Exercise and bone mass in mature premenopausal women

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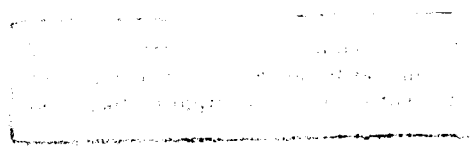

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DECLARATION

I, Lisa Kim Micklesfield, declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part thereof has been, is being, or is to be submitted for any other degree at this or any other University.

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SUMMARY

It is now well established that amenorrheic athletes have low vertebral bone density (Nilson and Westlin, 1971; Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Drinkwater et al., 1986; Nelson et al., 1986; Myburgh et al., 1993). Bone mineral density (BMD) in this population may be influenced by a variety of factors including current menstrual status, menstrual history, body mass, functional loading and calcium balance. Complex interrelationships between these variables may exist.

The long-term consequences of menstrual irregularities are still unknown, as most studies to determine the effect of amenorrhea on BMD in women athletes have been performed on young subjects (Drinkwater et al., 1984; Marcus et al., 1985; Drinkwater et al., 1986; Drinkwater et al., 1990). Drinkwater et al. (1986) and Lindberg et al. (1987) have also shown that the osteopenia induced by amenorrhea is, at least partially, reversible in athletes who have regained menses. The question is whether the resumption of normal menses in these women is sufficient to counterbalance all of the negative effect of their oligo/amenorrhea. A study by Marcus et al. (1985) found that bone mineral density of the lumbar spine in amenorrheic runners was lower than that in cyclic women, as well as sedentary, age-matched controls. Lack of regular exercise may therefore be less detrimental to lumbar spine BMD than a history of menstrual irregularity associated with running. However it is also important to consider the age at which this menstrual irregularity occurs as well as the age when bone mass is determined. There is still controversy surrounding the time at which peak bone mass is achieved (Buchanan et al., 1988; Bonjour et al., 1991; Szejnfeld et al., 1993). Lindsay et al. (1993) indicate that bone mass continues to increase up until the fourth decade. However, Recker et al. (1992) indicated that gain in bone mass ends earlier in the third decade, whereas Bonjour et al. (1991) have suggested that most bone is gained by the end of the second decade.

In study 1, we measured bone mineral density (BMD) in 25 premenopausal ultramarathon (56 km) runners aged 29 to 39 years and related risk factors for decreased BMD with actual BMD. Fifteen runners who had never had oligo/amenorrhea (R) were compared with 10 oligo-amenorrheic runners (OA): 4 oligomenorrheic, 2 amenorrheic and 4 with prior oligo/amenorrhea. Menstrual, dietary and training data were obtained. BMD of the lumbar spine (LS) and proximal femur (F) were measured by dual energy x-ray densitometry. Both groups had similar body mass (58 ±8 vs. 57 ±8 kg), running and dietary histories. F BMD was not different ($p=0.07$) and correlated only with BMI ($p<0.05$; $r=0.43$). LS BMD was lower in OA (0.946 ±0.098 $g.cm^{-2}$) than R (1.088 ±0.069 $g.cm^{-2}$; $p<0.001$). Menstrual History Index (MHI), (estimated $periods.yr^{-1}$ since age 13), was higher in R (11.6 ±0.6) than OA (9.4 ±2.1; $p<0.01$). LS BMD correlated with MHI ($p<0.0005$; $r=0.67$) and years oligomenorrheic ($p<0.01$; $r=-0.58$) but not years amenorrheic, parity, breastfeeding, diet or training. Lumbar spine BMD was significantly higher in R (1.088 ±0.069 $g.cm^{-2}$; $p<0.001$) than in subjects with a history of oligomenorrhea, but no history of amenorrhea (HO) (0.953 ± 0.024 $g.cm^{-2}$; $p<0.005$), as well as subjects who had a history of either amenorrhea or both oligomenorrhea and amenorrhea (HOA) (0.942 ± 0.131 $g.cm^{-2}$; $p<0.005$). The two subdivisions of the OA group were not significantly different to each other for lumbar spine BMD.

In the addendum to study 1, we completed the same tests on four additional subjects with a history of oligo/amenorrhea in order to provide a wider distribution of subjects in the OA group and to have a more even number of subjects with regular menses (R=15) and with current or previous menstrual irregularities (OA=14). There was still no difference in age (R:35.5 ± 3.2 vs OA: 36.2 ± 5.1 yr), body mass (R:58.3 ± 7.9 vs OA:56.6 ± 6.6 kg) and BMI (R:21.4 ± 2.4 vs OA: 21.0 ± 1.97 $kg.m^{-2}$) between the two groups. The results of the addendum to study 1 were able to provide further evidence of the significant relationship between overall MHI and LS BMD ($p=0.0001$; $r=0.67$). Lumbar spine BMD also correlated significantly with total number of years of amenorrhea ($p<0.01$, $r=-0.53$), total number of years of oligomenorrhea ($p=0.01$, $r=-0.44$), and total number of years of regular menstruation

($p<0.01$, $r=0.53$). However in the subsample of women with a history of menstrual irregularity, no particular menstrual variable correlated with lumbar spine BMD. LS BMD was lower in OA (0.951 ±0.085 $g.cm^{-2}$) than R (1.088 ±0.069 $g.cm^{-2}$; $p<0.001$), however there was no difference between the two groups for the left proximal femur ($p=0.08$). However, after division of the OA group into runners who had resumed regular menses (CR, n=6) and runners who were currently oligo/amenorrheic (COA, n=8), lumbar spine BMD of the regularly menstruating runners (R) was still significantly higher than *both* the other two groups (R: 1.088 ± 0.069 vs CR: 0.985 ± 0.110 $g.cm^{-2}$ vs COA: 0.926 ± 0.060 $g.cm^{-2}$; $p=0.0001$), and although the two groups were not different to each other.

In study 2, we repeated the tests on those runners who had previously participated in study 1 and who were still running, as well as on a control group of sedentary women. Twelve runners (R) and 8 sedentary controls (SC) who had always had regular menstrual periods were compared to 9 runners who had a history of oligo/amenorrhea (OA): 1 who was currently amenorrheic and 1 who was currently oligomenorrheic. Subjects ranged in age from 29-46 years. The oligo/amenorrheic runners were significantly younger than the sedentary control group (OA: 35.9 ± 4.4 vs SC: 41.6 ± 3.1 yr; $p=0.01$) but did not differ in age from the regularly menstruating group (R: 39.0 ± 3.8 yr). The sedentary control group had a higher body mass (SC: 63.3 ± 8.6 vs R: 56.3 ± 7.9; or OA: 54.4 ± 3.8 kg; $p<0.05$), BMI (SC: 23.9 ± 3.4 vs R: 20.4 ± 1.8; or OA: 20.0 ± 1.5 $kg.m^{-2}$; $p<0.05$), endomorphic component (SC: 6.3 ± 1.3 vs R: 4.0 ± 0.9; or OA: 3.5 ± 1.1; $p<0.001$) and fat mass (SC: 21.8 ± 4.8 vs R: 15.8 ± 3.2; or OA: 13.7 ± 2.7 kg; $p<0.001$) than the other two groups, as well as a lower measure of ectomorphy (SC: 1.7 ± 1.2 vs R: 3.2 ± 0.9; or OA: 3.2 ± 0.9; $p<0.01$). We therefore covaried for age and body mass when determining whether or not there were differences in BMD between the groups. LS BMD of OA was significantly lower than the LS BMD of the R and SC groups (OA: 0.948 ± 0.070 vs R: 1.043 ± 0.100; or SC: 1.094 ± 0.077 $g.cm^{-2}$; $p<0.05$), however the three groups did not differ for BMD of the proximal femur (total, neck, trochanter, and intertrochanter). There was no difference in the biochemical markers, osteocalcin and deoxypyridinoline/creatinine (Dpd/Creat), between the three groups. Lumbar

spine BMD correlated significantly with overall MHI ($p=0.001$, $r=0.45$), total number of years of amenorrhea ($p=0.01$, $r=-0.45$), and the total number of years of oligomenorrhea ($p<0.05$, $r=-0.38$). No dietary or training variables correlated with lumbar spine BMD for the whole sample of women. No other BMD parameters, including the total proximal femur, neck of the femur, greater trochanter, and the intertrochanteric space, correlated with any body composition, menstrual, training or dietary variables. Lumbar spine BMD for the sample of runners only, correlated significantly with total number of years of amenorrhea ($p<0.05$; $r=-0.44$) and estimated number of periods per year from 21-30 years ($p<0.05$; $r=0.44$). Lumbar spine BMD, as well as BMD of the proximal femur and the neck of the femur, for the sub-group of runners with current and/or prior oligo/amenorrhea was not significantly correlated with any of the menstrual variables. Change in BMD of the lumbar spine and the proximal femur was not physiologically significant or different between the groups. Change in lumbar spine BMD since the last study (three years previously), correlated significantly with change in mass since the last study ($p=0.05$; $r=0.36$), but was not significantly correlated with change in MHI, estimated portions of dairy products per week over the past year, current calcium intake, or total energy intake.

In conclusion, in mature women distance runners low LS BMD is related to a history of oligo/amenorrhea regardless of resumption of regular menstrual cycles in some subjects. Not only amenorrhea, but also prolonged oligomenorrhea may negatively influence peak adult bone mass. Lack of regular exercise is less detrimental to lumbar spine BMD than any history of menstrual irregularity associated with running. BMD may not increase substantially between 35-45 years of age regardless of menstrual status and menstrual history.

CHAPTER 1

Exercise and bone mass: a literature review

A. (i) BONE: HISTOLOGY & PHYSIOLOGY

There are 3 phases of bone development which all have important functions in maintaining the integrity of bone in order to provide a sturdy, but flexible, framework for the body. The first is bone growth which determines bone size, secondly modeling determines the shape of the bone, and thirdly remodeling which includes the ongoing processes of bone formation and resorption. The shape and size of the bone is maintained throughout the remodeling process which has two functions. It is required to maintain mineral homeostasis, as well as to prevent the accumulation of microfractures or fatigue damage (Frost, 1983). An annual turnover rate of approximately 15-30% is achieved by remodeling which is carried out by basic multicellular units (BMU) (Frost, 1973) .

The BMU consists of different cell types which each have a specific function but which work together to form lamellar bone. There are 4 steps involved in the bone remodeling cycle, (i) activation, the conversion of a part of the bone surface from a passive state to active remodeling, (ii) resorption, the eroding of the bone surface by osteoclasts over a 1-3 week period, (iii) reversal, occurs for a period of 1-2 weeks before, (iv) formation, which is the function of osteoblasts and is a two step process of bone matrix synthesis and subsequent mineralization (Frost, 1973).

Bone is a specialised connective tissue with important structural and functional capabilities (Baron, 1990). There are two types of bone which are composed of the same cells and the same matrix elements; however due to certain structural differences, they

have different functions. The external part of bones is formed by a thick and dense layer of calcified tissue called cortical, or compact, bone which encloses the medullary cavity where the bone marrow is found. Toward the epiphysis, the width of the cortical bone becomes progressively thinner and the internal space is filled with a network of thin, calcified trabeculae, the trabecular, or spongy, bone. The skeletal system consists of approximately 20% trabecular bone, which includes bones such as the vertebrae, while the appendicular skeleton consists primarily of cortical bone. An important structural difference between the two types of bone is that 80-90% of the volume of the cortical bone is calcified, while only 15-25% of the trabecular bone is calcified with the remaining volume occupied by the marrow. These structural differences have important functional repercussions. Cortical bone has a more mechanical and protective function, while trabecular bone has a more metabolic function due to the larger bone surface area to bone volume ratio. This results in trabecular bone being more sensitive to its environment, such as mechanical loading, serum calcium, cellular messengers, and in particular the hormonal status, including parathyroid hormone (PTH), Vitamin D and estrogen. This may explain the results of a study by Marcus et al. (1985) in which trabecular bone density was higher in a group of regularly menstruating athletes compared to a group of equivalently trained women with sustained amenorrhea, whereas cortical bone mass did not appear to be affected by the loss of menses and the resultant reduction in circulating estrogen. At any one time, most of the bone is in a quiescent state, with approximately 20% of trabecular bone and 5% of cortical bone in an active remodeling state (Frost, 1987).

Bone balance is the nett result of bone formation and resorption. During childhood, adolescence and early adulthood until peak adult bone mass is attained, a positive balance is generally maintained. However, a sustained negative balance, which normally occurs with aging, when resorption exceeds formation, will result in a progressive loss of bone.

Bone balance responds to mechanical and nutritional factors, such as exercise and diet, as well as biochemical and neurological factors (Dalsky, 1990). When estrogen is deficient, such as after menopause, the rate of bone loss is accelerated over and above that due to the normal bone loss which occurs with aging. Regular mechanical loading in the form of bending strains, within the elastic limits of deformation, should theoretically ensure that an appropriate level of bone mass is maintained by stimulating a higher rate of formation relative to resorption as has been shown in animal models (Raab-Cullen et al., 1994). Bone is a dynamic organ which can also alter its shape and size in order to adjust to the demands and level of strain placed on it. However, if environmental conditions are not favourable, such as during estrogen deficiency or calcium deficiency, the adaptation of the bone to the mechanical strain of weight-bearing may be negated. The most striking example of this is adolescent anorexia nervosa (Bachrach et al., 1990). Thus, although physical activity may be beneficial, in the presence of menstrual irregularity or a severely low energy intake, its benefits may not be sufficient to prevent a negative calcium balance and a nett resultant bone loss (Dalsky, 1990). In the same way, during prolonged intervals when mechanical strain is removed altogether, such as during bed rest, the bone readjusts to the lower level of strain by increasing the remodeling activity with an increase in resorption and no simultaneous increase in bone formation (Frost, 1987). Bone loss continues until a specific lowest possible baseline is reached which is genetically determined and which is preserved even under conditions promoting severe bone loss (Lanyon, 1987).

(ii) BONE MARKERS AND BIOCHEMISTRY

The mechanism of bone loss due to hypoestrogenism is an increased bone resorption rate relative to formation rate (Cann et al., 1984; Lutter, 1983). It is generally accepted that

hypoestrogenism causes a disturbance of calcium balance which is tightly coupled to parathyroid hormone (PTH) secretion (Heaney and Recker, 1982b). However, it has also more recently been shown that bone may have estrogen receptors which may therefore act directly on bone balance (Braidman et al., 1995). Hypoestrogenism can be prevented, or at least slowed down, by estrogen replacement therapy in postmenopausal women (Horsman et al., 1983; Lindsay, 1990). Bone turnover rate can be indirectly determined by biochemical markers of bone formation (eg. serum osteocalcin) and bone resorption (eg. urinary excretion of deoxypyridinoline) (Seibel et al., 1993), or directly by histological examination of iliac crest biopsies which provide information regarding the relative amounts of mineralised bone and unmineralised osteoid, active resorption and formation surfaces and the quantity of bone relative to the quantity of porous space in the specimen (Melsen and Mosekilde, 1981). In some countries iliac crest biopsies are used to assist in the physician's decision on appropriate treatment, however a comparison between the non-invasive and invasive methods of assessing bone formation and resorption (Delmas et al., 1991) found a poor comparison between the level of urinary collagen cross-links and the histologic parameters of resorption. This discrepancy may indicate a limitation of histomorphometric measurements: a small bone biopsy from a defined site may not represent other skeletal sites. Alternatively, a relative weakness of biochemical markers of bone turnover rate may be that they represent both cortical and trabecular bone turnover rather than specifically trabecular bone turnover which is of most clinical significance. In young females with menstrual irregularities neither direct, or indirect, markers of bone metabolism are routinely measured. It is also unknown how bone turnover rates may influence the recovery of bone mass following athletic amenorrhea.

