requirements, although this increase was insufficient. It is possible that some runners find it difficult to consume sufficient calories from their diet alone. However, the particularly large energy deficits suggest purposeful energy restriction or significant under-reporting. The most severe energy deficit was found for a male runner engaged in particularly high levels of exercising (cycling and running) whose daily energy expenditure was calculated to be 4633.6 kcal. It is possible, that in this case energy expenditure may have been over-reported and/or energy intake under-reported. On the other hand, it is known that some athletes become obsessive about exercise and are prone to developing disordered eating. This was not screened for in the current study, therefore cannot be ruled out.

Although under-reporting may have affected the results, there were several indications to suggest that many runners may have purposefully restricted dietary intake. Firstly, nearly half of the sample admitted that they were unhappy with their

body mass and indicated that they wanted to lose weight. Research elsewhere indicates that the incidence of clinical and non-clinical eating disorders is higher in athletes than in the general population (Beals and Manore, 1998; Sundgot-Borgen and Torstvert, 2004). Eating disorder inventories were not used for the current study so to not influence the recording of nutritional intake amongst the runners. In light of the particularly low energy intakes, the incidence of disordered eating was not assessed by the current study but can not be discarded.

Secondly, runners with the largest energy deficits also had significantly lower BMI and body fat, correlating positively with energy balance. This may be indicative of a potential chronic energy deficit and would suggest that the reported energy deficits in this sample of runners may be, at least in part, valid. Unfortunately, changes in body mass over time could not be monitored as this study was cross-sectional. However, it is possible that even if body mass was monitored there would be little change if BMR was reduced as an energy-conserving mechanism. Female runners with the greatest energy deficits, had BMI's indicative of chronic undernutrition. Seven female runners had a BMI less than 17.5 kg m^{-2}, meeting the diagnostic criteria for anorexia-nervosa. Thirteen runners had a BMI less than 18 kg m^{-2}. These subjects also had significantly greater energy deficits, higher weekly running mileage and fewer eumenorrhoeic years. Similarly, an earlier study found significantly reduced levels of the nutritional markers IGF-1 and T_3 (indicative of an energy deficit) in female runners who had a BMI less than 18 kg m^{-2} (Zanker and Swaine, 1998). The mean body fat Z-score was low in male and female runners and many had extremely low levels of body fat (as low as 4.4% in females and 5.4% in males). Such low body fat may reflect greater

utilisation of fat when glycogen stores are inadequate due to chronic negative energy balance.

The validity of the energy expenditure data was dependent on the accuracy of the computation of BMR. It is acknowledged that there may be a potential overestimation of energy expenditure arising from the computation of BMR. BMR was estimated using McArdle and Katch's formula, and then multiplied b a PAL value to account for normal, daily activities. However, the extra energy used for training was estimated by multiplying BMR by MET values, as described by Ainsworth *et al*, (1993). The overestimation may occur because of the two different formulae used and because of the addition of training energy expenditure to the 24 hour estimated energy expenditure.

The validity of the energy expenditure data is also dependent on the accuracy of the exercise records completed by each subject. As expected, runners provided very accurate records of their training over 7 days and were aware of their training schedule and competition programme. The majority provided detailed information concerning their heart rate during training and 'minutes per mile' running pace. This assisted in the quantification of energy expenditure from exercise. However, it is also recognised that many of the distance runners in this study will be highly efficient in terms of metabolic cost of exercise and BMR, thus the MET values may have resulted in the overestimation energy expenditure. It is possible that runners with low calorific intakes are using their energy more efficiently. Never-the-less elsewhere, energy conservation has been associated with reductions in IGF-1, which is implicated in disturbed bone turnover (Laughlin and Yen, 1996; Thong *et al.*, 2000; Loucks and

Thuma, 2003). Thus, even if energy deficit has been overestimated due to low BMR and energy efficiency, it is possible that this energy conservation may have a negative affect on bone. This could not be determined from the present study for there were no laboratory measures of BMR or biochemistry.

ii) Energy balance and BMD

This study differed to previous work in this area by investigating BMD in relation to energy balance. Previous studies have focused on the relation between menstrual dysfunction and BMD in athletes, or in terms of energy balance and availability, have assessed biochemical markers of bone turnover. In this study, after controlling for factors such as years of eumenorrhoea, BMI and calcium intake, energy deficiency was the dominant risk factor for low lumbar spine BMD in this sample of runners. Positive correlations were found between energy balance and lumbar spine BMD even when results were controlled for the various covariates (including years of eumenorrhoea in the female group). Lumbar spine BMD and to a lesser extent, total body and dual femur BMD declined linearly with increased energy deficiency. Although it was not possible to measure bone markers in this study, the findings would support the hypothesis that energy deficit is detrimental to bone as derived from observations of reduced bone formation and bone turnover in women with anorexia (Grinspoon *et al.*, 1996; Heer *et al.*, 2002), amenorrhoeic distance runners (Okano *et al.*, 1995; Zanker and Swaine, 1998), women undergoing an energy restriction trial (Ihle and Loucks, 2004) and male runners under settings of energy deficiency (Zanker and Swaine, 2000). Future, longitudinal studies using nutritional markers, multiple bone markers and measures of BMD to investigate energy and bone status in endurance athletes are warranted.

Although research concerning the effects of certain factors on bone structure and size continues to accumulate, it has been suggested that the primary effect of chronic energy restriction on bone is a reduction in bone size and that oestrogen deficiency reduces bone density (Karlsson *et al.,* 2000). However, the present study found no association between reduced bone size and energy deficit, but there was a significant positive correlation between lumbar spine and dual femur BMAD and energy balance. This suggests that energy deficit is related to a reduced bone density rather than bone size.

Although it has been hypothesised that athletes who restrict energy intake will consume insufficient calcium (Sundgot-Borgen *et al.,* 1993; Clarkson and Haymes, 1995) the runners in this study reported calcium intakes that were above the current RDA. This is in agreement with previous studies in male (Bilanin *et al.*, 1989) and female (Drinkwater *et al.,* 1984; Marcus *et al.,* 1985; Rencken *et al.,* 1996) runners. Furthermore, no correlations were observed between calcium intake and lumbar spine BMD, thus it would seem unlikely that calcium deficiency was causing low BMD at this site. Vitamin D deficiency is now recognised as a major risk factor for bone loss in people of all ages. However, although dietary intake of vitamin D in the sample of runners was marginal, it is very likely that these athletes received sufficient vitamin D through exposure to sunlight during training.

The main limitation of this study was that it could not be determined whether the athletes were being truthful when reporting their dietary intake. This is not so much a limit in that it is unavoidable in studies of self-report and recognised from the outset. However, in order to encourage complete and accurate reporting, runners were

informed that they would receive a dietary report following completion of the study. Many runners went into great detail with their nutritional records, providing food labels and recipe lists, even quantifying the amount of water they drank. Unfortunately, several runners did not provide sufficient information, therefore were contacted by telephone to obtain more accurate accounts of the types of food they consumed. Never-the-less, the majority of runners provided detailed weighed records which were easily computed into the nutritional analysis software. There may also have been incidents of over-reporting by some runners, particularly those who may have wanted to mask a potential eating disorder. However, again this cannot be determined from the current study. In summary, although under-reporting and high energy efficiency cannot be disregarded, in light of their body dissatisfaction and corresponding low BMI and body fat (more long term indicators of energy status) as discussed earlier, it is possible that the majority of runners were chronically energy deficient.

The failure to balance energy demands arising from vigorous, high volume training, with appropriate energy intake, leads to energy deficit. To conclude this chapter, the current study has identified a link between energy balance, BMI, body fat and lumbar spine BMD. The finding that the relationship between energy balance and BMD at the dual femur and total body was no longer significant after controlling for intakes of other nutrients emphasises that preliminary findings should not be taken at face-value and potential confounders should be addressed. The following chapter links the energy balance data with the results presented in chapters 4 to 6.