Serum osteocalcin, a biochemical marker of osteoblastic activity, is a small noncollagenous protein which is produced during bone formation, and is thus a useful

marker of bone formation rate (Gundberg, 1990). Although osteocalcin, also called bone gla protein (BGP), is found mainly in the skeleton, a small amount is present in the blood, and this may be due to its release from the extracellular matrix of bone or as a result of new protein synthesis. Specific disease states that are characterised by high bone turnover (eg. hyperparathyroidism), will result in abnormal concentrations of osteocalcin in the blood, which result from changes in either the rate of synthesis, or the rate of degradation, of the protein in bone tissue. Serum osteocalcin concentrations show large diurnal variation, with levels declining in the morning, reaching the lowest point in the afternoon, and then rising again to reach a peak at approximately 4.00 am (Gundberg et al., 1985). Serum osteocalcin concentrations are higher in infants and children than in adults with an increase occurring at puberty that corresponds to the adolescent growth spurt (Gundberg, 1990). Serum osteocalcin is significantly increased after menopause in women (Johansen et al., 1988) , however there does not appear to be any change with age in men (Gundberg, 1990). In premenopausal women if samples are taken at the same time of the day, there is no change in the biochemical markers of bone formation during the menstrual cycle (Schlemmer et al., 1993).

In young adults, serum osteocalcin varies between 2 and 12 $ng.ml^{-1}$ (Gundberg, 1990). Within this range, levels of osteocalcin may be influenced by several factors. Research by Kelly et al. (1991) on 70 pairs of postmenopausal twins found that serum osteocalcin levels are genetically influenced which would suggest that there is some genetic regulation of bone turnover rate. Surprisingly, a full 80% of the variance in serum osteocalcin in their study could be explained by genetic factors. These data have not been confirmed in young adults. However, other research has investigated the differences in basal and postexercise osteocalcin levels in athletic and non-athletic males, to determine the effect of short-term, moderate-intensity exercise on bone metabolism (Nishiyama et al., 1988). This study found that serum osteocalcin levels before a bout of exercise were

higher in the athletic group than the non-athletic group, and that the response to exercise differed between the groups. Osteocalcin levels remained the same immediately post-exercise in the athletic group and increased 60 minutes later, but in the non-athletic group the serum osteocalcin was significantly increased immediately post-exercise and reverted to pre-exercise levels 60 minutes later. However, the physiological significance of this difference in response to exercise is unknown.

More recently it has been discovered that pyridinoline and deoxypyridinoline are good markers of bone resorption (Delmas, 1993). Pyridinoline (Pyr) and deoxypyridinoline (D-Pyr) are cross-links which support the collagen chains within the extracellular matrix. Although pyridinoline cross-links are present in the matrix of bone and cartilage, they are also present in other connective tissue. However deoxypyridinoline cross-links occur in substantial amounts *only* in bone matrix (the ratio of Pyr/D-Pyr being 2:3). Urinary Pyr and D-Pyr are released in response to bone degradation by osteoclasts and urinary levels are increased by 50-100% during menopause, however they return to premenopausal levels within 6 months of hormone replacement therapy (Uebelhart et al., 1991). Total urinary Pyr and D-Pyr excretion also undergoes a circadian rhythm, with a gradual decline starting at 8:00 am, reaching a minimum at 5:00 pm-8:00 pm, and then slowly rising to reach a peak during the early morning (5:00 am-8:00 am). The decrease between 8:00 and 11:00 am is between 25-35% (Schlemmer et al., 1992). The similarity in diurnal variations of the pyridinium cross-links as well as osteocalcin, suggests that there is a nocturnal increase in both bone resorption and formation rates. No statistically significant changes during the menstrual cycle have been observed in the urinary biochemical markers of bone resorption (Schlemmer et al., 1993).

B. PHYSIOLOGICAL CHANGES IN BMD WITH AGE

(i) Attainment of peak bone mass

It is accepted that bone loss in the general population occurs with increasing age. However, there is controversy about when peak bone mass is achieved and when bone loss begins (Lindsay et al., 1993; Szejnfeld et al., 1993; Buchanan et al., 1988). It is important to note that there may also be a plateau in bone mass after peak bone mass is achieved and before bone loss begins. Several studies have found that increases in skeletal bone mass appear to dramatically slow down at the levels of both lumbar spine and the femoral neck at 15-16 yr of age in female adolescents (Bonjour et al., 1991; Theintz et al., 1992), and stress the importance of bone acquisition during adolescence (Katzman et al., 1991). Results from other data that support this hypothesis showed no significant age-related changes in bone density between 20 and 40 years of age (Armamento-Villareal et al., 1992; Lloyd et al., 1988), and suggest that a peak is reached earlier than age 35 as previously believed (Rodin et al., 1990). Nevertheless, longitudinal data should provide the best estimates of the age at which peak bone mass is achieved and Recker et al. (1992) have shown in 156 young women followed for up to 5 years that it occurs between 28 .3 and 29.5 years of age. The length of time that peak bone mass is maintained before bone loss begins is also subject to controversy. Some studies suggest that bone loss occurs in premenopausal women prior to the disruptions in ovarian function indicating the onset of menopause (Lindsay et al., 1993). However, other studies show no statistically significant decline in bone density with age during the premenopausal period (Szejnfeld et al., 1993). It is however important to bear in mind the limitations of bone densitometry in making the conclusion that bone mineral density is maintained up until a certain age, when in fact bone loss may occur in trabecular bone, but it is not being detected by densitometry which necessarily evaluates both trabecular

and cortical bone at the same time. A study by Recker et al. (1992b) on a group of 75 healthy women nearing menopause (all at least 46 years) found no significant changes (>1%/year) in lumbar spine BMD, and not much more for the forearm or total skeleton (%/year). The conclusion is that bone loss was not detectable in this longitudinal study in premenopausal women.

(ii) Definitions of osteoporosis

At any age, and at any particular site, a reduction in bone tissue is a result of an imbalance between bone formation and bone resorption rates, the two physiological processes that are necessary for bone remodelling. Osteoporosis can be defined as a condition of general skeletal fragility which increases the risk of non-traumatic fractures due to a reduction in bone mass and the disruption of normal skeletal microarchitecture. The risk of osteoporosis increases with age, particularly in females after the onset of menopause. However, osteopenia does occur in adolescents and may be a result of genetic abnormalities, immobilization, endocrine reproductive abnormalities and nutrition. The World Health Organisation has determined a bone density criterion for osteoporosis: "BMD below 2.5 SD from the average value predicted for a 25 year old Caucasian woman" (WHO study group, 1994).

(iii) Prevalence of osteoporosis

The prevalence of osteoporosis is very high. It is thought to affect 15-20 million individuals aged > 45 years in the United States (Matkovic et al., 1990). It has been estimated that by the age of 90 years as many as 32% of women and 17% of men will have sustained an osteoporotic hip fracture, and between 12 and 20% of this group will die of related complications (Marcus, 1989).

(iv) Prevention of osteoporosis

Bone loss occurs with age and places each individual, but particularly women, at an increased risk of certain fractures, such as hip, spine and wrist fractures. Bone loss is not apparent on routine x-rays until 30-50% of bone mass is lost. One study on 521 female volunteers over a 15 year span has shown that a single bone mass measurement can predict the probability of hip fracture (Hui et al., 1989). A later study by the same authors (Slemenda et al., 1990) went as far as saying that identifying risk factors for osteoporosis is of limited value in identifying women with low bone mass around the time of menopause and therefore actual measurement of bone mass is necessary. However, bone mass measurement is not a diagnostic test for fracture, rather reduced bone mass over a period of time should be considered a risk factor for future fractures (Johnston and Melton, 1990).

(v) Cortical vs trabecular bone loss

Postmenopausal bone loss amounts to approximately 1-2% per year, which appears to decrease at about 5-10 years after menopause (Eisman et al., 1991). Women with spinal osteoporosis lose similar amounts of cortical bone, but more trabecular bone, than women without spinal osteoporosis (Riggs et al., 1981). If bone loss occurs prior to menopause, it is thought to be primarily trabecular bone which is lost. Results of a cross-sectional study by Buchanan et al. (1988) found trabecular bone density to be significantly higher in the second (178 ± 8 mg/ml) and third (171 ± 6 mg/ml) decade than the fourth (158 ± 4 mg/ml) and fifth (140 ± 12 mg/ml) decades, while there was no difference in cortical bone between the third (0.711 ± 0.021 mg/ml), fourth (0.721 ± 0.012 mg/ml) and fifth (0.736 ± 0.012 mg/ml) decades. Similar results by Riggs et al. (1981) comparing 187

normal men and women (20-89 years), and 76 women and 9 men with vertebral fractures due to osteoporosis, concluded that in women cortical bone loss did not begin until age 50, and is accelerated from ages 51-66 years. In women cortical bone loss from the appendicular skeleton may be more closely related to estrogen deficiency resulting from menopause, than is trabecular bone loss from the axial skeleton which begins earlier. The primary mechanism for trabecular bone loss is less clear, and may include a decline in physical activity.

The influence of hormonal factors in relation to menopause cannot be ignored, and it is thought that menopause may accelerate the process of bone loss due to a deficiency of estrogen (Armamento-Villareal et al., 1992) even if bone is already in a negative bone balance prior to menopause. Thus, age at menopause is crucial in determining the onset of rapid bone loss (Lindquist et al., 1981). Lindquist et al. (1981) compared 5 groups of women from 3 age strata,(mean ages: 46, 54 and 62 years), with different menopausal status and found that bone mineral content of the third lumbar vertebra was higher in premenopausal, or recently postmenopausal, 54 year-old women (3.85 ± 0.62 g.cm^{-1}) than in women of the same age who had been postmenopausal for a longer time (3.46 ± 0.49 g.cm^{-1}). A similar trend was found for women aged 62 years. They thus concluded that there is a relationship between early menopause and accelerated trabecular bone loss.

(vi) Quantity of bone lost

It has been hypothesised that the cause of bone loss in most cases of postmenopausal osteoporosis is an increased bone resorption rate in the presence of a normal rate of formation. There are two hypotheses to explain why the incidence of osteoporosis is higher in women than in men. Riggs et al. (1981) hypothesise that women lose more bone. The cumulative loss of vertebral BMD from early adulthood to late old-age may be

as high as 47% in women and 14% in men (Riggs et al., 1981). Lindquist et al. (1981) propose that women have a lower bone mineral density than men because the female skeleton contains less bone than the male skeleton at skeletal maturity and therefore women start to lose bone from a lower initial bone density. This, along with the hormonal influence of menopause, will explain a lower BMD that occurs in older women. On the basis of data from Seeman et al. (1989) on a group of premenopausal daughters of women with and without postmenopausal osteoporosis, a similar conclusion can be made. This study concluded that the low bone mass found in patients with osteoporosis is not entirely due to excessive bone loss but rather to the attainment of a low peak bone mass. This is why it is important to ensure that the peak adult bone density attained is high, as a low vertebral BMD in young adulthood is an independent risk factor for a low vertebral BMD in later life. The identification of factors that influence the development of peak bone mass are vital in order to develop the most appropriate methods for maximising bone mass in the developmental period (Armamento-Villareal et al., 1992).

C. FACTORS THAT DETERMINE PEAK BONE FORMATION

From the previous discussion we conclude that peak bone mass, along with the age at onset of bone loss and the rate of bone loss contribute to the development of osteoporosis. A study by Riggs et al. (1981) supports the hypothesis that age related bone loss is fairly constant in most women and that a relatively low vertebral BMD in young adulthood is an *independent* risk factor for a relatively low vertebral BMD in later life. Therefore low peak bone mass is a major cause of osteoporotic fractures and consequently potential immense medical expense.

A combination of a low peak adult bone mass and an increased rate of bone loss at an early age could have disastrous effects, as the bone density at the fracture threshold will

be reached early and spontaneous fractures are likely to occur. To prevent the onset of osteoporosis and the resultant osteoporotic fractures, a high peak bone mass and the maintenance of this bone mass must be achieved. Much research has focused on the maintenance of bone mass after menopause, whereas less attention has been paid to gaining bone mass in the premenopausal period. It is now clear however that peak bone mass attained is very important and can be influenced by various factors. Some research has suggested that the average premenopausal female may be able to increase BMD in her lumbar spine by as much as 10-15% during the years preceding menopause (Kanders et al., 1988). This was achieved by a consistent increase in calcium intake of 200-400 mg/d (previous average calcium intake was about 600 mg/d) together with an increase in physical activity resulting in an increase in average energy expenditure of 400 $kcal.day^{-1}$. A study by Riggs et al. (1981) support the hypothesis that age related bone loss is constant in all women and that a relatively low vertebral BMD in young adulthood is an independent risk factor for a relatively low vertebral BMD in later life.

There are a variety of factors that influence the quantity of peak bone mass attained, the age at which bone loss begins, and the rate of bone loss. Some of these factors can be controlled by lifestyle, however factors such as age, gender and genetics are out of the individual's control. A large genetic component may determine the maximum bone mass achievable, however several nutritional and lifestyle factors will determine whether an individual is able to achieve this level or not. These factors include, amongst others, current menstrual status and menstrual history, habitual levels and type of physical activity, dietary calcium and Vitamin D intake, lactation and parity, and oral contraceptive use. Thus, it has been identified that detailed longitudinal studies of menstrual and other factors controlling bone mass in premenopausal women are required (Lindsay et al., 1993).

C.i **Genetic factors**

Genetic factors play a large role in determining achievable peak bone mass and the onset and rate of bone loss. Some data suggest that genetic potential has a relatively larger influence than environmental factors in the development of premenopausal bone mass. One study found that premenopausal women with a low bone mass had a positive maternal family history of osteoporosis, while neither dietary habits nor any other environmental factors were statistically significantly influencing bone mass (Armamento-Villareal et al., 1992). The researchers did however accept the limitations of a cross-sectional study in making these conclusions. Other data support the hypothesis that peak bone size, bone mass, and bone density in young women are strongly influenced by genetic information not only from the mothers but from fathers as well (Matkovic et al., 1990), and support the hypothesis that heredity may also be one of the main determinants of osteoporosis in elderly people. This study found a resemblance between the mean bone status of the parents and that of their daughters for bone size, bone mass, and bone density of the appendicular and axial skeleton. At the beginning (age: 14 years) and on conclusion (age: 16 years) of the trial, the bone variables of the daughters were expressed as percentages of the same variables of their mothers and it was concluded that by the age of 16 years, daughters had accumulated 90-97% of the bone mass of their premenopausal mothers. Another study reported a significantly lower lumbar spine BMD in the premenopausal daughters of osteoporotic mothers compared to other women of the same age (Seeman et al., 1989).

Twin studies may however be more effective in determining the influence of genetic factors since in family resemblance studies similarities may be attributed to physical activity and nutrition. A study by Pocock et al. (1987) in which they measured lumbar spine and proximal femur BMD and forearm bone mineral content in 38 monozygotic

and 27 dizygotic twins, found spine BMD to be more significantly correlated in the monozygotic twins than in dizygotic twins. A similar, but less significant, relationship was found in the proximal femur and in the forearm of premenopausal twins, suggesting that the bone mass of these two sites may be determined more by environmental factors than the lumbar spine.

Another genetic factor influencing bone mass appears to be a polymorphism in the vitamin D receptor. Ferrari et al. (1995) found that the rate of change in lumbar spine BMD was greater in 78% of subjects with homozygous alleles (*BB*), but in only 41% and 31% of subjects with the *Bb* and *bb* alleles respectively. The authors concluded that variability in BMD response to calcium intake is partly due to genetic factors.

Thus, as has previously been suggested (Eisman et al., 1991), it may be the interaction between environmental and lifestyle factors which allows full expression of bone mineral density.

C.ii **Training/physical activity**

It is unknown whether bone loss in athletic women follows a different trend relative to the general population. Studies with relatively small numbers of subjects, suggest that physical activity may prevent age-related premenopausal bone loss (Brewer et al., 1983; Davee et al., 1990). Indeed "age-related premenopausal" may be a misnomer and premenopausal bone loss may be simply due to age-related decline in physical activity. Brewer et al. (1983) concluded from their study comparing marathon runners to sedentary controls that bone mineralisation may be enhanced in premenopausal middle-aged (30-49 years of age) women who participate in moderate to intense exercise, with an inferred consequence being a lower risk of fracture later in life. Several studies have found that

athletes involved in weight-bearing activities, such as running, volleyball, basketball and gymnastics, have higher bone densities than non-athletes (Brewer et al., 1983; Marcus et al., 1985, Risser et al., 1990, Taaffe et al., 1995). A study by Bassey and Ramsdale, (1994), divided a group of healthy premenopausal women (n=27) into a test group who performed high-impact exercise and a control group who were on a programme of low-impact exercise. Results after 6 months on the programmes showed a significant increase in trochanteric bone density in the test group, and this was significantly different to the control group. For the next 6 months the two groups were crossed over, and the previous control group showed a significant increase in trochanteric density with high impact exercise, while the previous test group maintained their improvement relative to baseline. However, athletes who participate in activities that do not involve vertical weight-bearing activity, such as swimming, may not be protected from low bone mineral density of the lumbar spine (Risser et al., 1990; Taaffe et al., 1995). A contentious issue is still whether women with relatively higher BMD choose to participate in regular physical activity or whether their higher BMD is a result of their exercise habits. Although Smith and Gilligan (1987) have concluded from their review that weight-bearing activities such as walking, running, and racket sports seem to be more effective in maintaining integrity of the neck of the femur and the spine than nonweight-bearing activities such as bicycling and swimming, only longitudinal studies using exercise as intervention will be able to definitely solve this issue. Even then, variables such as previous habitual exercise patterns and starting level of BMD may affect the outcome of intervention studies.

The effect of physical activity on bone mass is not only relevant to the athletic population. Rather the effects of the normal range of activity most likely to be undertaken in daily living and leisure time is possibly even more important, since the athletic population is relatively small. A study by Kanders et al. (1988) on a sample of non-athletic women with a stable life-style showed a highly significant correlation

between BMD in the lumbar spine and overall level of physical activity. This relationship suggested that a slight increase in daily activity such as a 4 mile walk, could result in an increase of as much as 5% in vertebral BMD by the time of onset of menopausal bone loss. However, a limitation of the Kanders et al., (1988) study was that total energy expenditure was estimated without taking into account the effect of body weight, and when energy expenditure ($kcal.day^{-1}$) is divided by body weight there is no longer a significant regression between average energy expenditure and vertebral bone mineral density. A similar study by Halioua and Anderson (1989) investigated lifetime physical activity habits of a group of healthy premenopausal women (20-50 year; n=181) and found significant relationships between lifetime physical activity habits and BMD and BMC of the distal radius and the mid-radius. They concluded that good exercise habits during the formative years of adolescence and early adulthood should maximise the genetic potential for bone mass of the individual.

Although these cross-sectional studies can lay scientifically sound foundations for hypotheses, controlled intervention studies are required to substantiate them. However, most intervention studies to date have been done on postmenopausal women. For example Dalsky et al. (1988) found that in a group of healthy, sedentary, postmenopausal women who completed a 9-month program of weight-bearing exercise, BMC of the lumbar spine increased significantly. The increase in lumbar spine BMC was maintained with a long term program. However, after a 13 month period of detraining, mean BMC for the group had returned to just above baseline. Although these data were collected in postmenopausal women, they suggest that the skeleton is indeed responsive to physical activity, but that the level of activity should be maintained or the benefit will be lost. Snow-Harter et al. (1992) conducted an 8-month intervention trial on a group of 31 healthy, premenopausal women (mean age 20 years) who were randomly assigned to a control group, or to progressive training in jogging or weight-training. The study found a

significant increase in lumbar spine BMD in the runners and weight-trainers, in comparison to the control subjects in whom BMD did not change. There was no significant difference in lumbar spine BMD between the 2 exercising groups. Similar results were obtained in a 2-year intervention trial by Friedlander et al. (1995). One hundred and twenty seven women (ages of 20-35 years) were randomly assigned to either an exercise program that contained both aerobics and weight training, or to a stretching program. The results of this study showed a significant difference in BMD between the two groups, as well as a significant gain in BMD between the two groups. Results of a prospective study by Rockwell et al. (1990) concluded however that short term weight training may result in a decrease of vertebral bone mass in premenopausal women. This study compared the lumbar spine bone mass of 10 women (36.2 ± 1.3 years of age) on a weight training program with 7 sedentary women (40.4 ± 1.6 years of age). Although there was an increase in muscle strength at the end of the 9 month program, the lumbar spine bone density in the exercising women was significantly decreased.

If weight-bearing activity is such an important determinant of BMD it seems logical that body weight may also play a role in the attainment of peak bone mass, either as a function of a larger skeleton (bone mass) or in conjunction with weight-bearing exercise (bone density). Different studies have noted a positive relationship between several parameters of body stature and bone mineralisation (Brewer et al., 1983; Mazess and Barden, 1991; Warren et al., 1991; Jonnavithula et al., 1993; Young et al., 1994;), indicating that larger women do indeed have greater bone mass, and in particular a higher bone mineral density. Conversely, women with smaller stature have lower bone mass A study by Drinkwater et al. (1990) in athletic premenopausal women aged 18-38 years, found body weight to be a significant predictive variable for bone density at all of seven sites measured, including lumbar spine, femoral neck and shaft, tibia, fibula, and the distal radius and shaft of the radius. The authors suggested two other possible explanations for

this relationship. Firstly, the skeleton will respond to the stress placed on it by the additional body mass, and secondly, there is increased conversion of androgens to estrone in the adipose tissue of the heavier women. But body weight predicted BMD of weight-bearing as well as non weight-bearing sites in this study, indicating that the relationship is more complex than a simple gravitational response. A strong positive correlation between body weight and BMC has also been shown in a group of perimenopausal women (Lindquist et al., 1981). However, these authors suggested another potential variable which may explain this association: they hypothesised that there may be a later onset of menopause in women with a higher % body fat. It is therefore clear that the effect of body weight on BMD is multifactorial.

C.iii **Muscle mass/strength**

Similar to the positive relationship between body mass and bone mass, a positive correlation has been demonstrated between muscle mass and bone mass (Aloia et al., 1995). This may be due to the relationship between body mass and muscle mass and therefore merely another method of relating body size to bone mass. However, cross-sectional studies showing higher BMD in weight lifters than other athletes suggest that weight training may provide a better stimulus for improving bone status than running and swimming (Nilson and Westlin, 1971; Heinrich et al., 1990; Davee et al., 1990). In a group of regularly menstruating female resistance and endurance trained athletes, fat-free body weight was the best predictor of bone mineral content (Heinrich et al., 1990). Therefore, the relationship between muscle mass and bone mass may be due to skeletal loading during weight training or other activities that cause muscle hypertrophy. Indeed, bone remodeling has been shown to occur in response to site-specific mechanical loading (Davee et al., 1990; Madsen et al., 1993). For example, Davee et al. (1990), showed that lumbar BMD was greatest in women who supplemented their aerobic exercise with

muscle-building activities, including arm, leg, chest and back exercises. They concluded that as the lumbar spine is the site of maximal resistance loading by arm and leg lifts, as well as back strengthening exercises, bone remodelling occurs mainly at sites where mechanical loading is maximal. However, Snow-Harter et al. (1990) concluded that although muscle strength is the most significant independent predictor of BMD and may account for 15-20% of the total variance in bone density in young women, it is more complex than a simple site-specific relationship between the muscle attachments and the bone. In that study biceps strength was an independent predictor of BMD at the hip, and grip strength best predicted lumbar spine density. It is not clear whether this finding is best explained by the systemic hormonal effect of exercise, or whether the biceps curl is an exercise which is most representative of overall exercise habits influencing the whole body.

C.iv **Menstrual and medical factors**

a. Menstrual status: current and history

The rapid loss of BMD following menopause illustrates the fact that normal reproductive hormonal status is one of the most important requirements for maintenance of bone mineral density, and consequently is highly likely to influence the attainment of peak adult bone mass. Research in premenopausal women has found that estrogen status (determined by a combination of age at menarche, average length of menstrual cycles since menarche, and circulating estrogen levels) is the most important determinant of bone mass in Caucasian women (Lloyd et al., 1988; Armamento-Villareal et al., 1992). Values within the normal range for these factors, along with age, previous pregnancy and use of birth control pills, are determinants of reproductive maturity and have been shown to be protective against development of menstrual dysfunction in premenopausal women

(Loucks and Horvath, 1985). Female athletes are a population at risk of developing menstrual dysfunction (Speroff and Redwine, 1980; Schwartz et al., 1981), particularly those participating in sports that place emphasis on leanness (Rippon et al., 1988). Since one of the important potential benefits of regular physical exercise is increased bone mineral density, (Lane et al., 1990; Risser et al., 1990) it is incongruous that the beneficial effects of exercise are negated or reversed in those athletes who experience menstrual cycle disruption and certain hormonal deficiencies (Drinkwater et al., 1984; Lindberg et al., 1984; Drinkwater et al., 1986). Many studies have identified a significant, direct relationship between current menstrual status and bone mineral density in both sedentary and exercising subjects (Cann et al., 1984; Drinkwater et al., 1984; Lindberg et al., 1984; Marcus et al., 1985; Nelson et al., 1986; Cook et al., 1987; Drinkwater et al., 1990; Warren et al., 1991; Jonnavithula et al., 1993; Myburgh et al., 1993; Rutherford, 1993; Micklesfield et al., 1995)(See Table 1.1). These studies unanimously indicate that amenorrhea is associated with lower lumbar spine BMD than in both control athletes (Drinkwater et al., 1984; Lindberg et al., 1984; Marcus et al., 1985; Nelson et al., 1986; Drinkwater et al., 1990; Warren et al., 1991; Jonnavithula et al., 1993; Myburgh et al., 1993; Rutherford, 1993) and sedentary controls (Cann et al., 1984; Lindberg et al., 1984; Marcus et al., 1985; Jonnavithula et al., 1993; Rutherford, 1993).

It is important to note that not only amenorrhea is associated with low BMD. Many female runners who may be classified as eumenorrheic, due to a regular number of cycles per year, may be experiencing short luteal phases, anovulation or both (Shangold et al., 1979). A study by Prior et al. (1990) on 66 premenopausal (21-42 years) women of varying activity levels, found that the inadequate production of progesterone, which occurs in cycles of short luteal phases and anovulatory cycles, was associated with accelerated bone loss, despite normal production of estradiol and the presence of regular

menses. Also Lloyd et al. (1988) showed that even moderate oligomenorrhea (6-7 menses/year) is sufficient to cause reduced lumbar spine BMD. This has also been shown by Cook et al. (1987) and Drinkwater et al. (1990). The significance of this bone loss has been highlighted in a study by Myburgh et al. (1990) which showed that athletes with stress fractures had significantly lower lumbar spine BMD, a higher incidence of current menstrual irregularity and a lower incidence of oral contraceptive use than the age- and exercise-matched control athletes who had never had a bone injury.

Some cross-sectional studies have also looked in more detail at the overall history of menstrual dysfunction instead of focusing only on the current status (Drinkwater et al., 1990; Grimston et al., 1990a; Myburgh et al., 1993; Micklesfield et al., 1995). Drinkwater et al. (1990) found a significant relationship between a score (category 1-9) derived from combining current and previous menstrual status and current lumbar spine BMD. Those subjects who scored 1 (always regular) tended to have the highest BMD and those subjects scoring 8 (current amenorrhea with a history of oligomenorrhea) and 9 (primary amenorrhea) tended to have the lowest BMD. Interestingly, a study by Myburgh et al. (1993) which calculated the number of years of amenorrhea, the number of years of regular menstruation and the estimated total number of periods missed found that the most robust predictor of lumbar spine BMD was the number of years of regular menstruation. In that study no significant relationship between lumbar spine BMD and years of amenorrhea, or total periods missed since menarche, was found. Their explanation for this was that the BMD deficit noted in amenorrheic women may not necessarily only reflect bone loss, but may also be due to inadequate bone gain during adolescence and early adulthood. Other methods of quantifying menstrual history have estimated the overall average number of periods per year, so that years of regular menses are taken into account (Grimston et al., 1990a; Micklesfield et al., 1995). The calculated

menstrual history indexes support the findings of both Drinkwater et al. (1990) and Myburgh et al. (1993).

It is clear that women athletes with menstrual irregularities are at risk of not achieving their peak adult bone mass due to a combination of factors relating to their current and previous menstrual status, such as age at menarche, average length of menstrual cycles, use of birth control pills and previous pregnancy.

b. Trabecular bone vs cortical bone

Several studies have found a significant difference in trabecular bone density between regularly menstruating athletes and athletes with secondary amenorrhea, with no difference in cortical bone mass between the two groups (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Nelson et al., 1986; Rutherford, 1993)(See Table 1.1). It is likely that bone mineral deficits are greater in trabecular bone than in compact bone due to the greater surface-to-volume ratio in trabecular bone. The bone surface is exposed to the hormonal milieu and similar to the hypoestrogenic state associated with menopause, lower circulating estradiol levels have been found in amenorrheic young women compared to women with regular menstrual periods (Cann et al., 1984; Drinkwater et al., 1984; Nelson et al., 1986). A positive linear correlation between lumbar spine BMD and serum estradiol (Nelson et al., 1986) supports this hypothesis. A study by Young et al. (1994) concluded that cortical bone in weight-bearing regions of the body will benefit more from weight-bearing exercise and suffer less from hypoestrogenism. Young et al. (1994) postulate that cortical bone may be more responsive to mechanical loading as it is normally adjacent to, or receives, direct muscle insertions while trabecular bone is more central and encased by cortical bone. Cortical bone may be less sensitive to estrogen deficiency than trabecular bone because it has a lower inherent turnover rate. Conflicting

results from a study by Myburgh et al. (1993) found that cortical bone is not necessarily protected from mineral loss as BMD of the femoral mid-shaft and neck (where the proportion of cortical bone may be as high as 50%) was significantly lower in the amenorrheic group than the regularly menstruating group, despite similar body weight.

Trabecular bone is more sensitive to hypoestrogenism, while cortical bone may be more responsive to mechanical loading rather than estrogen deficiency.

c. Age at menarche

Delayed menarche and intensive training at a young age may also contribute to subsequent development of amenorrhea and a higher incidence of bone injuries, as some studies have shown a later age at menarche in amenorrheic runners (Nelson et al., 1986; Drinkwater et al., 1990; Rutherford, 1993; Warren et al., 1991; Sanborn et al., 1987; Myburgh et al., 1993). Marcus et al. (1985) showed that the amenorrheic athletes in their study had commenced training within 1 year of menarche, whereas athletes with regular menstrual cycles did not begin training for competition until approximately 5 years later. These findings may be explained by the hypothesis that intensive endurance training affects the immature hypothalamic-pituitary-gonadal function more than it would once regular menstrual cycles have been established (Marcus et al., 1985). Delayed menarche in a normal, non-athletic population of women may also result in reduced BMD. In a study by Armamento-Villareal et al. (1992) an inverse relationship between vertebral bone density and age at menarche was found. This study showed that reaching sexual maturity earlier in life will increase the exposure of the skeletal tissue to the beneficial effects of estrogen. Similarly Bachrach et al. (1990) have shown that BMD is highly significantly lower in amenorrheic anorexic teenagers than in age-matched controls.