Chapter 8

Energy balance, BMD and inter-relationships

The aetiology of reduced BMD in distance runners may involve complex inter-relationships between factors such as those identified by the current research. Indeed, chapter 7 has demonstrated that the main factor associated with BMD in distance runners was energy balance, whilst chapters 4 to 6 indicate that lumbar spine BMD in these athletes is also related to training volume, BMI and body composition and in the female runners, number of eumenorrhoeic years. It is possible that these findings are manifestations of an underlying energy deficit, induced by a dietary energy intake insufficient to match high quantities of training. This chapter draws together and evaluates the main findings from chapters 4 to 7, with a major emphasis on energy balance. Furthermore, given the potential inter-relationships between variables, stepwise multiple regression analyses were performed to determine which variables best predicted BMD and BMAD.

8.1 Results

8.1.1 Energy balance, menstrual status and BMD

(i) Predictors of menstrual status

To investigate which variables best predicted amen/oligomenorrhoea in the female runners, logistic regression was performed. The dependent variable 'menstrual status' was defined by coding eumenorrhoeic runners as '0' and amen/oligomenorrhoeic

runners as '1'. Oral contraceptive users were not included in the regression analysis. Potential covariates were entered (BMI, body fat, mileage, years training, age at menarche, energy balance, energy expenditure, dietary fat intake and calcium intake). The best predictors of menstrual status were dietary fat intake and percent body fat. This model correctly classified 86.4% of the female runners into their respective menstrual groups. When dietary fat intake was removed from the logistic equation, energy balance and percent body fat became the most predictive covariates of menstrual status (78.8%). It was possible that the effect of dietary fat intake was mediated by energy balance therefore partial correlations were computed with the total number of eumenorrhoeic years as the dependent variable. Energy balance was shown to be unrelated to total number of eumenorrhoeic years, when controlling for age and dietary fat intake ($p=0.476$). When controlling for energy balance, dietary fat positively correlated with eumenorrhoeic years ($r =0.342$; $p<0.01$) and when controlling for energy intake, the coefficient was slightly greater ($r =0.423$; $p<0.001$). Collectively, these findings suggest that dietary fat intake and percentage body fat mediate the observed relationship between energy balance and amen/oligomenorrhoea.

ii) Energy balance and BMD

Chapter 5 compared the BMD of female runners by menstrual status and unexpectedly found significantly reduced lumbar spine BMD in runners who used the oral contraceptive pill. These deficits were of a similar magnitude to those observed for amen/oligomenorrhoeic runners. In this chapter, differences in energy status and BMD T-scores between the menstrual groups were evaluated. The results are shown

in Table 8.1. Figure 8.1 is an error bar chart demonstrating the differences in energy balance between the three menstrual groups.

As shown in Table 8.1 the mean energy intakes in amen/oligomenorrhoeic runners and oral contraceptive users were lower than in eumenorrhoeic runners and energy expenditures were higher ($p<0.01$). Energy deficits were greater in amen/oligomenorrhoeic runners and the oral contraceptive group compared to eumenorrhoeic runners ($p<0.01$).

Table 8.1: Nutritional and bone status (mean and SEM) of female runners by menstrual status group

	Eumenorrhoeic (n=34)	Amen/oligomenorrhoeic (n=17)	Oral contraceptive users (n=14)
Energy intake (kcal day^{-1})	2084.7 (84.4)	1417.9 (128.4)†	1564.8 (49.6)†
Energy intake per kg body mass (kcal day^{-1})	38.5 (1.4)	28.9 (2.3)†	29.8 (1.4)†
Energy expenditure (kcal day^{-1})	2368.4 (48.3)	2439.7 (65.9)	2590.4 (57.7)*
Energy balance (kcal day^{-1})	-293.9 (108.9)	-1022.1 (85)†	-1007.4 (62.1)†
Fat intake (g day^{-1})	67.5 (4.2)	31.8 (4.4)†	43.4 (2.3)†
BMI (kg m^{-2})	19.8 (0.3)	18.2 (0.3)†	19.2 (0.3)
Body fat %	18.3 (0.6)	13.6 (1.3)†	15.3 (1.5)†
Lumbar spine T-score	-0.4 (0.3)	-1.4 (0.2)†	-1.1 (0.1)†
Dual femur T-score	0.7 (0.1)	0.0 (0.2)†	0.5 (0.2)
Total body T-score	0.5 (0.1)	-0.2 (0.2)†	0.5 (0.1)

* *greater than eumenorrhoeic group, $p<0.01$*
† *less than eumenorrhoeic group, $p<0.01$*

Oral contraceptive users and amen/oligomenorrhoeic runners had a lower BMI, dietary fat intake and percent body fat than eumenorrhoeic runners ($p<0.01$). As evaluated in Chapter 5, these runners also ran significantly more miles per week.

Seven (23.3%) eumenorrhoeic runners were also found to be osteopenic at the lumbar spine (the observed range was -1.0 to -2.4). Interestingly, subsequent analysis revealed that eumenorrhoeic runners with osteopenia had greater energy deficits (-1128.9 kcal day^{-1}) compared to eumenorrhoeic runners with normal BMD (-77.4 kcal day^{-1}) ($p<0.001$).

Overall, energy deficiency was found to be the major factor predictive of reduced BMD amongst female runners and may at least in part, explain the lower lumbar spine BMD in oral contraceptive users and in eumenorrhoeic runners.

8.1.2 Energy balance, training, performance level and BMD

(i) Training characteristics

It was shown in chapters 4 and 6 that weekly running mileage negatively correlated with lumbar spine BMD in male and female runners. Coefficients for the relationship between energy balance and lumbar spine bone status ($r =0.624$) were larger than those computed for weekly running mileage ($r = -0.371$). High weekly running mileage was negatively associated with energy balance ($r = -0.392$; $p<0001$) and positively associated with energy expenditure ($r =0.352$; $p<0.001$), suggesting that an energy deficit in this group is likely to be accompanied by higher volumes of running

due to higher levels of energy expenditure. Although a positive association was observed between energy intake and energy expenditure ($r = 0.268$; $p<0.001$), the results indicate that the amount of energy consumed by the runners was insufficient to match their high energy expenditures.

As discussed in chapter 6, male and female runners who did not have at least one rest day from training per week had significantly lower lumbar spine and total body T-scores and BMD than those who did have a rest day. ANOVA also revealed that these runners ran more miles per week (65.6 *v* 49; $p<0.001$) and had greater energy deficits (-1119.9 *v* -448.8 kcal day^{-1}; $p<0.001$) than runners who included a rest day in their weekly training schedule. Thus, high weekly running mileage and training schedules that do not include regular recovery days are associated with significant energy deficit and reduced lumbar spine BMD.

(ii) Performance level

Differences in energy status and lumbar spine T-score between elite, county and club level female and male runners are shown in Tables 8.2 and 8.3 respectively. ANOVA followed by the Games-Howell post-hoc test were used to test for differences whilst accounting for variations in group size. Dual femur and total body results have not been included for there were no differences between groups [1].

[1] Results have been provided in Chapter 6.

Table 8.2: Energy (kcal day^{-1}) and lumbar spine bone status of female runners by performance level (mean and SEM)

	Elite (n=31)	County (n=20)	Club (n=14)
Energy intake (kcal day^{-1})	1721.6 (89.2)*	1790.2 (137.9)	1979.8 (141.9)
Energy expenditure (kcal day^{-1})	2566.7 (47.6) †‡	2323.5 (54.7)	2302.1 (58.2)
Energy balance (kcal day^{-1})	-836.9 (91)*	-533.3 (166.4)	-347.2 (139.6)
Lumbar spine T-score	-1.2 (0.1)*	-0.7 (0.2)	-0.3(0.2)
BMI (m kg^{-2})	18.8 (0.2)	19.5 (0.3)	19.8 (0.4)
Body fat %	14.0 (0.8)*	18.2 (0.9)	19.2 (1.2)
Body Fat Z- score	-1.8 (0.1) §	-1.3 (0.2)	-1.2 (0.3)
Weekly running mileage	58.2 (2.5) †‡	47.5 (2.5)	42.0 (1.9)

* *Elite < Club, $p<0.005$*
† *Elite > Club, $p<0.005$*
‡ *Elite > County, $p<0.05$*
§ *Elite < County, $p<0.005$*

In male and female runners, weekly running mileage correlated positively with energy expenditure ($r = 0.465$; 0.542; $p<0.01$), which was higher in the elite groups ($p<0.05$). Elite male and female runners also had lower energy intakes and a greater energy deficit compared to club level runners ($p<0.005$).