Therefore both adequate nutritional status and adequate estrogen is important for bone mineralisation during the rapid growth phase.

d. Resumption of menses

It is apparent that young women with a history of irregularity generally have lower BMD than age-matched controls. The question is whether the resumption of normal menses in these women is sufficient to counterbalance the negative effect of transient hypoestrogenism within the fertile period (Drinkwater et al., 1990; Armamento-Villareal et al., 1992). Drinkwater et al. (1986) have shown that after resumption of regular menses, previously amenorrheic athletes show a significant increase in lumbar spine BMD over a 15 month period, with no change in the cyclically menstruating women over this period. However, the level of lumbar spine BMD attained by the previously amenorrheic athletes was still significantly lower than control athletes. The resumption of menses in this group of previously amenorrheic runners coincided with a decrease in mileage due to injury or illness, and the substitution of running with other activities. There was also a significant increase in body weight during the 15 month period and five of the previous nine amenorrheic athletes reported an increase in consumption of dairy products and/or calcium supplementation over the previous year. Similarly, research by Lindberg et al. (1987), also over a 15 month period in which previously amenorrheic athletes took supplemental calcium and reduced their running distance, showed that along with an increase in body weight, increased estradiol levels and eumenorrhea, there was a 6,6% increase in lumbar spine bone mineral density. Our study (Micklesfield et al., 1995) in somewhat older women showed that any history of menstrual irregularity was more predictive of BMD than was current menstrual status, indicating that BMD may never be regained in sufficient quantity to match women who have always been

eumenorrheic. If these data are repeatable, it is unknown why BMD does not recover to normal premenopausal levels.

e. Oral contraceptive use, lactation history and parity

Other factors related to reproductive history such as use of oral contraceptives and parity may have a positive influence on peak adult bone mass, although a long history of lactation may be a negative influence. Several studies have found no relationship between the use of oral contraceptives and BMD (Hreshchyshyn et al., 1988; Lloyd et al., 1989; Mazess and Barden, 1991). Oral contraceptive use in women athletes has been shown to protect against the development of stress fractures and other musculoskeletal injuries (Lloyd et al., 1985; Myburgh et al., 1990), as well as early age-related bone loss (Recker et al., 1992a) and may therefore have an effect on achieving peak bone mass. It is still uncertain however whether oral contraceptives used specifically to correct menstrual irregularities will improve bone mass or merely prevent bone loss.

In a study by Lindquist et al. (1981) on women from three different age strata (46, 54 and 62 years), there was no relationship between parity and bone mineral content. Sowers et al. (1993) found that women who breastfeed for more than 6 months have reduced BMD of the lumbar spine and the femoral neck. This is in contrast to research by Koetting and Wardlaw (1988) who found no association between a history of long-term lactation and low bone density in the lumbar vertebrae and the femoral neck. However, this discrepancy in results may be explained by the fact that BMD of the lumbar spine has been shown to return to baseline levels within 12 months of stopping breastfeeding (Sowers et al., 1993). The return of BMD to baseline levels may also be influenced by dietary calcium intakes. Athletic women tend to have paradoxically low energy intakes and some studies have also documented low intakes of calcium (Nelson et al., 1986;

Myburgh et al., 1988; Myburgh et al., 1990). There is as yet little information regarding the combination of breastfeeding and dietary habits of mature athletic women.

f. Training and menstrual function

A relationship has also been found between distance run, ie. mileage per week, and chronic menstrual dysfunction (Schwartz et al., 1981; Lindberg et al., 1984; Drinkwater et al., 1984; Drinkwater et al., 1986; Cook et al., 1987; Drinkwater et al., 1990). A study by van Gend and Noakes (1987) found that all of the amenorrheic and 3 of the oligomenorrheic women in their study were running over 75 km/wk, while no one who ran under 35 km/wk experienced any changes in their menstrual pattern. Other studies have not found a relationship between menstrual irregularity or amenorrhea and weekly mileage (Speroff and Redwine, 1980; Nelson et al., 1986; Myburgh et al.; 1993). However, a study by Schwartz et al. (1981) found that the group of amenorrheic runners had been running for a longer period of time than those runners with regular cycles. Running mileage should however not be considered in isolation, as chronic menstrual dysfunction may be the result of a combination of lifestyle factors associated with running, such as sustained heavy training, frequent intensive competition and chronic low body weight due to a kilojoule-restricted diet. Indeed, Myburgh et al. (1992) have shown that an athlete's risk of menstrual dysfunction increases with the presence of more risk factors and that no single risk factor is more important than another. Therefore, those factors which promote menstrual dysfunction may also indirectly promote bone loss and prevent the achievement of the genetically predetermined potential peak adult bone mass

C.v **Nutrient intake**

a. Kilojoule intake

As stated above, women athletes report a reduced kilojoule intake relative to their high energy expenditure (Marcus et al., 1985; Nelson et al., 1986; Van Gend and Noakes, 1987). Our previous research has found a significant relationship between total energy intake and the Menstrual History Index, a measure of menstrual irregularity, and therefore suggests that there is a relationship between current energy intake and the history of menstrual dysfunction which may be related to a history of chronic undernutrition (Micklesfield et al., 1995). Results of a study by Drinkwater et al. (1984) showed a daily energy intake of 6817 kJ in a group of amenorrheic athletes compared to 8253 kJ in their control group of eumenorrheic athletes, a difference ($p<0.06$) which closely approached the level of significance ($p=0.05$) established for the study. Marcus et al. (1985) also reported a difference in energy intake between their group of amenorrheic runners (5342 kJ) and their age-matched controls (7203 kJ), however the large variance in the data prevented these values from reaching statistical significance. Thus, previous and current energy intake, specifically in women athletes, may also indirectly influence peak bone mass.

b. Calcium intake

Adequate calcium intake during the adult years is necessary to maintain calcium balance and a strong skeleton and therefore calcium deficiency contributes to bone loss (Heaney et al., 1982a). Not only current calcium intake but also the history of calcium intake, ie. during adolescence and in the early 20s, is important in the attainment of peak adult bone mass (Halioua and Anderson, 1989). Calcium intake is an important determinant of peak

bone mass among young adults and it has been hypothesized that calcium influences skeletal calcium retention during bone growth (Matkovic et al., 1990). Calcium intake may also be particularly important in exercising adults since Myburgh et al. (1990) have shown that athletic subjects (men and women) with stress fractures had a significantly lower current intake of dietary calcium and also a lower estimated intake of dairy products since leaving school, than did control athletes who had never had a bone injury. Even shin soreness without frank tibial stress fractures have been associated with low dietary intakes of calcium (Myburgh et al., 1988). However it would appear that the relationship is not simple. A cross-sectional study by Kanders et al. (1988) on 60 premenopausal women, with stable endocrine status, found that although there was not a significant linear relationship between lumbar spine BMD and calcium intake, when the sample of women were divided into those with a calcium intake above the RDA (800 mg/d) and those with a calcium intake below the RDA, both vertebral and radial BMD were significantly greater in the high calcium intake group. Although Grimston et al. (1990b) found that current calcium intake was not shown to be related to current BMD, they did find a positive relationship between estimated history of calcium intake during the formative years of bone growth, and current BMD of the lumbar spine. Calcium requirements increase with the onset of puberty at which time calcium intake is often reduced (Heaney, 1982a), placing the individual at risk of inadequate calcium retention resulting in reduced peak bone mass and the subsequent risk of developing osteoporosis in later life. Other studies have found no association between calcium intake and BMD and/or BMD changes (Mazess and Barden, 1991; Micklesfield et al., 1995).

It is important to remember that effective calcium intake is not only a result of adequate calcium in the diet. A study by Heaney and Recker (1982b) found that the most prominent determinant of calcium balance was calcium absorption, with dietary calcium intake being the next prominent factor. Thus, factors such as absorption efficiency,

retention efficiency, various nutrient-nutrient, drug-nutrient, and disease-nutrient interactions all affect the availability and utilization of the calcium taken in in the diet (Heaney, 1982a). Therefore, in healthy women excessive intake of certain nutrients such as nitrogen, phosphorus and caffeine, as well as the abuse of certain drugs such as diuretics, antacids and alcohol, may impact negatively on calcium absorption.

c. Vitamin D and other nutrients

Calcium balance (body retention) is determined not only by calcium intake and net calcium absorption but also by urinary excretion (Matkovic et al., 1990). Urinary calcium does not necessarily increase with an increase in calcium intake, so an increased intake is still a major determinant of calcium balance. However, not only current calcium intake, but also Vitamin D intake has been identified as a major determinant of calcium balance in sedentary women (Fehily et al., 1992). Vitamin D deficiency is one of several factors that decrease dietary calcium absorption from the gut, and increase excretion of calcium in the urine or faeces or both. This may be due to a fall in circulating calcitriol or 1,25 dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] levels, the hormonal form of Vitamin D. Two schools of thought exist regarding the mechanism of how this occurs (Heaney et al., 1982a). One interpretation assumes that the reduced secretion of calcitriol which occurs with age, deficiencies of gonadal steroids, or other factors, results in a malabsorption of calcium and thus reduced serum calcium. This stimulates an increase in PTH secretion in order to increase the renal production of calcitriol, however the elevated levels of PTH result in bone resorption and consequent bone loss. The other hypothesis assumes an imbalance between bone resorption and bone formation due to factors such as reduced physical activity and hypoestrogenism. Because calcium released from the skeleton is greater than calcium taken up by the skeleton, PTH secretion, as well as calcitriol synthesis, are reduced. The result will then be reduced calcium absorption from the

skeleton. Heaney et al., (1982a) concluded however that both these hypotheses may in fact be correct and applicable to different individuals.

Some research has found that a high protein intake in excess of dietary need may enhance bone loss through increased urinary calcium excretion due to reduced retention of absorbed calcium (Heaney and Recker, 1982b). There is also a strong relationship between caffeine consumption and bone mineral density, as a high caffeine intake has been found to increase the risk of osteoporotic fractures in middle-aged women as a result of increased urinary calcium excretion (Hernandez-Avila et al., 1991). Other dietary factors that may influence calcium balance include nitrogen and phosphorous (Heaney et al., 1982b). The intake of these variables is strongly related to each other, therefore an increase in the intake of one will result in an increase in the intake of the other factor. For example, with an increase in the intake of dairy products, there is an increase in the intake of calcium, nitrogen and phosphorus. It seems that increasing phosphorous alone has no influence on calcium balance, however nitrogen and caffeine have a negative effect on calcium balance due to an increase in urinary calcium excretion, rather than calcium absorption efficiency. The study concluded that a 50% increase in nitrogen intake above the group mean intake value (10.9 ± 2.5 g/d), an intake which is not extreme, would be predicted to result in calcium balance shift of -0.032 g/d, while for caffeine, the corresponding calcium balance shift would be predicted to be -0.006 g/d. A negative balance of 0.040 g/d may explain the 1-1.5% loss in skeletal mass per year noted in premenopausal women. Large amounts of dietary fibre have also been found to have an affect on calcium balance, however it is unlikely that the fibre content of most Western diets is sufficient to cause negative calcium balance, however some individuals may be at risk (Heaney et al., 1982a).

d. Nutrition and hormones

Nutrient status may also affect hormonal influences on the remodeling process.(Dalsky, 1990). Under conditions of inadequate calcium intake, blood calcium levels are maintained through PTH stimulation of calcium withdrawal from bone, resulting in an increase in remodeling activity and a negative bone balance. Alternatively, estrogen withdrawal produces an increase in the calcium intake requirement due to lower intestinal absorption and increased urinary calcium loss (Heaney et al., 1982a; Drinkwater et al., 1984; Nelson et al., 1986). The calcium intake levels recommended by the Consensus Conference on Osteoporosis conducted by the National Institutes of Health are higher than the previous RDA of 800 mg/d. Rather 1 000 mg/d for men and premenopausal women and 1 500 mg/d for estrogen-deficient women has been recommended.

C.vi **Combination of the factors**

A combination of the various factors and their influence on BMD must be considered. However, although there is some interaction between the three major factors that influence bone health namely, physical activity, hormonal status and nutrition, each should be seen as playing an independent role in the attainment of bone mass, and neither is more important than the others. Research using an avian model has provided evidence to suggest that exercise training in the presence of low calcium intake, may minimise the adaptation of the bone to exercise training (Lanyon, 1986). During calcium deficiency, physical activity resulting in mechanical loading should decrease resorption and turnover, however when calcium deficiency is severe, physical activity may not be sufficient to prevent a negative calcium balance from developing. In contrast, some research has suggested that *adequate* levels of both will produce maximal bone mineralisation, which will not be increased further even by higher calcium intakes or physical activity levels

(Halioua and Anderson, 1989). There may also be an additive effect between exercise and calcium as some research has shown a significant difference in mean vertebral BMD between women who had both an active life-style and high calcium intake, and those who were both inactive and calcium deficient (Halioua and Anderson, 1989). However, other research has found no significant interaction of calcium and exercise with BMD, although results did show a tendency for negative bone changes over 2 years in the least active subjects (Mazess and Barden, 1991).

It has already been established that women who develop amenorrhea as a result of excessive exercise have reduced bone mass (Cann et al., 1984; Drinkwater et al., 1984; Drinkwater et al., 1990; Armamento-Villareal et al., 1992; Micklesfield et al., 1995). Estrogen may therefore be potentially more powerful in determining bone mass than physical activity, even in premenopausal women (Aloia et al., 1988), unless very high impact exercise is performed regularly for several hours per day such as in elite gymnasts (Robinson et al., 1995). However, although bone mineral density is compromised in women with exercise-induced amenorrhea, it is higher than in women with other causes of amenorrhea eg. oophorectomy (Genant et al., 1982; Young et al., 1994). Similarly, Marcus et al. (1985) showed that although amenorrheic runners had bone density which was lower than eumenorrheic women and age-matched controls, it was higher than in runners with secondary amenorrhea who were less physically active. Thus, the mechanical loading characteristics of exercise may have some protective effect on bone in the presence of menstrual irregularity and may reduce the impact of amenorrhea on bone mass (Jonnavithula et al., 1993), particularly if adequate calcium is absorbed.

D. INTERVENTION

Most of the factors which influence BMD and which were discussed above are easy to manipulate, by either dietary supplementation or pharmacological intervention with hormone replacement therapy. It is therefore necessary to identify populations at risk of premature bone loss and to base treatment and education programmes on sound scientific evidence. Although interventions may seem insignificant, such as the intake of a particular dietary variable, it has been shown that a high protein intake can result in an average negative calcium balance of 84 mg/d which is greater than the negative calcium balance of 40 mg/d which has been calculated to result in a loss in skeletal mass of 10-15 % per decade (Heaney and Recker, 1982). This alone, or in combination with other factors, will have a large impact on the risk of developing osteoporosis in later life. In contrast, findings from a study by Recker et al. (1992a) in which it was determined that bone gain continues in women up until a point close to 30 years of age, suggests that fracture risk late in life can be reduced significantly by modest increases in physical activity and calcium intake under the age of 30 years.

For highly competitive amenorrheic premenopausal athletes who may be resistant to reducing training and gaining weight to regularise their menstrual cycles, estrogen replacement therapy is recommended. Although not yet scientifically proven it is perhaps prudent to institute estrogen therapy at an early stage since it is still unknown whether or not bone loss due to prolonged menstrual irregularity can ever be fully regained prior to menopause. Until data are available to definitely show that women in their late premenopausal years who had menstrual irregularity in their twenties, can regain their bone mass, it must be assumed that such women are at risk of developing osteoporosis in later life. However, the data does suggest that regular weight-bearing exercise or weight-training, or both, in conjunction with adequate calcium intake should benefit the

attainment of peak adult bone mass in regularly menstruating women who have a family history of osteoporosis or other significant risk factors for low bone mass.

However, no controlled intervention trials have done in amenorrheic women to confirm the benefits of weight-bearing exercise, an increase calcium intake and estrogen therapy. Neither have any studies investigated bone mineral density changes in athletic women in their mid-thirties to forties, to determine whether the effects of prior menstrual irregularity are still significant in that late premenopausal period.

Therefore the main aims of this thesis were to:
(i) determine whether mature women athletes who have experienced prior menstrual irregularities are still at risk of a reduced bone mineral density in the late premenopausal period;
(ii) compare risk factors for reduced bone mineral density between exercising women with a history of menstrual irregularity, exercising women with no history of menstrual irregularity, and sedentary controls with no history of menstrual irregularity.

TABLE 1.1: SUMMARY OF LITERATURE ON THE RELATIONSHIP BETWEEN MENSTRUAL STATUS AND BONE MINERAL DENSITY

AUTHOR	SUBJECT AGE RANGE	ACTIVITY OF ATHLETE	BMD PROCEDURE	SEDENTARY *Regular* (SED_R)	SEDENTARY *Amenorrheic* (SED_A)	REGULARLY MENSTRUATING ATHLETES (R)	OLIGOMEN. ATHLETES (O)	AMEN. ATHLETES (A)	p-value
Cann et al. 1984	16-49 yrs	Amount of exercise not controlled	Computed tomography (mg/cm^3) L_1, L_2	165.8 ± 4.2 (n=50)	22-29% of control values (n=38)				$p<0.001$ SED_R vs SED_A
Drinkwater et al. 1984		Running Rowing	DPA L_1-L_4 (g/cm^2)			1.30 ± 0.03 (n=14)		1.12 ± 0.04 (n=14)	$p<0.01$ R vs A
Lindberg et al. 1984		Running	SPA Distal radius (g/cm^2)	0.56 ± 0.05 (n=14)		0.56 ± 0.07 (n=15)	0.50 ± 0.07 (n=5)	0.46 ± 0.07 (n=11)	$p<0.05$ R vs A $p<0.05$ SED_R vsA
Marcus et al. 1985	18-29 yrs	Running	Computed tomography (mg/cm^3) L_1, L_2	166 ± 4 (n=20)		182 ± 5 (n=6)		151 ± 8 (n=11)	$p<0.02$ R vs A $p<0.05$ SED_R vsA
Nelson et al. 1986	24-30 yrs	Running Rowing	DPA L_1-L_4 (g/cm^2)			1.196 ± 0.025 (n=17)		1.099 ± 0.027 (n=11)	$p<0.05$ R vs A
Cook et al. 1987	15-44 yrs	Running	DPA L_1-L_4 (g/cm^2)			1.226 ± 0.082 (n=17)	1.131 ± 0.112 (n=19)		$p<0.01$ R vs O
Drinkwater et al. 1990	18-38 yrs	Running Ballet	DPA L_1-L_4 (g/cm^2)			1.27 ± 0.02 (n=21)	1.18 ± 0.02 (n=55)	1.05 ± 0.04 (n=21)	$p<0.01$ R>O>A

Table 1 cont.

AUTHOR	SUBJECT AGE RANGE	ACTIVITY OF ATHLETE	BMD PROCEDURE	SEDENTARY *Regular* (SED_R)	SEDENTARY *Amenorrheic* (SED_A)	REGULARLY MENSTRUATING ATHLETES (R)	OLIGOMEN. ATHLETES (O)	AMEN. ATHLETES (A)	p-value
Warren et al. 1991	13-29 yrs	Ballet	DPA L_1-L_4 (g/cm^2)	1.266 ± 0.134 (n=30)	1.154 ± 0.211 (n=17)	1.283 ± 0.187 (n=29)		1.118 ± 0.105 (n=22)	$p<0.05$ R vs A $p<0.05$ SED_R vs A
Jonnavithula et al. 1993	13-29 yrs	Dancing	DPA L_1-L_4 (g/cm^2)	1.30 ± 0.10 (n=9)	1.13 ± 0.21 (n=4)	1.25 ± 0.20 (n=12)		1.05 ± 0.09 (n=5)	$p<0.05$ R vs A $p<0.05$ SED_R vs A $p<0.05$ R vs SED_A $p<0.05$ SED_R vs SED_A
Myburgh et al. 1993	20-41 yrs	Running	DEXA L_1-L_4 (g/cm^2)			1.050 ± 0.11 (n=9)		0.928 ± 0.056 (n=12)	$p<0.01$ R vs A
Rutherford et al. 1993	20-52 yrs	Running Triathlon	DEXA L_1-L_4 (g/cm^2)	Lunar database for 1 000 healthy British women		1.180 ± 0.02 (n=16)		1.071 ± 0.02 (n=15)	$p<0.01$ R vs A $p<0.01$ SED_R vs A
Micklesfield et al. 1995	25-39 yrs	Running	DEXA L_1-L_4 (g/cm^2)			1.088 ± 0.07 (n=15)	0.923 ± 0.07 (O/A) (n=6)		$p<0.001$ Rvs O/A

CHAPTER 2

Bone Mineral Density in mature, premenopausal, ultramarathon runners.

STUDY 1

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i. INTRODUCTION

It is now well established that amenorrheic athletes have low vertebral bone density (Cann et al., 1984; Drinkwater et al., 1984; Drinkwater et al., 1986; Marcus et al., 1985; Myburgh et al., 1983; Nelson et al., 1986; Nilson and Westlin, 1971). As a result of the association between osteopenia and an increased incidence of stress fractures in athletes (Myburgh et al., 1990), and since osteoporosis may be a potential long-term complication (Marcus et al., 1985), there has been a growing interest in the identification of the etiological factors associated with this form of osteopenia.

Bone mineral density in this population may be influenced by several variables such as current menstrual status, menstrual history, body mass, functional loading and calcium balance. Complex inter-relationships between these variables may exist. It has been suggested that physical activity, especially if it is weight-bearing, may act to increase BMD (Aloia, 1981; Marcus et al., 1985; Nilson and Westlin, 1971), and may therefore offset bone loss induced by oligo/amenorrhea. However, large training volumes and inadequate energy intake, are also associated with oligo/amenorrhea. However, large training volumes and inadequate energy intake, are also associated with oligo/amenorrhea

(Loucks, 1990; Rippon et al., 1988), low endogenous estrogen concentrations (Drinkwater et al., 1986), low calcium intake (Nelson et al., 1986) and consequently, osteopenia (Lutter, 1983). Some previous studies have found that both amenorrheic and oligomenorrheic runners ran greater distances each week than eumenorrheic runners (Drinkwater et al., 1984), while other studies failed to demonstrate differences in training patterns between these groups (Marcus et al., 1985). Some (Drinkwater et al., 1984; Marcus et al., 1985; Nelson et al., 1986), but not all, studies have shown that amenorrheic runners have a significantly lower reported energy intake than do eumenorrheic runners.

The long-term consequences of oligo/amenorrhea are still unknown, as most of the studies to determine the effect of amenorrhea on BMD in women athletes have been performed on young subjects (Drinkwater et al., 1984; Drinkwater et al., 1986; Drinkwater et al., 1990; Marcus et al., 1985). The average age of the amenorrheic subjects used in these studies ranged from 20 to 27 years. Results from our laboratory have shown (Watkin et al., 1991) that dietary intakes did not influence menstrual function in mature, ultramarathon runners (average age 34 years), indicating that the risk factors for low BMD may be different in somewhat older athletes. Also, Drinkwater et al. (1986) showed that the osteopenia induced by amenorrhea is, at least partially, reversible in athletes who regained menses. Both the resumption of menstrual cycles and the increase in BMD in these athletes followed a change in a variety of interrelated risk factors for low BMD, namely a decrease in training, an increase in body weight, and an increase in consumption of dairy products or calcium supplements or both. This may indicate that somewhat older athletes with a prior history of oligo/amenorrhea may be at less risk for osteoporosis in later life than previously expected. However, this is as yet not substantiated by sufficient scientific evidence.

Therefore the aim of this study was to relate risk factors for a reduced bone mineral density, especially delayed menstrual cycles, to actual BMD, in a group of older, premenopausal female ultramarathon runners.

ii. HYPOTHESIS OF STUDY 1

The hypothesis of this study was that any history of menstrual dysfunction will influence lumbar spine bone mineral density.

iii. MATERIALS AND METHODS

All women, between 25 and 39 years of age, who had entered a 56 km ultramarathon road race and were living in the Cape Town metropolitan area and surrounding rural districts, were contacted and invited to complete a questionnaire. Eighty of the 152 women returned the questionnaire. Forty women were randomly selected from these respondents and were invited to participate in the study. The study was approved by the Ethics and Research Committee of the University of Cape Town Medical School, and written informed consent was obtained from all subjects.

Subject details including age, height, highest and lowest adult weights, as well as training histories, menstrual history, and use of oral contraceptive medications, were obtained from an initial questionnaire. Subjects who were currently taking oral contraceptives (n=15) were excluded from further analysis. Of these 15 subjects, only 3 had a prior history of oligo/amenorrhea. Body Mass Index and change in Body Mass Index (DBMI) were calculated from the following equations:

$$BMI = mass * height^{-2} \ (kg.m^{-2})$$

$$DBMI = \text{highest adult BMI} - \text{lowest adult BMI} \ (kg.m^{-2})$$

Body composition was measured according to the method of Durnin and Wormesley (1974). Skinfold measurements were taken on the right side at the triceps, biceps, subscapular and suprailiac sites. Fat mass, % body fat and fat free mass were calculated. Somatotype was assessed using the Heath-Carter method (Heath and Carter, 1967).

Daily energy expenditure ($MJ.d^{-1}$) was estimated for each of the subjects (Blair et al., 1985). All the subjects also completed a 3-day dietary record for two week days and one week-end day. These data were analysed using the food quantities tables of the National Research Institute for Nutritional Diseases (Parow, South African Medical Research Council Publications Unit, 1986) and a computerised dietary analysis programme (Floro Diet Data Programme, P.O. Box 6138, Durban, 4000). Total energy (kJ), calcium (mg), phosphorus (mg), protein (g), fat (g), carbohydrate (g), and dietary fibre (g) intakes per day were determined.

Venous blood samples were drawn during the mid- to late-follicular phase of the menstrual cycle (days 8 - 12) and serum estradiol concentrations were determined by means of a specific radioimmunoassay performed on ether extracts of the plasma samples. The extraction and assay procedures are modifications to the methods of Abraham et al. (1976). A 2 hour urine collection was obtained from each subject for the first two hours after waking and analysed via atomic absorption spectrometry for urinary calcium concentration (Willis, 1961).

An osteodensitometry scan was performed in the Department of Nuclear Medicine, Groote Schuur Hospital using a Hologic QDR-1000 (version 4.20) dual energy x-ray bone densitometer (Hologic Inc., Waltham, Massachusetts). Scans were made of the lumbar spine and the left proximal femur. Average bone mineral densities ($g.cm^{-2}$) were

determined for lumbar vertebrae 1 through 4 and the total proximal femur, Ward's triangle, femoral neck, greater trochanter, and the intertrochanteric space.

During this period of the study the subjects were interviewed to obtain more detailed information concerning menstrual history and intake of dairy products during the following time periods: 13-20 yr.; 21-30 yr. and 31 to current age. Parity and breastfeeding details were also obtained by interview. The information was used to calculate the following:

(i) Menstrual History Index (modified from Grimston et al. (1990a)) was calculated to determine the estimated number of periods per year since age 13:

Menstrual History Index = (11.5*R + 7*O + 1.5*A)/(C-13)

where: R= number of years of regular menstrual cycles (defined as 10-13 menstrual periods per year and assuming an average of 11.5 periods.yr^{-1});

O= number of years of oligomenorrhea (defined as 4-9 menstrual periods per year and assuming an average of 7 periods.yr^{-1});

A= number of years of amenorrhea (defined as 0-3 menstrual periods per year and assuming an average of 1.5 periods.yr^{-1});

C= current age.

(ii) The Menstrual History Index was then divided into the estimated number of periods per year for the following three time periods:

- 13-20 years of age
- 21-30 years of age (or 21-present age)
- 31-39 years of age (or 31-present age).

As above, it was assumed that eumenorrhea = 11.5 menstrual cycles per year;

oligomenorrhea = 7 menstrual cycles per year; and amenorrhea = 1.5 menstrual cycles per year.

(iii) Dairy product intake was reported as the estimated number of portions consumed per week during junior school, high school, on leaving school, and over the past year. One portion was equivalent to one cup of milk, 175 ml of yoghurt, a rounded scoop of ice-cream, a 250 g tub of cottage cheese, and a cheese meal or sandwich.

Data analysis

Data are presented as means ± standard deviations (SD). Unpaired Student's *t*-tests were performed to compare data from runners who had always had regular menstrual cycles (R, n=15), and those with current or previous menstrual irregularity (OA, n=10), defined for this study as oligo/amenorrhea (0-9 periods.yr^{-1}). The Kolmogorov Smirnov 2-sample test was performed for the following non-parametric variables: estimated number of periods per year between the age of 21 and 30 years, and above 30 years of age. Kruskal-Wallis tests were performed to compare the BMD data of three groups after dividing the subjects into those women who had always had regular menstrual function (R), those who had oligo/amenorrhea in the past but who had now resumed regular menstrual cycles (CR; n=4), and those women who were currently oligo/amenorrheic (COA; n=6). Kruskal-Wallis tests were also performed to compare the BMD data of three groups after dividing the subjects into those women who had always had regular menstrual cycles (R), those who had a history of oligomenorrhea with no history of amenorrhea (HO; n=4), and those women who had a history of either amenorrhea or both amenorrhea and oligomenorrhea (HOA; n=6). Pearson-product moment correlation coefficients were calculated to determine which variables were significantly correlated with BMD, as well as between selected variables to determine the possibility of an

indirect effect on BMD. Multiple linear regression was performed to develop prediction equations for bone mineral density of the lumbar spine and the left hip. This was done in several steps: first, five variables were entered into a stepwise selection for each of the four main categories, namely, physical characteristics (mass; height; fat mass; BMI; DBMI), menstrual variables (estimated no. periods.yr^{-1} for 13-20 yr of age and 21-30 yr of age; MHI; menarche; years amenorrheic), training variables (training index; estimated energy expenditure per day; personal best race time: 56 km and 21.1 km; years of marathon training) and dietary variables (calcium index, total energy intake, calcium intake, fibre intake, urinary calcium). The variables with the best F-values from each of the four main categories were then entered into another stepwise selection to determine the best overall predictor of BMD at the lumbar spine and the femoral neck. Subsequently Pearson-product moment correlation coefficients were also calculated to determine the relationships between LS BMD and MHI, years of regular menstrual cycles, oligomenorrhea and amenorrhea in those 10 subjects with a history of menstrual irregularity.

iv. RESULTS

Comparison of groups

Fifteen subjects had always been regular (R, 10-13 periods.yr^{-1}) and 10 had a history of oligo/amenorrhea, (OA, 0-9 periods.yr^{-1}). Subjects ranged in age from 29 to 39 years of age. The regular (R) and oligo/amenorrheic (OA) group did not differ in age (R: 36 ±3 vs. OA: 35 ±4 yr.), (mean ± SD), body mass (R: 58 ±8 vs. OA: 57 ±8 kg), body mass index (BMI) (R: 21.4 ±2.4 vs. OA: 20.9 ±2.0 kg.m^{-2}) or estimated body fat (R: 29 ±4 vs. OA: 29 ±4 %) (Table 2.1). The DBMI (highest - lowest adult BMI) was also not significantly different, although the OA group tended to have a greater range of values. Other anthropometric measurements were also similar in both groups: endomorphy (R: 4.4 ±1.3 vs. OA: 4.6 ±1.3); mesomorphy (R: 3.5 ±1.1 vs. OA: 3.6 ±1.1); ectomorphy (R: 2.9 ±1.3 vs. OA: 3.1 ±1.0). Current weekly training distances (R: 68 ±21 vs. OA: 76 ±33 km.wk^{-1}) and years of running training (R: 4.1 ±2.3 vs. OA: 3.8 ±3.6 yr.) were also similar between the two groups (Table 2.1). Total daily estimated energy expenditure (R: 14 ±3 vs. OA: 15 ±3 MJ.d^{-1}) was similar between R and OA.