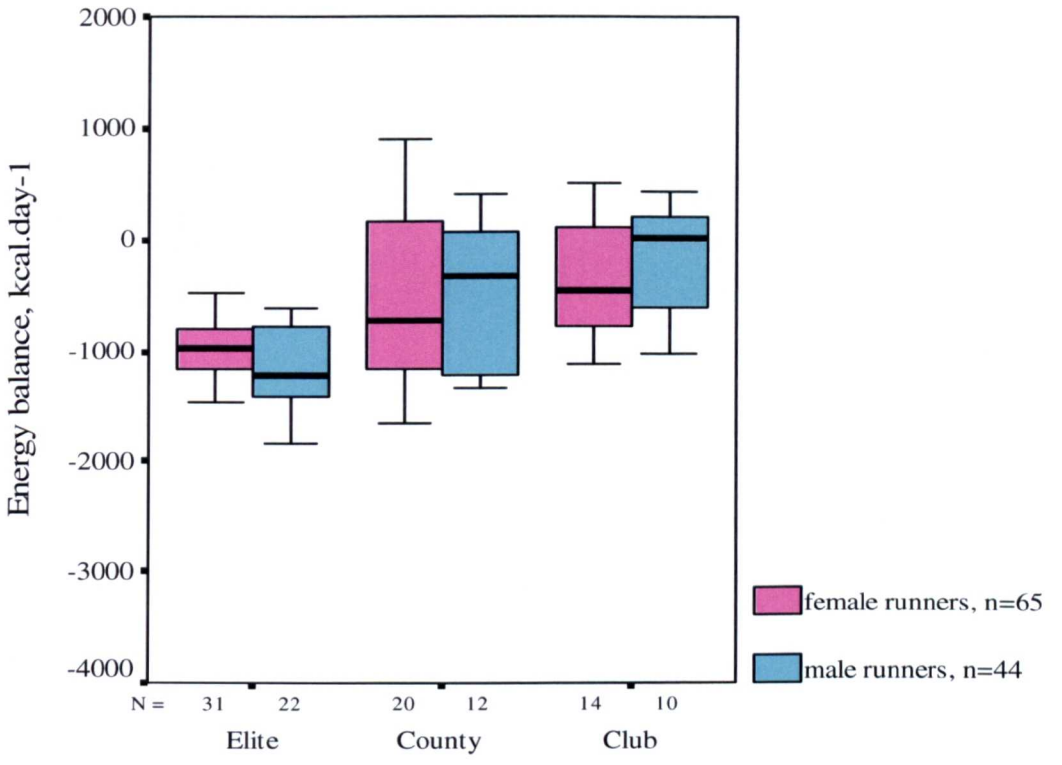
Table 8.3: Energy (kcal day^{-1}) and lumbar spine bone status of male runners by performance level (mean and SEM)

	Elite (n=24)	County (n=10)	Club (n=10)
Energy intake (kcal day^{-1})	2286.4 (79)*	2728.2 (276.8)	2843.9 (134)
Energy expenditure (kcal day^{-1})	3409.2 (54.6)†‡	3275.1 (183.4)	3011.9 (120.5)
Energy balance (kcal day^{-1})	-1130.9 (67.2)*	-546.9 (352.4)	-167.9 (166.3)
Lumbar spine T-score	-1.1 (0.1)*	-0.5 (0.3)	-0.4 (0.2)
BMI	20.6 (0.2)§	22.1 (0.4)	21.5 (0.4)
Body fat %	9.1 (0.7)*§	13.6 (1.2)	13.9 (1.8)
Body Fat Z- score	-1.5 (0.1)§	-0.7 (0.3)	-0.9 (0.3)
Weekly running mileage	71.6 (3.6)†‡	61.0 (1.7)	47.0 (4.4)

* *Elite < Club, p<0.005*
† *Elite > Club, p<0.005*
‡ *Elite > County, p<0.05*
§ *Elite < County, p<0.005*

It was established in chapter 6 that male and female elite runners ran significantly more miles per week and had significantly lower lumbar spine BMD and T-scores than club runners. Weekly running mileage was negatively associated with lumbar spine T-score in male and female runners (r =-0.517; -0.435; p<0.001), but subsequent analysis revealed that energy balance was the strongest predictor of lumbar spine T-score (r =0.715; 0.861; p<0.001). The differences in energy balance between the three performance groups are illustrated in Figure 8.2.

Figure 8.2: Box-plot illustrating differences in energy balance between runners grouped by performance level



8.1.3 Multiple regression

Stepwise multiple regression analyses were performed to determine which factors best predicted BMD at the various sites in male and female distance runners. All potential covariates were entered into the equation and were removed by order of least significance. The following covariates were chosen for inclusion in the regression analysis, based on the established correlations between these variables and BMD in runners: *age, energy balance, weekly running mileage, eumenorrhoeic years (female runners), age of menarche (female), BMI, body mass, percent body fat, body fat Z-score, lean body mass, rest days (categorical), dietary fat intake and calcium intake.*

(i) Female runners

In female runners, multiple regression analyses indicated that energy balance and body mass were the strongest predictors of lumbar spine BMD, accounting for 79.4% of variation in BMD at this site ($p<0.001$). Energy balance and body mass best predicted lumbar spine BMAD, accounting for 64% of variation ($p<0.001$) suggesting that body mass independent from bone size predicts BMD. Total number of eumenorrhoeic years, were only significant once energy balance was removed from the equation.

Again, the regression analysis showed that energy balance and BMI was predictive of BMD at the dual femur. However, this model could only account for 33.3% of variation in BMD ($p<0.005$). BMI and energy balance also best predicted dual femur BMAD although could only account for about one quarter of variation ($p<0.01$). Dietary fat intake, weekly running mileage and BMI were the strongest predictors of total body BMD in female runners explaining 60.9% of BMD variation of this site ($p<0.005$).

(ii) Male runners

In male runners, lumbar spine BMD was best predicted by a positive effect from energy balance and BMI and a negative effect from years of training. This model accounted for 68% of variation in BMD ($p<0.001$). The only significant predictor of lumbar spine BMAD in male runners was energy balance which could explain 76.4% of variation at this site ($p<0.001$).

Total number of years in training (-) best predicted dual femur BMD (31.2%) and together with body mass, best predicted dual femur BMAD (34.3%; both $p<0.01$). Total body BMD was best predicted by total number of years in training (-), BMI, dietary fat intake and percentage body fat, accounting for 58.8% of variation in BMD at this site ($p<0.005$).

Overall, the multiple regression analysis confirmed the significant association between energy balance and lumbar spine BMD and BMAD in both male and female runners. The previously identified correlation (chapter 4 and 6) between weekly running mileage and lumbar spine BMD became less significant when considering energy balance. The results also support the significant interactions between body mass and energy balance on BMD. Body mass and/or BMI were universal predictor(s) of BMD at the lumbar spine, dual femur and total body.