Of the 10 subjects with a history of oligo/amenorrhea (OA), 6 were still currently irregular, while 4 had regained regular menstrual cycles. Of these 4, 3 had regained regular menses greater than 10 years prior to the study and the other subject had regained regular menses 4 years prior to the study. OA subjects had a history of amenorrhea for a total of 3.2 ±3.7 yr (mean ±SD) and oligomenorrhea for 2.7 ±2.2 yr. The MHI was significantly lower in OA than R (R: 11.6 ±0.6 vs. OA: 9.4 ±2.1, $p<0.01$). Four of the OA subjects had a MHI score between 6 and 7. They had all had amenorrhea, ranging from 3-7 years in total. However the 2 with the lowest BMD values also had a history of oligomenorrhea for 5-6 years. The MHI of OA subjects was significantly different from R subjects between age 21 and 30, and above age 30 years (both $p<0.0001$) (Table 2.2).

Parity and months of breastfeeding were similar in R and OA (R: 1.6 ±1.0 vs. OA: 1.2 ± 1.7 children and R: 6.2 ±7.9 vs. OA: 10.7 ±14.3 months). Only one woman was currently breastfeeding her 3 year old child. No woman had been pregnant for at least 3 years, and the age since the last pregnancy ranged between 3 and 16 years. The normal range of estradiol, during the follicular phase of the menstrual cycle, is 100-700 $pMol.l^{-1}$, for this laboratory. Only two women had levels below the minimum, and thus had hypoestrogenic amenorrhea at the time of the trial. The other 4 women in the currently oligo/amenorrheic group were currently oligomenorrheic. Mean estradiol levels were not significantly different between the two groups (see Table 2.2).

The mean BMD of the lumbar spine in this sample was 1.032 ±0.105 $g.cm^{-2}$ and that of the left proximal femur was 0.935 ±0.118 $g.cm^{-2}$. The range of BMD values for the lumbar spine varied from 84% to 112% of age-matched normals (reference curve for American females, Hologic QDR-1000, October 1984). The range of BMD values for the left hip varied from 86% to 124% of age-matched normals (reference curve for females, Hologic QDR-1000, October 1984). The mean BMD of the lumbar spine for R was 1.088 ±0.069 $g.cm^{-2}$ and for OA was 0.946 ±0.098 $g.cm^{-2}$ ($p<0.001$), and BMD of the left proximal femur was 0.964 ±0.133 $g.cm^{-2}$ for R and 0.889 ±0.083 $g.cm^{-2}$ for OA ($p=0.07$). Neither total proximal femoral BMD, nor BMD at the femoral neck, trochanter or Ward's triangle were different between the two groups (Table 2.3).

After division of the OA group into those who were currently oligo/amenorrheic (COA) and those who were currently regular (CR) but previously oligo/amenorrheic, we found that BMD of the lumbar spine was significantly lower in COA subjects compared to R (R:1.088 ±0.069 vs. COA: 0.923 ±0.069 $g.cm^{-2}$; $p<0.001$), as well as showing a similar trend in CR compared to R (R: 1.088 ±0.069 vs. CR: 0.982 ±0.136 $g.cm^{-2}$; $p<0.07$).

Body mass was not significantly different between these groups (R: 58.3 ±7.9 vs. CR: 54.5 ±9.3 vs. COA: 59.1 ±6.7 kg). Neither was DBMI different between these groups (R: 4.1 ±2.6 vs. CR: 4.0 ±1.4 vs. COA: 5.7 ±4.3 $kg.m^{-2}$).

The OA subjects were also subdivided into those who had a history of oligomenorrhea, but no history of amenorrhea (HO) and those who had a history of either amenorrhea or both oligomenorrhea and amenorrhea (HOA). Lumbar spine BMD was not different between HO subjects (0.953 ±0.024 $g.cm^{-2}$) and HOA subjects (0.942 ±0.131 $g.cm^{-2}$). Both these groups were significantly lower than R: those with a history of oligomenorrhea, but no history of amenorrhea (HO; $p<0.005$) and those with a history of amenorrhea (HOA; $p<0.005$) (see Fig. 2.1).

There were no significant differences between groups for current reported daily energy and calcium intakes (R: 7164 ±1894 vs. OA: 6479 ±1557 $kJ.d^{-1}$ and R: 709 ±459 vs. OA: 774 ±350 $mg.d^{-1}$) (Table 2.4), or for any other measured component of the diet, except for fibre intake which was significantly higher in OA ($p<0.05$). Urine calcium excretion measured from a 2-hour morning urine collection, was not different between the two groups (see Table 2.4).

Analysis by correlation

Lumbar spine BMD for the whole sample of women did not correlate with the total number of years of amenorrhea, but correlated significantly with the total number of years of oligomenorrhea ($p<0.01$, $r= -0.58$), total number of years of regular menstrual cycles ($p=0.01$, $r=0.50$) and with the overall MHI ($p<0.0005$, $r= 0.67$) (see Fig. 2.2). Lumbar spine BMD for the whole sample of women also correlated significantly with the estimated number of periods per year from (a) 13-20 years of age ($p<0.05$, $r=0.39$), (b) 21- 30 years of age ($p<0.01$, $r=0.59$), and (c) 31 to 39 years of age ($p<0.01$, $r=0.52$). No

dietary, training or body composition variable correlated with lumbar spine BMD. Femoral BMD was significantly correlated with BMI ($p<0.05$, $r=0.43$), but no other variable.

Despite small subject numbers and low statistical power, lumbar spine BMD of those women with a history of menstrual irregularity correlated significantly with the total number of years of oligomenorrhea ($p<0.05$, $r= -0.53$) and the years of regular menstrual cycles ($p<0.05$, $r= 0.60$). The correlation between LS BMD and the overall MHI ($r= 0.48$) approached significance, but LS BMD did not correlate with the total number of years of amenorrhea ($r= -0.38$) (see Fig. 2.3).

In addition to determining those factors directly associated with BMD, we also determined which risk factors for low BMD were also related to each other. The MHI was negatively correlated with total energy expenditure per day ($p<0.01$, $r= -0.51$) (see Fig. 2.4), indicating that a history of fewer menstrual cycles per year was associated with higher activity levels. Total energy expenditure was also positively correlated with BMI ($p<0.01$, $r= 0.53$) and DBMI ($p<0.001$, $r= 0.62$), indicating that those subjects who were currently heavier relative to their height, or whose body mass had fluctuated to the largest extent in the past, were physically more active.

The variables with the best F-values for the stepwise multiple regressions performed by category were entered into the final stepwise multiple regression in order to determine the best single predictor of LS BMD. These variables were: MHI, BMI, DBMI and mesomorphy. The best single predictor of the BMD of the lumbar spine was MHI (stepwise multiple regression: $r^2=0.45$, $p<0.001$). The formula which best predicted variability in BMD of the lumbar spine was:

$$\text{BMD lumbar spine} = (\text{MHI} * 0.04) + 0.59 \qquad (r^2 = 0.45)$$

Table 2.1 Physical characteristics and training variables of ultramarathon runners who had always had regular menstrual cycles and who had either current or prior oligo/amenorrhea, or both.

	Always regular (n=15)	Current or prior oligo/amenorrhea (n=10)
Age (yr)	35.5 ±3.2	34.8 ±4.3
Mass (kg)	58.3 ±7.9	57.2 ±7.7
Height (cm)	165.2 ±6.5	165.4 ±6.8
BMI ($kg.m^{-2}$)	21.4 ±2.4	20.9 ±2.0
DBMI ($kg.m^{-2}$)	4.1 ±2.6	5.0 ±3.4
Body fat (%)	29 ±4	29 ±4
Fat mass (kg)	16.6 ±4.1	16.6 ±3.3
Lean body mass (kg)	41.1 ±5.8	40.7 ±5.7
Running training		
- minimum $km.wk^{-1}$	44 ±14	58 ±29
- maximum $km.wk^{-1}$	68 ±21	76 ±33
Personal best race time (min)		
56 km	334 ±18	322 ±42
21.1 km	104 ±8	98 ±12
Marathon training (yr)	4.1 ±2.3	3.8 ±3.6
Energy expenditure ($MJ.d^{-1}$)	14 ±3	15 ±3

Data are presented as means ±SD. Statistical analysis: unpaired *t*-test.
Abbreviations: BMI = Body Mass Index; DBMI = Delta Body Mass Index

Table 2.2 Variables related to the past and present menstrual and reproductive status of ultramarathon runners who had always had regular menstrual cycles and who had either current or prior oligo/amenorrhea, or both.

	Always regular (n=15)	Current or prior oligo/amenorrhea (n=10)
Menarcheal age (yr)	12.9 ±1.3	13.0 ±1.8
Menstrual History Index (periods.yr^{-1} since 13 yr)	11.6 ±0.6	9.4 ±2.1**
Estimated no. periods per year:		
13-20 yr of age	12.3 ±2.2	9.8 ±2.1***
21-30 yr of age #	11.5 ±0	10.0 ±2.1 ***
31-39 yr of age #	11.5 ±0 (n=14)	8.5 ±3.5 (n=8) ***
Total no. periods	261 ±42	209 ±66 *
Years amenorrheic##	0	3.2 ±3.7
Years oligomenorrheic##	0	2.7 ±2.2
Serum estradiol (pMol.l^{-1})	608 ±367	395 ±397
Months of breastfeeding	6.2 ±7.9	10.7 ±14.3
Number of children	1.6 ±1.0	1.2 ±1.7

Data are presented as means ±SD. Statistical analysis by unpaired *t*-test except for variables marked #: Kolmogorov test and ##: no statistical test.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

Table 2.3 Bone mineral densities of ultramarathon runners who had always had regular menstrual cycles and who had either current or prior oligo/amenorrhea, or both.

	Always regular (n= 15)	Current or prior oligo/amenorrhea (n=10)
Lumbar Spine ($g.cm^{-2}$)	1.088 ±0.069	0.946 ±0.098*
Femur:		
Neck ($g.cm^{-2}$)	0.872 ±0.110	0.817 ±0.088
Trochanter ($g.cm^{-2}$)	0.740 ±0.121	0.671 ±0.057
Intertrochanter ($g.cm^{-2}$)	1.115 ±0.150	1.032 ±0.108
Total hip ($g.cm^{-2}$)	0.964 ±0.133	0.889 ±0.083
Wards triangle ($g.cm^{-2}$)	0.702 ±0.133	0.673 ±0.085

Data are presented as means ±SD. Statistical analysis: unpaired *t*-test.
* $p<0.001$

Table 2.4 Dietary intake and history of dairy product intake of ultramarathon runners who had always had regular menstrual cycles and who had either current or prior oligo/amenorrhea, or both.

	Always regular (n=15)	Current or prior oligo/amenorrhea (n=10)
Total energy ($kJ.d^{-1}$)	7164 ±1894	6479 ±1557
Calcium ($mg.d^{-1}$)	709 ±459	774 ±350
Phosphorus ($g.d^{-1}$)	1089 ±409	1022 ±381
Protein ($g.d^{-1}$)	65 ±21	52 ±17
Fat ($g.d^{-1}$)	69 ±30	51 ±25
Carbohydrate ($g.d^{-1}$)	172 ±57	198 ±35
Fibre ($g.d^{-1}$)	14 ±6	19 ±6 *
Urinary calcium ($mmol.2\ hr^{-1}$)	0.25 ±0.19	0.21 ±0.14
Portions of dairy products per week:		
Past year	5.2 ±4.6	7.7 ±5.5
On leaving school	5.9 ±8.0	6.3 ±6.3
During high school	6.5 ±5.21	7.2 ±5.8
During junior school	7.5 ±5.7	6.1 ±4.5
Total portions/4	6.2 ±4.7	6.6 ±4.5

Data are presented as means ±SD. Statistical analysis: unpaired *t*-test.

* $p<0.05$

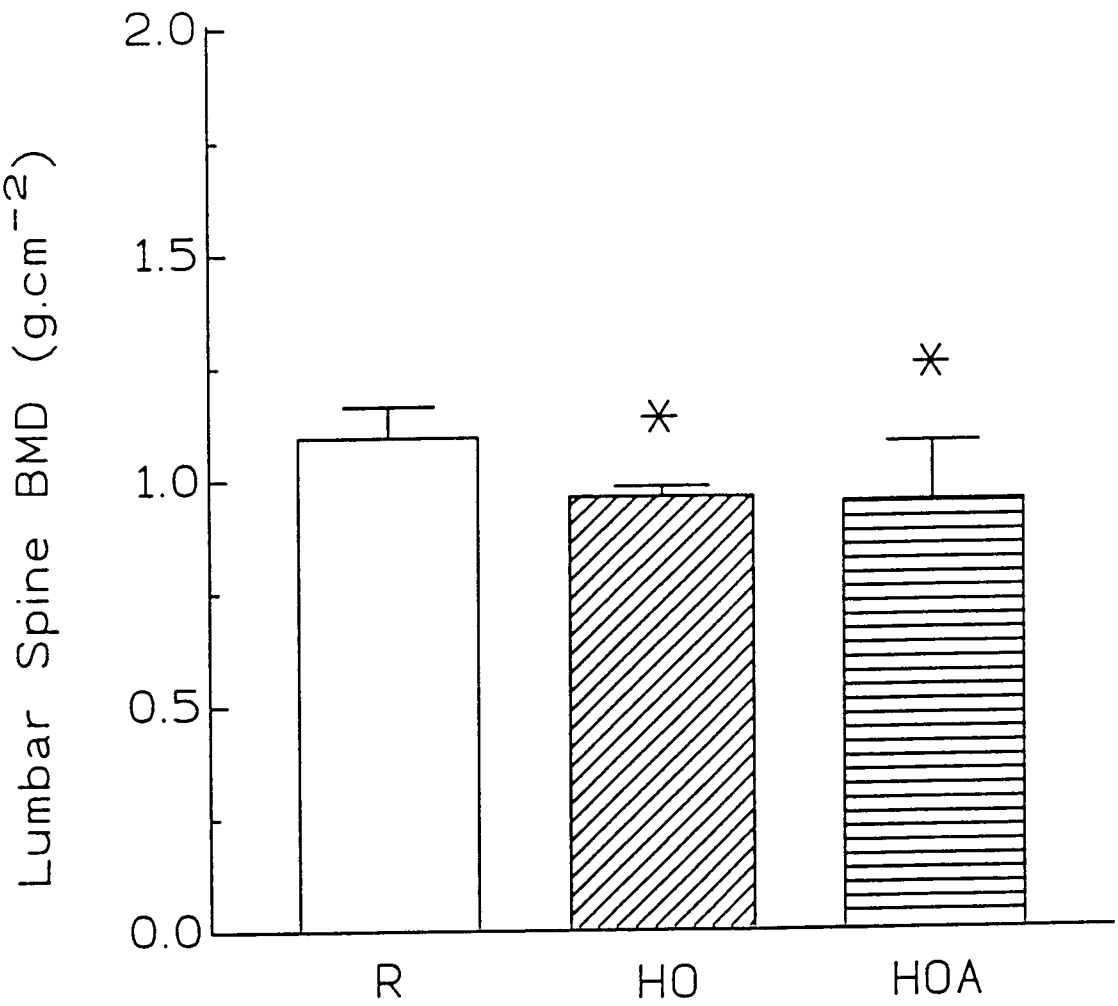

Fig 2.1: Lumbar spine bone mineral density (BMD) of premenopausal long distance runners who were divided into 3 groups according to previous menstrual status. Data presented as mean ±SD. Statistical analysis by Kruskal-Wallis test. Subjects who had a history of oligomenorrhea with no history of amenorrhea (HO; n=4) were significantly (* $p<0.005$) lower than regular subjects (n=15). Subjects who had a history of either amenorrhea or both amenorrhea and oligomenorrhea (HOA; n=6) were also significantly (* $p<0.005$) lower than regular, but not different from HO.

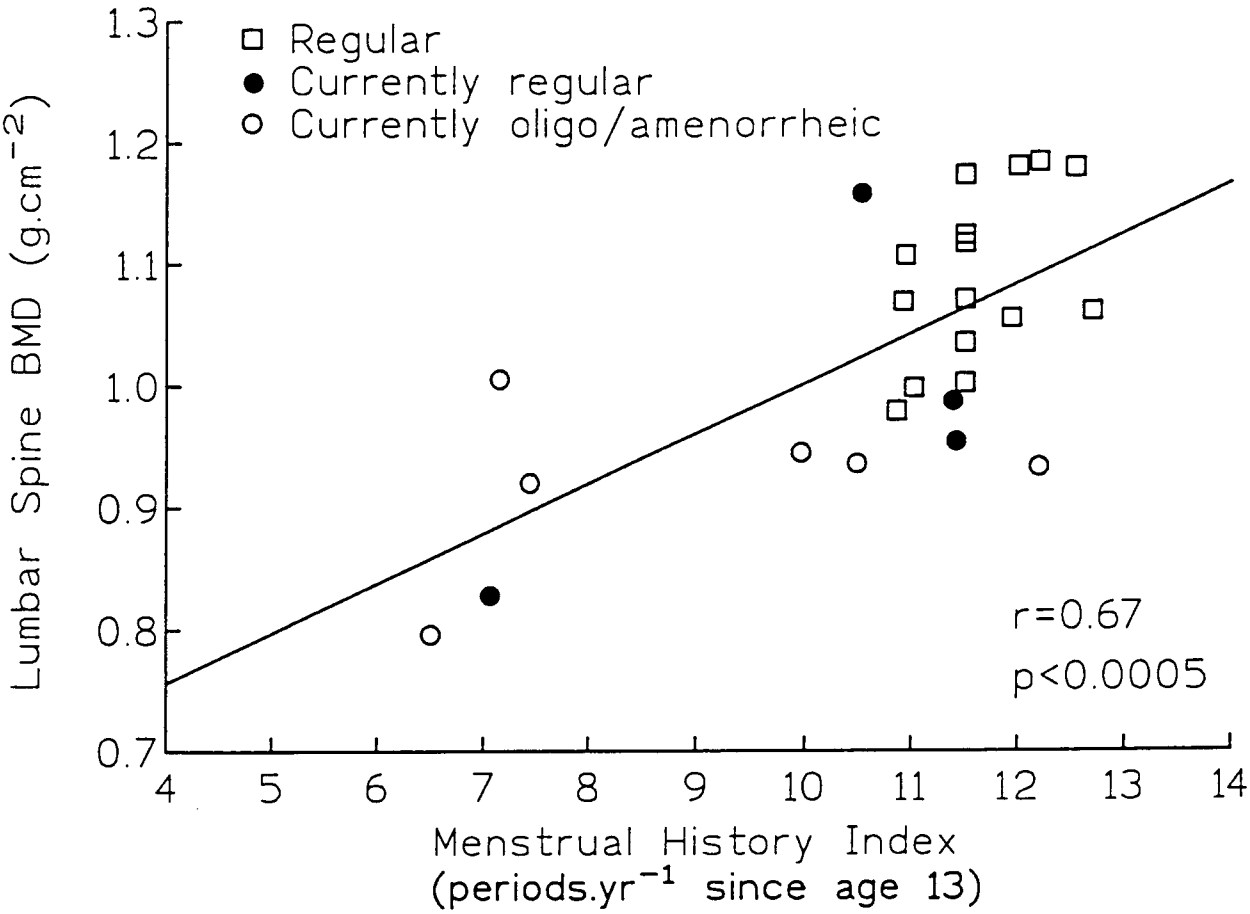

Fig 2.2: Lumbar spine bone mineral density (BMD) of premenopausal long distance runners relative to their Menstrual History Index, which represents an estimation of the average number of menstrual periods per year since age 13 years. A Pearson-product moment correlation coefficient of r=0.67 was highly significant (p<0.0005).

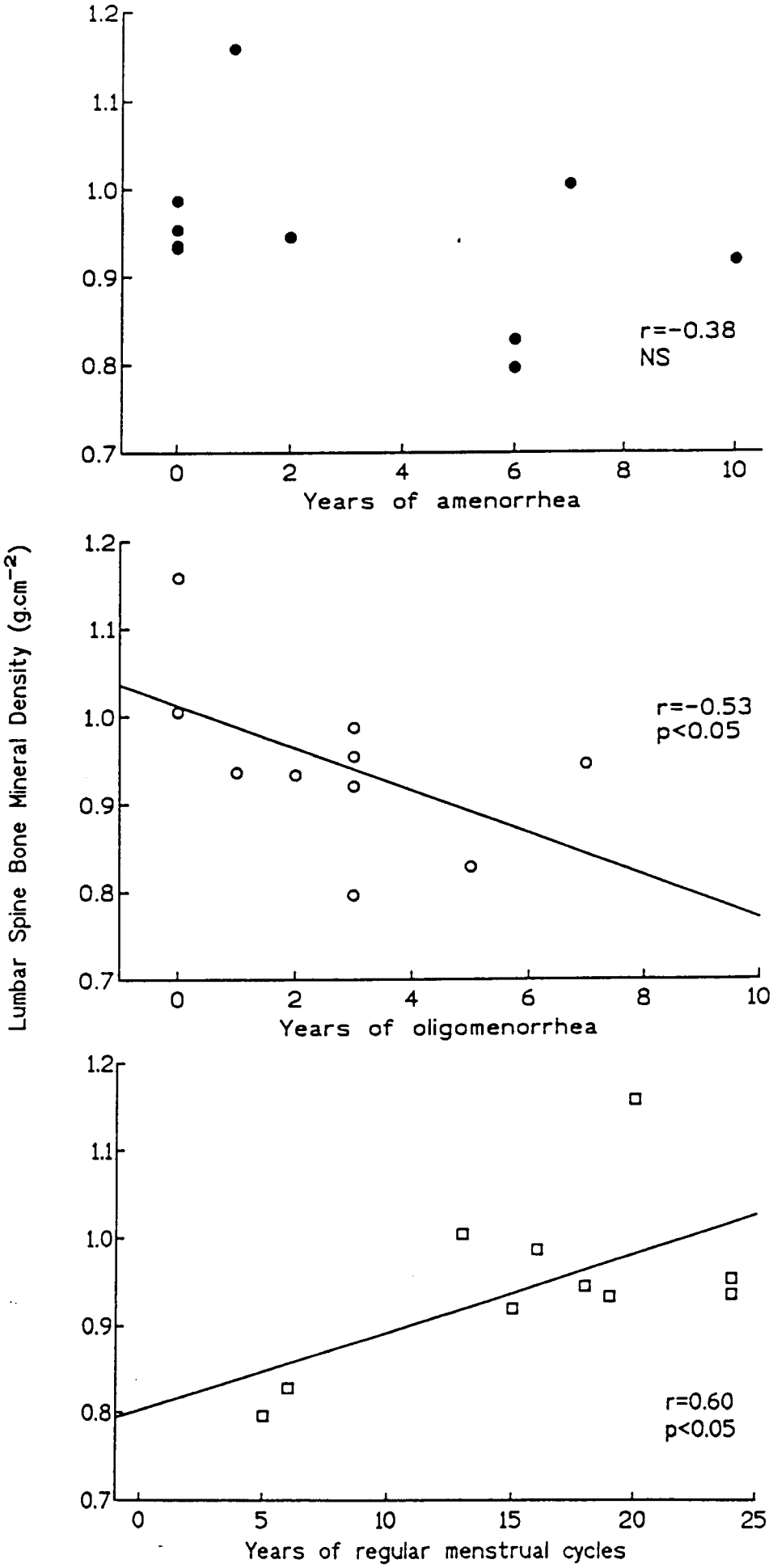

Fig 2.3: The lumbar spine BMD of those subjects with a history of oligo/amenorrhea (n=10) was correlated with the reported years of regular menstrual cycles, oligomenorrhea and amenorrhea. Pearson-product moment correlation coefficients showed that the relationship between LS BMD and years of amenorrhea did not reach significance in this small group of subjects, but that there was a significant ($p<0.05$) positive correlation between LS BMD and years of regular menstrual cycles and a significant negative correlation between LS BMD and years of oligomenorrhea ($p<0.05$).

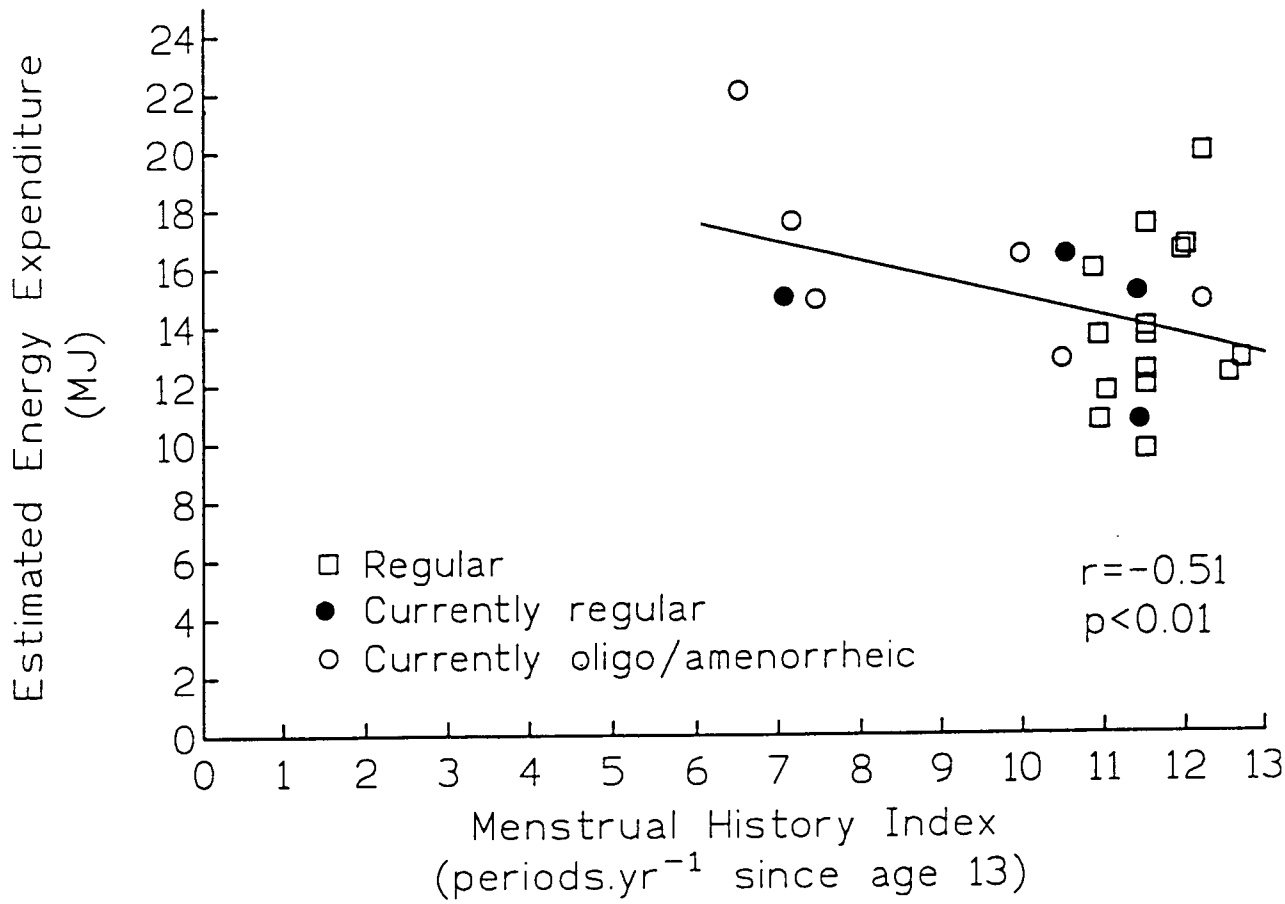

Fig 2.4: Menstrual History Index (MHI) of premenopausal long distance runners relative to their estimated total energy expenditure per day. A Pearson-product moment correlation coefficient of r= -0.51 was significant ($p<0.01$).

iv. DISCUSSION

The major contributions of this study were to show that in mature, premenopausal, long distance runners (i) a history of oligo/amenorrhea influences present bone mineral density (BMD) of the lumbar spine regardless of resumption of regular menstrual cycles in some subjects, and (ii) a history of oligomenorrhea, without any history of amenorrhea, is significantly related to low lumbar spine BMD.

These data were collected from a cross-sectional sample of endurance trained women. When interpreting the data, the cross-sectional nature of the study must be taken into consideration. However, several previous studies which have also compared BMD of groups of runners on a cross-sectional basis have provided important insights into factors related to premature osteopenia in athletes (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Drinkwater et al., 1990; Myburgh et al., 1990; Myburgh et al., 1993).

The athletes recruited for this study were generally older, heavier and "slower" than the subject samples used in other similar studies. Although all the women had completed an ultramarathon there was a large range in mass, % body fat and race times between the athletes. Few of the subjects were training at a competitive level and there was no selection criteria based on performance as was used in the study by Marcus et al. (1985) in which all the subjects had to have completed a marathon race within 3 hours as well as running distances totalling more than 65 km/week. Average % body fat and weight of the amenorrheic subjects in the Marcus et al. (1985) study was 10.0 ± 1.1% and 53.8 ± 1.6 kg respectively, while the subjects with current or prior oligo/amenorrhea in this study had an average % body fat of 29.0 ± 4% and average weight of 57.2 ± 7.7 kg. Although personal best 10 km racing times were not recorded in this study, personal best race time for a half marathon of 104 ± 8 mins and 98 ±12 mins for eumenorrheic and

oligo/amenorrheic runners were obtained. Extrapolation of the personal best 10 km times of eumenorrheic and amenorrheic athletes in a study by Drinkwater et al. (1984) of 46 ±1.5 mins and 42 ± 5 mins respectively would be generally faster than the athletes in our study.

Many studies have found menstrual irregularity, particularly amenorrhea, to be associated with low lumbar spine BMD (Cann et al., 1984; Drinkwater et al., 1984; Drinkwater et al., 1990). In our study only 2 of the OA group (20%) were currently amenorrheic and hypoestrogenic, 4 were oligomenorrheic (40%) and 4 had resumed regular menstrual cycles (40%); however lumbar spine BMD of the OA group was still significantly lower than that of the R group. The longitudinal study of Drinkwater et al. (1986) showed that resumption of menses in previously amenorrheic athletes is beneficial to lumbar spine BMD. Our cross-sectional data indirectly supports this evidence, since the lumbar spine BMD of our currently regular subjects was less different from R ($p<0.07$) than that of our currently oligo/amenorrheic subjects ($p<0.001$). However, further research is required since only 4 subjects in our study had resumed regular menses, one of whom still had a very low LS BMD despite four consecutive years of regular menses (See Fig. 2.2).