8.2 Discussion

This chapter has presented several key findings. Firstly, the predominant determinant of lumbar spine BMD in runners was energy balance. Correlations were less significant between energy deficiency and BMD at the dual femur and total body to suggest that other factors may be more influential. Secondly, differences in energy balance could account for lower lumbar spine BMD in elite *versus* club runners and between female amen/oligomenorrhoeic runners and oral contraceptive users *versus* eumenorrhoeic runners. Thirdly, energy deficiency was not the primary predictor of amen/oligomenorrhoea in the female runners.

i) Energy balance, menstrual status and BMD

Although not a main aim of this work, the strongest predictors of overt menstrual status were investigated by using logistic regression. The current consensus throughout the literature is that menstrual disorders in female athletes are caused by energy deficiency (Yeager *et al.,* 1993; De Souza and Williams, 2004) and such evidence has been causally-inferred (Williams *et al.,* 1995; Loucks and Thuma., 2003). With these advancements came the gradual opinion that the association between body fat and menstrual function (Frisch and McArthur; 1974; Linnell *et al.,* 1984) was of less importance, due to the lack of rigorous evidence. With respect to the current consensus, it could be argued that the observed association between energy deficiency and bone loss may in part, be mediated by oestrogen deficiency. However, the most significant predictors of amenorrhoea in female runners were dietary fat intake and body fat percentage, even when controlled for energy intake. These results conflict with the majority of the literature, although a recent report made a similar finding (Cobb *et al.,* 2003). Elsewhere, lower body fat levels and markedly lower dietary fat intakes have been found to accompany low plasma leptin levels in elite amenorrhoeic runners and it is possible that reduced availability of leptin is involved in the hypothalamic dysfunction that leads to menstrual dysfunction (Laughlin and Yen, 1997; Thong *et al.*, 2000). It is unclear what role dietary and body fat may have in the aetiology of menstrual dysfunction. However, it may possibly be linked to the hormone, leptin. As an emerging issue from the current study, this requires further research, potentially in the form of intervention trials.

Concerning BMD, it was found that the major factor predictive of low BMD in female runners was energy deficiency. This is in contrast to the common assumption

throughout the literature has been that the instigating factor for bone loss in female endurance athletes is oestrogen suppression, characterised by amenorrhoea. Indeed, when energy balance was removed from the regression equation, total number of eumenorrhoeic years became the strongest predictor of BMD. These results agree with Cobb *et al* (2003), who found that high eating restraint scores in female distance runners were more predictive of low BMD than were menstrual disorders. The results would also support reports of reduced bone formation and turnover in amenorrhoeic athletes, as opposed to increased bone resorption and turnover that would be expected due to an oestrogen deficiency (Zanker and Swaine, 1998). Thus, it is suggested that bone loss in athletes may primarily arise from energy deficiency and the contribution of menstrual dysfunction may be secondary.

Although the efficacy of the oral contraceptive pill is unclear, it was unexpected that the oral contraceptive pill users would have a similarly low lumbar spine BMD as the amen/oligomenorrhoeic group. This indicates that the oral contraceptive pill had been unsuccessful in preventing bone loss in the runners of this study. A potential explanation for this finding is energy deficiency, which was virtually undistinguishable from that observed in the amen/oligomenorrhoeic runners. In the literature women with anorexia nervosa (Kreipe *et al.,* 1993; Grinspoon *et al.,* 2002) and amenorrheic runners have been found to be less responsive to oestrogen therapy but display an improvement in BMD with increased nutrition, a reduction in training and a small degree of weight gain (Warren and Holderness, 1992; Hergenroeder *et al.,* 1995; Keen and Drinkwater, 1997; Gibson *et al.,* 1999; Hawkins *et al.,* 1999; Braam *et al.,* 2003). In summary, the result emphasise the importance of nutrition rather than of oestrogen therapy for the maintenance of BMD in female runners.

An interesting finding was that although lumbar spine BMD was significantly lower in oral contraceptive users than in eumenorrhoeic runners, dual femur and total body BMD were significantly greater than in amen/oligomenorrhoeic runners. As the oral contraceptive users and amen/oligomenorrhoeic runners were very similar in all other variables, these results suggest that BMD at more weight-bearing sites may be protected from bone loss in oral contraceptive pill users, but that the oral contraceptive pill cannot protect against bone loss at the lumbar spine. These findings are particularly thought-provoking and it is clear that future research using larger subject samples and biochemical tests, are required. The current study was limited in that no biochemical measurements of hormones were taken, thus the true oestrogen status of the oral contraceptive users was unknown. A further limitation is that causal inferences cannot be made on the basis of cross-sectional research. It is likely that the extent of bone mineral deficiencies in female runners is dependent on the extent and duration of energy deficiency and whether or not this is accompanied with amenorrhoea. Thus, longitudinal research is required and follow-up work would be useful.

Overall, although energy balance did not appear to be implicated in menstrual disorders, it was found the primary determinant of lumbar spine BMD in female runners, even after controlling for total number of eumenorrhoeic years. The oral contraceptive pill appears to be ineffective in protecting the lumbar spine from bone loss related to energy deficiency.

ii) Energy balance, training, performance and BMD

The inverse relationship between weekly running mileage and lumbar spine BMD in male and female runners was discussed in chapter 6 and supports previous observations in male (Bilanin *et al.,* 1989; Hetland *et al.,* 1993b) and female (Winters *et al.,* 1996; Burrows *et al.,* 2003) distance runners. Those who ran more miles per week and who included no regular rest day in their training schedules had higher energy expenditures, greater energy deficits and lower levels of body fat. These runners also had significantly lower lumbar spine BMD. Thus, it is possible that energy deficiency may account for reduced lumbar spine BMD in runners with high volume training. In support of this hypothesis, elite male and female runners ran more miles per week had greater energy deficits, lower body fat and lower lumbar spine BMD than club runners. The higher volume training schedules undertaken at elite level may contribute to bone loss in runners, again, due to high energy expenditures that are not met by adequate energy intakes. This is reflected in the observation that the negative relationship between weekly running mileage and lumbar spine BMD became less significant when conducting regression analyses that included energy balance. Thus, high weekly running mileages may only be detrimental to lumbar spine BMD when athletes are in negative energy balance. This conclusion is based on observational data taken at one point in time, therefore can not be used in its totality to infer definite causal relationships. Thus, future longitudinal interventions are required to confirm this hypothesis.

The negative association between total number of years in training and BMD at all sites suggests that long-term participation in endurance running may be potentially

harmful to skeletal health. However, as this study was cross-sectional, again, this observation requires clarification from future longitudinal, prospective studies.

The observation that dietary fat intake was predictive of total body BMD in male and female distance runners may reflect a potential association with leptin, particularly as percentage body fat was also predictive of total body BMD in the male runners. As discussed in chapter 2, accumulating evidence suggests that leptin has an important role in bone although further research is still required to clarify this.

Energy balance, BMI, body mass and percent body fat were major predictors of BMD in male and female runners. Energy balance was the most robust predictor of lumbar spine BMD and accounted for differences between elite and club runners, as well as amenorrhoeic runners and oral contraceptive users compared to eumenorrhoeic runners. Energy deficiency could not predict amen/oligomenorrhoea but was the primary determinant of low BMD in female runners and total number of eumenorrhoeic years was secondary. It should be considered that the energy balance data represents a short term indice, thus has inherent limitations. Like BMD, body mass and BMI are potentially more useful as long term markers of energy status, and were found to be universal predictor(s) of BMD at the lumbar spine, dual femur and total body.

Chapter 9

Conclusions, implications and recommendations for future research

9.1 Conclusions

This thesis was concerned with investigating BMD in distance runners. After reviewing the literature it was found that few studies had investigated BMD in male runners (Bilanin *et al.,* 1989; Mac Dougall *et al.,* 1992; Hetland *et al.,* 1993b; Bennell *et al.,* 1996; MacKelvie *et al.,* 2000) and none had compared bone status between male and female runners. The literature is also dominated by the hypothesis that the primary cause of bone loss in female athletes is oestrogen deficiency, characterised by amenorrhoea (Drinkwater *et al.,* 1984; Marcus *et al.,* 1985; Myerson *et al.,* 1992; Pettersson *et al.,* 1999), and this hypothesis has shaped the majority of previous studies, leaving little room for consideration of other factors. Accumulating evidence from studies using biochemistry indicate that energy deficiency can suppress bone formation and lead to an imbalance in bone turnover (Grinspoon *et al.,* 1996; Zanker and Swaine, 1998; 2000; Heer *et al.,* 2002). However, the relationship between energy balance and BMD had previously not been investigated.

Therefore, there were two aims to this research: first, to determine whether male distance runners are at a comparable risk for bone loss compared to their female counterparts and second, to determine if there was a relationship between reported energy balance and BMD. From these aims, several research questions were devised

and conclusions were provided at the close of the respective chapters (chapters 4 to 8). The current chapter collates the main conclusions, with reference to the research aims and questions.

The first aim was to investigate and compare the prevalence of low BMD in male and female endurance runners. As the first to do so, this study confirms the numerous reports of low BMD in female runners (Marcus *et al.,* 1985; Micklesfield *et al.,* 1995; Rencken *et al.,* 1996) but reveals a similar degree and incidence of bone mineral deficiencies in male runners. With reference to the first research question, *"What proportion of male and female runners have low BMD?"*, lumbar spine osteopenia was found in 36.4% of male runners and 41.6% of female runners, indicating at least a 2.5-fold higher prevalence than expected for the general female population. Of particular interest and concern, was the finding that male runners had significantly lower lumbar spine T-scores compared to eumenorrhoeic runners. The possible implications of such findings are discussed in section 9.2.

There were also similarities between the sexes regarding the competitive level most at risk, relationships with certain variables and the distribution of BMD throughout the skeleton. Firstly, low lumbar spine BMD was most severe in male and female runners who competed at elite level and the strongest predictor (before energy balance) of low lumbar spine BMD, was a high weekly running mileage. Secondly, the distribution of BMD throughout the skeleton was similar in male and female runners. BMD was reduced in the lumbar spine and arm, whereas in the dual femur and legs, BMD tended to be above normal and for the total body, BMD was normal. These results indicate that as a weight-bearing exercise, running may offer a degree of protection

from bone loss at lower extremity and more cortical, sites recipient of greater mechanical loading. This protection does not appear to extend to the lumbar spine where significant bone loss is evident. The results also emphasise the importance of measuring BMD at multiple sites in runners, for measuring only total body or lower limb BMD may give false reassurance.

In conclusion, osteopenia and low lumbar spine BMD in distance runners is not gender-specific. As osteopenia during young adulthood increases the risk of developing osteoporosis and fracture in later life (Cummings *et al.,* 1993; Marshall *et al.,* 1996; IOF, 2004), these athletes may be seriously jeopardising their long-term skeletal health. The results also raise the question, 'is there a mechanism of bone loss that applies to both male and female athletes?'

While it was not possible to conduct biochemical analyses of sex steroid hormones, previous work to date has shown that BMD is related to oestrogen deficiency in female runners (Marcus *et al.,* 1985) but is unrelated to sex steroids in male runners (Hetland *et al.,* 1993b). Self-reported amenorrhoea and fewer eumenorrhoeic years were found to be risk factors for low BMD in female runners at all sites, particularly at the spine. With reference to the second research question, "*Is BMD related to overt menstrual status in female runners?*" – the answer was found to be 'yes', but to a certain extent. For in contention with the theory that an absence of regular menstruation is the major risk factor for bone loss in athletic women, lumbar spine BMD and T-scores were significantly lower in the oral contraceptive using runners, with the mean T-score for this group indicative of osteopenia. In fact, reduced BMD at this site was of a similar magnitude to that observed in amen/oligomenorrhoeic

runners. This provides additional evidence that the oral contraceptive pill may be of limited efficacy for the protection of spinal bone loss in female athletes. It also emphasises the need for a randomised intervention trial, particularly as the oral contraceptive pill is routinely offered as a preventative strategy against bone loss in amenorrhoeic active females. Furthermore, 7 eumenorrhoeic runners had lumbar spine osteopenia and although it is possible that these runners may have had asymptomatic menstrual disorders, evidence elsewhere has shown that such disorders have little effect on BMD (De Souza *et al.*, 2003).

By taking an alternative stance to previous studies of BMD in athletes, the second aim of this research was to determine if there was a relationship between reported energy balance and BMD in distance runners and the final research question asked, *"Is there a relationship between energy balance and BMD?"* Although the data was based on self report, a relatively large sample of runners was used to improve the validity of the results. This work has demonstrated a link between calorific deficiency and low BMD. After controlling for dietary calcium and fat intake, the correlation between energy balance and BMD remained significant for the lumbar spine but not for the dual femur or total body. It is possible that energy deficiency is directly implicated in the aetiology of bone loss in runners, for research elsewhere indicates that metabolic disruptions arising from energy deficiency, such as low IGF-1 are associated with reductions in bone formation and in bone turnover (Grinspoon *et al.*, 1996; Zanker and Swaine, 1998; 2000). The finding that the relationship between energy balance and BMD at the dual femur and total body was no longer significant after controlling for intakes of other nutrients emphasises that preliminary findings should not be taken at face-value and potential confounders should be addressed. Overall, it is concluded

that energy balance was a major determinant of BMD and T-scores in the runners studied.

In this study, energy deficiency was found to be the underlying determinant of low lumbar spine BMD between various groups of runners: in elite compared to club level runners and in oral contraceptive pill users compared to eumenorrhoeic runners. In answer to the third research question, *"Is BMD related to training characteristics and performance level?"* reduced lumbar spine BMD and T-scores were detected in male and female elite runners compared to club runners and was first attributable to higher volume training schedules. This would agree with previous studies that higher weekly running mileages may be detrimental to BMD (Hetland *et al.*, 1993b; Winters *et al.*, 1996; Warren *et al.*, 2003). However, after assessing energy balance, it was concluded that the greater energy deficit in elite compared to club runners was more important. Elite runners were engaged in greater energy-demanding schedules which increased the incidence for negative energy balance, although they also tended to consume fewer calories than club runners which contributed to the magnitude of energy deficit. Thus, it is concluded that the relationship between greater training volume and reduced lumbar spine BMD in this study may be mediated by energy deficit, and that energy deficiency is a risk factor for low BMD.

Despite the oral contraceptive pill offering athletes a regular dose of oestrogen and ensuring menstrual regularity, oral contraceptive users had significantly lower lumbar spine BMD and T-scores than eumenorrhoeic runners. These runners were subsequently found to have greater energy deficits and a trend for lower BMI and body fat than eumenorrhoeic runners. Thus, it is suggested that normal bone health at

the lumbar spine may require adequate energy availability and stores, despite oral contraceptive pill use.

The observation that seven regularly menstruating runners had lumbar spine ostopenia may also be explained by their greater energy deficits, lower body fat levels and lower BMI compared to eumenorrhoeic runners with normal BMD. This would agree with Miller *et al* (2004) who recently found reduced BMD in regularly menstruating energy deficient women. Moreover, energy balance was found to be a more powerful predictor of lumbar spine BMD and T-scores in all female runners, than total number of eumenorrhoeic years. The latter factor only became significant when energy balance was removed from the regression equation. Thus, in this study it emerged that energy balance was the strongest predictor of lumbar spine BMD and T-scores in female runners.

Other conclusions drawn from this research include the positive influence of BMI and body mass at all skeletal sites, possibly reflecting the influence of greater magnitude during skeletal loading or the contribution of nutritional status. In male runners, those who had been training for a greater number of years had lower dual femur and total body BMD to suggest that over a longer period of time, running may become of little stimulus to bone at weight-bearing sites. By conducting multiple regression, dietary fat intake positively correlated with total body BMD and T-scores, in male and female runners. The role of body fat and dietary fat to bone status may be a useful avenue for future research.

Whilst conducting this research, several additional findings were made. It was found that dietary fat intake and body fat were more predictive of amenorrhoea than energy balance. In fact, energy balance was shown to have no relation with amenorrhoea and total number of eumenorrhoeic years after controlling for fat intake. This disagrees with the concept that energy deficiency is the primary cause of menstrual disorders in athletes, and it is concluded that dietary and body fat may be more influential. This may suggest a role for leptin, which has been shown elsewhere to be significantly reduced in amenorrhoeic athletes (Laughlin and Yen, 1997; Thong *et al.*, 2000).

Although BMD was the main bone measurement outcome, data regarding bone size were also used to provide an insight into the bone status of these athletes. As expected male runners had a larger bone area than female runners which could account for their lower BMAD. However, it was particularly of interest that despite larger amounts of exercise, elite female runners had a smaller bone area than club level runners. There was also a trend for reduced bone size in elite male runners. This in addition to their reduced BMD at the lumbar spine would increase their susceptibility to fracture in later life. This aside, it can not be discounted that elite runners may have had smaller bones due to self-selection into this performance category. The smaller bones of elite runners may be of an advantage in distance running where lightness is often beneficial to performance, thus running *per se* may not be associated with reduced bone size. The reductions in BMD as well as bone area at the lumbar spine in elite runners indicates that this site may be particularly susceptible to future fracture for although bone area was also smaller at the dual femur in elite runners, BMD at this site was normal. Thus, it appears that running is associated with reduced BMD but smaller

bone size may represent a performance-related advantage and as a consequent the reason why elite runners were found to have smaller bones.

Overall, it is concluded that energy balance was significantly and positively associated with BMD in distance runners. Runners with more severe energy deficits and corresponding low BMI were found to be osteopenic at the lumbar spine. As a potential mechanism of athletic bone loss, energy deficit may explain why the problem of low bone density in runners appears to be more widespread than once thought. There was a similar prevalence of compromised lumbar spine BMD in male as in female runners and low BMD in female runners was not exclusive to those who had overt menstrual disorders. Regular menstruation did not infer normal bone status and the oral contraceptive pill did not appear to be sufficient to maintain BMD.

Both male and female distance runners are at risk for significant energy deficits. Energy balance was shown to be the major determinant of lumbar spine BMD in male and female runners and in female runners energy balance was more predictive of lumbar spine BMD than menstrual status. The low BMI and body fat in runners with higher energy deficits and lower BMD may be more long term parameters of chronic energy deficiency compared to the short term energy balance data. The originally observed (chapter 4 and 6) negative relationship between weekly running mileage and lumbar spine BMD became less significant when conducting regression analyses that included energy balance (chapter 8). Thus, high weekly running mileages may only be detrimental to lumbar spine BMD when athletes are in negative energy balance. This conclusion is based on observational data taken at one point in time, therefore can not be used in its totality to infer definite causal relationships. Thus, future longitudinal

interventions are required to confirm this hypothesis. Low BMD in distance runners is serious, increasing risk of stress fractures and potentially leading to the development of premature osteoporosis. Low lumbar spine T-scores indicative of osteopenia in male and female athletes who should be aiming to maximise peak bone density are likely to have damaging implications for long-term skeletal health. The results of this current study would imply that the avoidance of energy deficit may be important in preventing bone loss in athletes.

9.2 Strengths and limitations of the research

Throughout chapters 4 to 8, the strengths and limitations of this research have been discussed and this conclusive section will summarise these and provide additional considerations. Firstly, the cross-sectional design had inherent strengths and weaknesses. In using this design, it could not be ascertained when exactly bone loss occurred in these runners and how rapidly this was progressing. In order for a negative factor to have a significant effect on BMD sufficient exposure to this factor is required. This study could not determine how long runners with low BMD had been in negative energy balance, this could only be achieved by a longitudinal study with additional methods such as indirect caloriometry and body mass monitoring. Furthermore, the results from this study cannot imply causation. Although a significant association was found between energy balance determined from self-report and BMD, it can not be concluded that energy deficit was the cause of low BMD in this group. Such assumptions can only be made following a randomised controlled intervention. On a more positive side, the cross-sectional design enabled the comparison of male and female distance runners which provided some interesting, useful and relevant data and fulfilled the first main aim of the study. It enabled

comparisons of reported energy intakes, expenditures and balance between various groups such as elite compared to club level athletes and between female runners grouped by overt menstrual status. This design was also more feasible for the time-scale allocated to this work and for the number of subjects studied.

Secondly, the recruitment of subjects for this study may have influenced who replied to the advertisements and who participated. Those runners who were concerned about their health due to stress fracture injuries would have been more likely to respond to the poster advertisements. As low BMD was found to be significantly associated with stress fracture occurrence, this may have resulted in a degree of volunteer bias with more people with low BMD applying to participate in the study. Furthermore, in hindsight, it may have been worthwhile including a non-active, healthy control group in order to compare parameters such as bone size, BMAD and energy balance with the runners. Strengths of the subject sample were that it comprised of runners from different performance levels, i.e. elite, county and club and of runners from all over the UK. Furthermore, in contrast to previous studies, female subjects were not selected according to their menstrual status which reduced the level of ascertainment bias. By taking this approach, it was found that this benefited the research by enabling a more holistic insight into the bone health of runners.

Thirdly, as discussed in chapters 3 and 7, menstrual status data in female runners, energy intake and energy expenditure data were attained from self-report. There are methodological limitations inherent to the honesty and accuracy of the participants. An important consideration is that the potential for under-reporting or indeed over-reporting cannot be discounted. Nevertheless, the majority of athletes completed

detailed reports and there were positive associations of BMI and body fat with energy balance to support energy balance results. Other methodological limitations were that biochemistry was not used to determine sex hormone status in male and female runners or bone turnover, although with respect to the aims of the research these measurements were not the main priority. However, it would have been useful to assess nutritional markers such as serum IGF-1 and leptin concentrations. Unfortunately in this study, it was not feasible to do so.

Finally, caution should be applied when interpreting T-scores in young male and female adults for this parameter of bone status was originally intended for use in postmenopausal women. Notwithstanding this, the use of T-scores in this study enabled bone status to be compared between male and female runners and provided a useful insight into the current and potential future bone status of these athletes.

9.3 Implications

There were several conclusions from this research that may have relevant clinical and research implications for the field of osteoporosis, as well as for sports medicine, coaches and the athletes themselves.

By providing new data concerning BMD in male compared to female runners, this study adds to the current literature, and may have important implications for future research and preventative strategies. The awareness of the osteoporosis risk is currently only promoted amongst female athletes and screening for osteoporosis only occurs in female athletes who experience prolonged amenorrhoea. The female athlete

triad model was devised to define the problem of low BMD and risk of osteoporosis in female amenorrhoeic runners (Yeager *et al.,* 1993; Otis *et al.,* 1997). However, the results of this study disagree with the triad and suggest that the problem of low lumbar spine BMD in runners is more widespread than previously thought, occurring in male runners and in female runners using the oral contraceptive pill. There currently exists no male-version of the Triad model and the role of the oral contraceptive pill is unclear. Furthermore, most publications aiming to raise awareness of bone health amongst athletes have focused on females, such as the NOS *Fit but Fragile* publication (NOS, 1999). If the findings from the current study are confirmed in subsequent research, prevention strategies to target all competitive endurance athletes and revision of the female athlete triad model may be required.

Low T-scores indicative of osteopenia in athletes, who should be aiming to maximise PBD, may have damaging implications for long term skeletal health.
The clinical relevance of this is that these athletes may become osteoporotic at an earlier age and have an increased risk of fracture later in life. BMD is an important predictor of future fracture risk in both sexes and vertebral fractures occur with a higher incidence earlier in life than other types of osteoporotic fractures. Thus, it is possible that continuing exposure to the cause of their bone mineral deficiencies may predispose these runners to vertebral fracture, in the foreseeable future. In the near term, low BMD may predispose athletes to an increased risk of stress fractures and the results support this.

The relationship between energy balance and BMD indicates that energy balance may be an underlying factor mediating bone loss in athletes regardless of sex and in

females, regardless of menstrual status. This would agree with research elsewhere that has been conducted using biochemical tests of bone turnover (Zanker and Swaine, 1998; 2000). This would have important implications for treatment and preventative strategies in that bone loss may be avoided with adequate nutrition. Indeed nutritional assessments and advice would be cost-effective and compared to oral contraceptive administration and in some cases HRT, would have no unwanted side-effects to general health in female runners. Moreover, the results would indicate that any individual if in negative energy balance, may be at risk for bone mineral deficits and thus this may apply to other energy deficient sports persons including ballet dancers, rowers and endurance cyclists.

A final word.... Participation in running is not discouraged as there are many benefits associated with regular exercise. However, the results of this study indicate the importance of adequate nutrition in terms of energy balance. Pursuit of thinness, even if for performance reasons, at expense of bone health is discouraged. If the results of the current study are clarified, the practical implications are that distance runners and indeed endurance athletes, may be able to protect their skeletal health by optimising energy intake to fuel their high energy expenditures during training. Many athletes may unknowingly be energy deficient due to large energy expenditures and potential appetite suppression. In such cases, a dietary and training assessment should be able to determine approximately how much energy is required. For situations in which runners are apprehensive about taking on board extra calories, dietary counselling would be required. This would be particularly useful for those runners who purposefully restrict their dietary intake.

9.4 Recommendations for future research

A shift in paradigm may be required, from that which previously focused on the role of menstrual dysfunction in the bone health of female athletes, to that of bone loss in all endurance athletes, regardless of gender, and with a greater emphasis on energy balance and availability. A number of recommendations for future research have emerged from the current work.

- Further research comparing bone status in male and female athletes from various sports.
- High weekly running mileages may only be detrimental to lumbar spine BMD when athletes are in negative energy balance. Thus, future longitudinal interventions are required to substantiate this hypothesis.
- Longitudinal research into the role of energy balance in BMD, preferably to monitor body mass, BMR, energy efficiency and bone turnover.
- Are there any longitudinal changes in BMD and bone turnover in distance runners who improve their energy balance by reducing training, withdrawing from competitive sport or increasing energy intake?
- A randomised intervention trail of the oral contraceptive pill in athletic women (measuring multiple skeletal sites and regular hormonal assays).
- The role of leptin and the relationship with body fat, in menstrual dysfunction and bone metabolism in athletes.
- Bone structure and bone quality in athletes compared to less active controls.

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Appendix 1

Ethical approval letter

The Leeds Teaching Hospitals NHS
NHS Trust

Local Research Ethics Committee
6th Floor, Wellcome Wing, The General Infirmary at Leeds,
Great George Street, Leeds LS1 3EX. Telephone 0113 3926788

Miss K Hind
PhD Student
Centre for Bone and Body Composition
University of Leeds
Ground Floor, Wellcome Wing
Leeds General Infirmary

Enquiries to: Anne Ward
Direct Line: 0113 392 6788
Our Ref: 02/103
Your Ref:
Date: 12 February 2003

Dear Miss Hind

Re: 02/103: Energy balance and bone mineral density in male and female distance runners

Thank you for your letter of the 3rd February 2003 clarifying that Leeds University will provide indemnity for this study and enclosing an information sheet reflecting this

I am now able to give full approval by chairman's action for this study to proceed. This approval is subject to the conditions of approval set out below.

Yours sincerely

Dr J Puntis
Chairman
Leeds (West) Research Ethics Committee

Encs. Application to LTH Trust MEC

Documents approved:
Protocol Version 2, November 2002
Volunteer information sheet and consent form Version 3, January 2003
National Training Scheme for Bone Densitometry certificates
Recruitment poster
Certificate of the University of Leeds liability insurance dated 30th January 2003
LTH Trust MEC approval, dated 3rd February 2003

List of members present and those who submitted written comments:
Dr J Puntis (Chairman)
Miss C Bedford

Appendix 2

Bone densitometry operator certification

The Bone Densitometry Forum of the National Osteoporosis Society

Ionising Radiation (Medical Exposure) Regulations 2000

Certificate

Name: Ms Karen Hind

This is to certify that the above named attended a course on

Date: 8th May 2002

to fulfil the requirements for adequate theoretical training as an

Operator

laid down in Schedule 2 of the

Ionising Radiation (Medical Exposure) Regulations 2000
as applied to bone densitometry

Signed: David Pye

David Pye PhD, MIPEM
Course Supervisor

Signed: Ignac Fogelman

Professor Ignac Fogelman, MD
On behalf of the NOS Bone Densitometry Forum

National Osteoporosis Society

Operator's Course approved by the Institute of Physics and Engineering in Medicine – Ref. 01/01

Certificate No: O12/01 – 147

breakfree campaign

National Osteoporo Society

Camerton, Bath BA2 0PJ
tel: 01761 471771
fax: 01761 471104
helpline: 01761 472721
website: www.nos.org.uk
e-mail: info@nos.org.uk

22nd July 2002
Our Ref: HMW/JAR

Ms Karen Hind
Phd Student - Academic Medical Physics
University of Leeds
Leeds General Infirmary
Wellcome Wing
Great George Street
Leeds
LS1 3EX

Dear Karen

National Training Scheme for Bone Densitometry – Examination Result

Your results in the recent examination are as follows:-

Core: **Pass**
DXA: **Pass**

The decision of the examination board is final. The National Osteoporosis Society will not enter into discussion with candidates about individual exam performance.

For the final stage of certification, you must submit a portfolio in your chosen technique by 8th November 2002.

Congratulations on your success!

With best wishes.

Yours sincerely

HM Warren

Heidi-Mai Warren
Training Scheme Co-ordinator

Appendix 3

QUESTIONNAIRE: TRAINING, DIET AND BONE HEALTH

NAME.....

ADDRESS.....

.....

.....

.....

TELEPHONE NO: **Home**.....

Mobile.....

EMAIL ADDRESS: .....

D.O.B.....

SEX: **Male** ☐ **Female** ☐

RACE: **Caucasian** ☐ **Asian** ☐ **Afro-Caribbean** ☐

Other (please state) .....

HEIGHT..... **WEIGHT** .....

CURRENT EMPLOYMENT.....

LAST EMPLOYMENT.....

1.0 FAMILY BONE HEALTH

1.1 HAS ANYONE IN YOUR FAMILY EVER BEEN DIAGNOSED WITH OSTEOPOROSIS?

YES ☐ NO ☐

If 'yes' please give details.....
.....
.....

1.2 HAS ANYONE IN YOUR FAMILY EVER SUFFERED A FRACTURE AFTER A MINOR FALL?:

HIP? ☐ SPINE? ☐ WRIST? ☐

Please give details.....
.....

2.0 ATHLETE'S BONE HEALTH

2.1 HAVE YOU EVER HAD A BONE SCAN? IF SO, PLEASE GIVE DETAILS (year, type of scan, reason, result) .....
.....
.....

2.2 HAVE YOU EVER SUFFERED A STRESS FRACTURE (S)? IF SO, PLEASE GIVE DETAILS.....
.....
.....

3.0 MENSTRUAL FUNCTION (female athletes only)

3.1 ARE YOU OR HAVE YOU EVER BEEN PREGNANT?

YES ☐ NO ☐

3.2 DO YOU USE THE ORAL CONTRACEPTIVE PILL OR HRT?

YES ☐ NO ☐

If 'yes' please give details (type, duration, reason)
.....
.....
.....

3.3 AT WHAT AGE DID YOU START YOUR PERIODS?.....

3.4 ARE YOUR PERIODS CURRENTLY REGULAR?

YES ☐ (move to 3.9) NO ☐ (move to 3.5)

3.5 ARE YOUR PERIODS ABSENT?

YES ☐ NO ☐

3.6 HOW LONG HAVE YOUR PERIODS BEEN ABSENT/IRREGULAR (please detail)?
.....
.....
.....

.....
.....

3.7 JUST BEFORE THEY BECAME ABSENT/IRREGULAR HAD YOU: (please tick and give details)

- Suddenly increased your training? ☐
  .....
- Experienced any emotional stress? ☐ (job, family, illness, anxiety)
  .....
- Experienced weight loss? ☐ If so, how much weight was lost?
  .....
- Embarked on a weight loss diet? ☐
  .....

3.8 HAVE YOU ADDRESSED THE ISSUE OF YOUR ABSENT/IRREGULAR PERIODS?

YES ☐ NO ☐

If 'yes', please give details.....
.....
.....
.....

3.9 HAVE YOU PREVIOUSLY EXPERIENCED IRREGULAR PERIODS?

YES ☐ NO ☐

If 'yes' please give details (age, number missed).....
.....
.....
.....
.....

4.0 COMPETITION

4.1 PLEASE STATE YOUR COMPETITION DISTANCE(S).
.....
.....

4.2 ARE YOU CURRENTLY COMPETING?

YES ☐ (move to 4.3) NO ☐ (move to 4.4)

4.3 AT WHAT LEVEL DO YOU COMPETE? (please tick and move to 4.5)

- CLUB ☐
- REGIONAL ☐
- NATIONAL ☐
- INTERNATIONAL ☐
- WORLD ☐

4.4 WHY ARE YOU NOT COMPETING AT PRESENT? (injury, break)
.....
.....

4.5 WHAT IS THE HIGHEST LEVEL YOU HAVE COMPETED AT AND WHEN WAS THIS?.....

.....
.....

4.6 PLEASE STATE YOUR PERSONAL BEST TIMES WHERE APPLICABLE:

- 1500 METRES .....
- 3000 METRES .....
- 5000METRES TRACK/ROAD .....
- 10,000METRES TRACK/ ROAD .....
- 5MILES .....
- 10MILES .....
- HALF MARATHON .....
- MARATHON .....

5.0 TRAINING

5.1 DURING YOUR YEARS AT SCHOOL/COLLEGE, DID YOU REGULARLY PARTICIPATE IN ANY SPORTS (excluding P.E. lessons)?

.....
.....
.....
.....

5.2 AT WHAT AGE DID YOU BEGIN REGULAR RUNNING TRAINING?

.....

5.3 ON AN AVERAGE WEEK, HOW MANY DAYS DO YOU RUN?

.....

5.4 ON AN AVERAGE WEEK, HOW MANY MILES DO YOU RUN?

.....

5.5 DO YOU HAVE A REST DAY/ REST PERIOD? (if so, please give details)

.....
.....

5.6 DO YOU EVER TRAIN TWICE A DAY? (if so, please give details)

.....
.....

5.7 HOW OFTEN DO YOU PARTICIPATE IN: (please state whether sessions are per week, 2 weeks, monthly or yearly)

- EASY RUNNING? .....
- STEADY RUNNING?.....
- THRESHOLD/TEMPO RUNNING?.....
- ROAD INTERVALS?.....
- OFF-ROAD (grass, trail) INTERVALS? .....
- TRACK INTERVALS?.....
- SPEED TRAINING? .....
- WEIGHT TRAINING? .....
- CIRCUIT TRAINING?.....
- CROSS TRAINING?.....

5.8 DO YOU PARTICIPATE IN ANY OTHER SPORTS? (if so, please give details)
.....
.....

5.9 HOW DO YOU PERCEIVE YOUR CURRENT TRAINING REGIMEN IN TERMS OF INTENSITY AND VOLUME?
EASY ☐ OK ☐ DEMANDING ☐
VERY DEMANDING ☐

5.10 DO YOU HAVE ANY BIOMECHANICAL PROBLEMS? IF SO, PLEASE GIVE DETAILS. (ie. over pronate, supinate).....
.....
.....

5.11 IF YOU ANSWERED 'YES' TO THE ABOVE QUESTION, HAVE THESE PROBLEMS BEEN LINKED TO ANY PREVIOUS INJURY, AND HAVE THEY SINCE BEEN ADDRESSED? (i.e. orthototics)
.....
.....

6.0 BODY WEIGHT AND BODY IMAGE

6.1 WHAT ARE THE HIGHEST AND LOWEST BODY WEIGHTS YOU HAVE BEEN OVER THE LAST 5 YEARS?
HIGHEST .....
LOWEST .....

6.2 DO YOU TRY TO KEEP YOUR WEIGHT BELOW A CERTAIN VALUE?
(if so, what is this value?) .....

6.3 HOW WOULD YOU BEST DESCRIBE YOUR BUILD?
VERY THIN ☐ THIN ☐
AVERAGE ☐ ABOVE AVERAGE ☐

6.4 ARE YOU HAPPY WITH YOUR PRESENT BODY WEIGHT?
YES ☐ NO ☐
If 'no', why and what would you change? .....
.....
.....
.....

7.0 NUTRITION

7.1 PLEASE STATE ANY NUTRITIONAL SUPPLEMENTS YOU USE ON A REGULAR BASIS.....
.....

7.2 DO YOU FOLLOW A SPECIFIC SPORTS DIET? (if so, please give details, i.e. high protein, high carbohydrate).....
.....
.....

7.3 DO YOU FOLLOW A WEIGHT LOSS DIET? (if so, please give details, i.e. avoidance of fats, number of calories, duration of restriction) .....

.....
.....

7.4 DO YOU RESTRICT CERTAIN FOODS FROM YOUR DIET? (if so, please state which foods are restricted) .....
.....
.....

7.5 HOW MANY PORTIONS OF THE FOLLOWING DO YOU CONSUME PER DAY OR PER WEEK?

- FRUITS AND VEGETABLES .....
- DAIRY PRODUCTS .....
- BREAD, CEREAL, PASTA, RICE .....
- RED MEAT .....
- WHITE MEAT .....
- SWEET FOOD *(candy, chocolate, desserts)*.....
- TEA/COFFEE .....
- COLA .....
- OTHER FIZZY DRINKS .....
- HIGH ENERGY SPORTS DRINKS .....
- MILK .....

7.6 HAVE YOU EVER HAD OR DO YOU CURRENTLY SUFFER FROM AN EATING DISORDER?

YES ☐ NO ☐

7.7 IF YOU ANSWERED 'YES' TO THE ABOVE, HOW LONG HAVE YOU SUFFERED AN EATING DISORDER FOR, AND WAS IT (HAS IT BEEN) CLINICALLY DIAGNOSED?.....
.....
.....
.....

8.0 MEDICATION

8.1 ARE YOU CURRENTLY TAKING ANY MEDICATION? (If so please give details).
.....
.....

8.2 IF YOU HAVE ASTHMA, DO YOU USE ANY OF THE FOLLOWING INHALERS?

- AEROBEC AUTO-HALER ☐
- ASMABEC CLIC-HALER ☐
- BECLOMETHASONE ☐
- BECODISK ☐
- FLIXOTIDE ☐
- PULMICORT TURBO-HALER ☐
- QUAR BECONASE ☐
- NASCORT ☐
- NASANEX ☐
- NASOBEX ☐
- RHINCORT ☐
- SYNTARIS ☐
- ZANIVENT ☐

Appendix 4

(i) One day example of the dietary record.

Monday .....(Date)

Time	**Food/drinks consumed**	**Brand name** (i.e. McVites, Heinz, etc) **& nutritional infor if known** (per 100g)	**Weight/ measure** (grams, ml, pints)…	**Or..estimated portion size** (i.e. average, large, sml glass, or i.e. 2 bananas)

Please continue on the back of this sheet if required.

(ii) One day example of the training/exercise record.

Monday .....(Date)

Description of training	**Location** (i.e. road, track, grass, trail)	**Total duration** (mins/hours) and mileage	**Intensity** (mile/repetition pace, heart rate)	**Perceived intensity** (good, tired, ok)
AM *(please state the time of training)* **Details**				
PM (Please state the time of training) **Details**				