Since the women in our study were all mature (29-39 years of age) and at, or close to their peak adult bone mass, our data can be interpreted to imply that the peak adult bone mass of the lumbar spine of women with a history of oligo/amenorrhea may never reach that of women who have always had regular menstrual cycles. It is not possible to distinguish whether this is due to bone loss or a lack of bone accretion, or both. Nevertheless, it has been suggested previously that factors which influence peak adult bone mass are similar to the risk factors for postmenopausal osteoporosis (Ott, 1990), including, in particular, a low level of circulating estrogen. To determine whether a history of amenorrhea is more detrimental to lumbar spine BMD than is a history of

oligomenorrhea, we divided the oligo/amenorrheic group into those who had current or prior amenorrhea (HOA) and those who had current or prior oligomenorrhea, but no history of amenorrhea (HO). There was no difference in the lumbar spine BMD between the HOA and HO groups and they were both highly significantly ($p<0.005$) (see Fig. 2.1) lower than the group who had always had regular menstrual cycles. Therefore, a history of oligomenorrhea should not be considered to be less detrimental to lumbar spine BMD than a history of amenorrhea.

There was a large difference between subjects in the length of time of oligo/amenorrhea which ranged from 1 to 13 years. We, therefore, pooled all the athletes into one group and related their BMD and risk factors for bone loss to their overall Menstrual History Index. The Menstrual History Index (MHI) is a measure of the average number of periods per year from age 13 to current age. As such it provides a numerical score which is influenced by the number of years of amenorrhea, oligomenorrhea and regular menstrual cycles. In contrast to the menstrual index of Grimston et al. (1990a), it also takes into account the periods missed by subjects with a menarche later than age 13 years and the "additional periods" of those subjects with a menarche earlier than age 13 years. The range of MHI scores for subjects in the R group was between 10.9 and 12.7 periods per year. This variation from the value of 11.5 (as the assumed number of periods per year during years reported as regular) is due to differences in menarcheal age. The range for subjects in the OA group was between 6.5 and 12.2 periods per year. This may be an overestimation, due to the assumptions of 1.5 and 7 periods per year for amenorrhea and oligomenorrhea respectively. However, the average for the oligo/amenorrheic group (9.4 $\pm$2.1) cannot be considered to be particularly low. Hence, these subjects had all menstruated regularly for considerable periods of time. In this regard, it is interesting to note that menstrual *regularity* (MHI is an index of the number of periods experienced rather than the number of periods missed) correlated better ($r=0.67$) with LS BMD than

did either years of amenorrhea (not significant) or years of oligomenorrhea (r= -0.58). This result may have been influenced by the large number of subjects who had always had regular menstrual cycles. We therefore further investigated this important finding by correlating LS BMD with MHI and the components of MHI in only those subjects who had experienced oligo/amenorrhea. These results showed that in this small group of subjects, years of regular menstrual cycles and years of oligomenorrhea correlated significantly with LS BMD ($p<0.05$), but years of amenorrhea did not (see Fig. 2.3). Nevertheless, the influence of the time periods of regularity were not sufficient to bring their LS BMD to the same level as the R group, despite 3 of the subjects reporting oligo/amenorrhea greater than 10 years prior to the study.

The MHI does not take into account at what age the menstrual irregularity was experienced or the time span since the last episode of menstrual irregularity. On average, the reproductive age of our subjects spanned over 20 years. We subdivided this age span into 3 time periods (13-20 yr of age, n=25 subjects; 21-30 yr, n=25 subjects and above 30 yr, n=22 subjects) to determine whether or not oligo/amenorrhea during any particular age was more closely related to bone loss, than the overall MHI. The number of periods per year for the decades from age 20 years to 30 years, and from 30 years to 39 years (or to current age), were both significantly lower in OA than R. Although the correlations between BMD of the lumbar spine and the estimated number of periods per year for the three specified age ranges were good, the best simple correlation was between overall MHI and BMD of the lumbar spine. These data show that the entire menstrual history is the important determinant of LS BMD.

Differences in LS BMD between groups R and OA could not be ascribed to differences in parity or lactation history. Sowers et al. (1993) found that women who breastfed for more than 6 months had reduced BMD of the lumbar spine and the femoral neck, but that

BMD of the lumbar spine returned to baseline levels within 12 months of stopping breastfeeding. In the present study there was no relationship between months of breastfeeding and BMD of the lumbar spine or the proximal femur. However, a subject who had been breastfeeding continuously for 3 years and was also amenorrheic had the third lowest spinal BMD, suggesting that breastfeeding may have been another factor, in addition to her prior history of oligo/amenorrhea, explaining her particularly low BMD.

Although menstrual history is the primary determinant of lumbar spine BMD, other variables such as body mass and weight-bearing may impact on BMD, particularly of the lower limbs. In the present study, the BMD of the left proximal femur tended to be lower in group OA vs. group R ($p=0.07$), but was not significantly correlated with any of the variables related to menstrual history, including the MHI. Although no significant difference was recorded between groups OA and R in the proximal femur and the other components of the hip, this may be due to small sample size, and therefore low statistical power, rather than the identity of the two groups. Recently, two studies have reported lower BMD in the femoral shaft of runners with amenorrhea or oligo/amenorrhea (Drinkwater et al., 1990; Myburgh et al., 1993). Drinkwater et al. (1990) reported lower femoral shaft BMD (measured below the lesser trochanter) in runners who had never had regular menstrual cycles and who had histories of oligomenorrhea or amenorrhea, or both. However, the difference was no longer significant when the data were corrected for lower body mass in the oligo/amenorrheic group. Nevertheless, Myburgh et al. (1993) reported lower proximal femur and mid-femoral shaft BMD in amenorrheic athletes who were very well matched with their control group for body mass. Major differences between the subjects in those studies and our current study, are the severity of menstrual irregularity, which was less severe in the current study, as well as the average body mass, which was higher in this study. Since femoral BMD was significantly related to BMI in our current study, it would appear that not only absolute body mass, but also body mass

relative to height are factors influencing lower limb BMD. Fat free body mass has previously been found to be the most significant predictor of bone mineral content in a group of athletes from a variety of sports (Heinrich et al., 1990), but was not related to femoral BMD in our study. Neither was mesomorphy, a measure of relative muscularity, related to BMD. This is probably because the subjects in our study were relatively homogenous compared with those in the study by Heinrich et al. (1990). The mechanism whereby higher body mass and BMI may influence BMD of the lower limbs is by imposing a larger mechanical load during weight-bearing exercise. According to Wolff's Law, bone mass will increase to meet the demands imposed by mechanical loading (Bassett, 1971), and longitudinal studies have now provided evidence that exercise training is, indeed, a stimulus for increasing bone mineral density (Dalsky et al., 1988; Margulies et al., 1986) and preventing bone loss (Krolner et al., 1983). In our subjects, it may be that the combination of relatively high body mass and weekly training load were sufficient to offset the influence of a history of oligo/amenorrhea on bone loss of the proximal femur. Further research is required to definitively prove this hypothesis.

It has previously been shown that several different risk factors, including combinations of low BMI, large amounts of exercise, low energy intake and vegetarianism, have an additive negative effect on menstrual regularity (Myburgh et al., 1992). In the present study, we determined those variables which were statistically related to Menstrual History Index and thus also indirectly influenced lumbar spine BMD. Total estimated energy expenditure per day was significantly negatively correlated with the MHI (Fig. 4). This implies that those women who were currently more physically active had the longer history of oligo/amenorrhea and that the currently less physically active women were more likely to have had a longer history of menstrual regularity. Therefore, although weight-bearing activity is associated with increased bone density (Dalsky et al., 1990; Risser et al., 1990), excessive exercise may be associated with a higher incidence of

oligo/amenorrhea, resulting in reduced BMD. Thus, this study supports the concept that physical activity leading to high daily energy expenditure, coupled with insufficient energy intake, has an indirect effect on BMD through an influence on menstrual regularity.

The positive correlation between total energy expenditure and DBMI (defined as highest adult BMI - lowest adult BMI), suggests that those subjects with the highest level of daily physical activity may be using exercise as a mechanism to control their body mass. In addition, it is highly likely that our subjects experienced a certain amount of energy drain, since the discrepancy between total energy expenditure and the reported energy intake was high. However, there was no relationship between % body fat and MHI, thus supporting the data of several other studies (Drinkwater et al., 1984; Loucks et al., 1984; Marcus et al., 1985; Sanborn et al., 1987; Rutherford, 1993;) who found no association between athletic amenorrhea and body fat. The effect of exercise and dietary patterns on oligo/amenorrhea therefore does not necessarily manifest in a low % body fat, but may manifest in large fluctuations in body mass.

In summary, this study of mature, premenopausal runners investigated the relative importance of various risk factors for osteopenia in predicting the current bone status. The data from this study indicate that a history of amenorrhea is less important than the overall history of oligomenorrhea and amenorrhea, as well as the number of years of regular menstrual cycles since age 13 years, in determining current bone status of the lumbar spine. Childbearing and breastfeeding histories were unrelated to BMD.

Although physical activity can be beneficial to bone health, this study suggests that even short episodes of oligo/amenorrhea are likely to have a negative influence on peak adult bone mass. Although these conclusions are significant in the understanding of osteopenia, they need to be substantiated by studies with larger sample sizes.

ADDENDUM TO STUDY 1

Fig 2.2 shows that only 4 of the 10 OA subjects had a low MHI. Four additional subjects were included with those in study 1 in order to determine if the conclusions made in Study 1 would still hold with a larger number and a wider distribution of subjects in the OA group.

i MATERIALS AND METHODS

Several women from a local running club who were participating in competitive marathon running were contacted and invited to take part in the study. Of the 25 women who were contacted, 5 women responded. It is not known whether the non-responders did not fit the inclusion criteria or whether they were unconcerned about their BMD, while those who did respond may have had musculoskeletal complaints that they wanted investigated. Inclusion criteria included i) being between the age of 29 and 45 yr, ii) being premenopausal, iii) not currently taking oral contraceptives and iv) having experienced current or previous menstrual irregularities. Of these 5 women, only 4 women completed all the tests necessary for inclusion in the study.

Information concerning menstrual history was obtained using the same forms as those filled in by subjects in study 1 in order to determine number of years of regular menstruation, number of years of oligomenorrhea, number of years of amenorrhea, and consequently the Menstrual History Index (MHI). All subjects had an osteodensitometry scan which was performed in the Department of Nuclear Medicine, Groote Schuur Hospital using a Hologic QDR-1000 (version 4.20) dual energy x-ray bone densitometer (Hologic Inc., Waltham, Massachusetts). Scans were made of the lumbar spine and the left proximal femur. Average bone mineral densities ($g.cm^{-2}$) were determined for lumbar vertebrae 1 through 4 and the total proximal femur, Ward's triangle, femoral neck, greater trochanter, and the intertrochanteric space. One subject did not return her questionnaire and was excluded from analyses.

Data Analysis

Unpaired Student's t-tests were performed to compare data from runners who had always had regular menstrual cycles (R, n=15), and those with current or previous menstrual irregularity (OA, n=14). Pearson-product moment correlation coefficients were calculated to determine which menstrual variables were significantly correlated with lumbar spine BMD. These

menstrual variables were entered into a stepwise multiple regression to determine the best single predictor of lumbar spine BMD. Kruskal-Wallis tests were performed to compare the lumbar spine BMD data of three groups after dividing the subjects into those women who had always had regular menstrual function (R), those who had oligo/amenorrhea in the past but who had now resumed regular menstrual cycles (CR, n=6) and those women who were currently oligo/amenorrheic (COA, n=8). Kruskal-Wallis tests were also performed to compare the lumbar spine data of three groups who had always had regular menstrual cycles (R), those who had a history of oligomenorrhea with no history of amenorrhea (HO, n=7), and those women who had a history of either amenorrhea or both amenorrhea and oligomenorrhea (HOA, n=7).

ii RESULTS

With the inclusion of the 4 extra subjects, data was now available on fifteen subjects who had always had regular menstrual cycles (R) and fourteen subjects with a history of oligo/amenorrhea (OA). Subjects ranged in age from 29-45 years. There was still no difference in age (R:35.5 ± 3.2 vs OA: 36.2 ± 5.1 yr), body mass (R: 58.3 ± 7.9 vs OA: 56.6 ± 6.6 kg) and BMI (R: 21.4 ± 2.4 vs OA: 21.0 ± 2.0 $kg.m^{-2}$) between the two groups, and none were significantly different from values in the original study.

Of the four subjects who had been added to the oligo/amenorrhea (OA) group, two were still currently irregular, while two had regained regular menses. The overall MHI was significantly lower in OA than R (R: 11.6 ± 0.6 vs OA: 9.1 ± 2.2, $p < 0.001$).

The mean BMD of the lumbar spine for R was 1.088 ± 0.069 $g.cm^{-2}$ and for OA was 0.951 ± 0.085 $g.cm^{-2}$ ($p<0.001$) and BMD of the left proximal femur was 0.964 ± 0.133 $g.cm^{-2}$ for R and 0.891 ± 0.071 $g.cm^{-2}$ for OA ($p=0.08$).

Lumbar spine BMD for the whole sample of women (1.022 ± 0.10 $g.cm^{-2}$) correlated significantly with overall MHI ($p=0.0001$, $r=0.67$) (see Fig 2.5), as well as total number of years of amenorrhea ($p<0.01$, $r= -0.53$), total number of years of oligomenorrhea ($p=0.01$, $r=-0.44$), and total number of years of regular menstruation ($p<0.001$, $r=0.61$). The best single predictor of the BMD of the lumbar spine was MHI (stepwise multiple regression: $r^2=0.45$, $p=0.0001$).

For the sample of women with a history of menstrual irregularity (OA: n=14), lumbar spine BMD did not correlate significantly with overall MHI ($r=0.47$), years of amenorrhea ($r=-0.41$), or years of oligomenorrhea ($r=-0.12$), while there was a significant relationship between lumbar spine BMD and years of regular menstruation ($p<0.05$, $r=0.59$)(see Fig 2.6).

After division of the OA group into those who were currently oligo/amenorrheic (COA) and those who were currently regular (CR) but previously oligo/amenorrheic, we found that BMD of the lumbar spine was significantly higher in the regularly menstruating runners (R) who had never experienced any menstrual irregularities compared to the COA subjects (R: 1.088 ± 0.069 vs COA: 0.926 ± 0.060 $g.cm^{-2}$; p=0.0001) as well as when compared to the CR subjects (R: 1.088 ± 0.069 vs CR: 0.985 ± 0.110 g.cm-2; p=0.0001). Lumbar spine BMD was not different between the two subgroups COA and CR.

After division of the OA subjects into those who had a history of oligomenorrhea but no history of amenorrhea (HO) and those who had a history of either amenorrhea or both oligomenorrhea and amenorrhea (HOA), we found that BMD of the lumbar spine was significantly lower in HOA subjects compared to R (R: 1.088 ± 0.069 vs HOA: 0.938 ± 0.120 $g.cm^{-2}$; p<0.001), as well as in the HO group compared to R (R: 1.088 ± 0.069 vs HO: 0.965 ± 0.030 $g.cm^{-2}$; p<0.001). Lumbar spine BMD was not different between the two subgroups HO and HOA (see Fig 2.7).

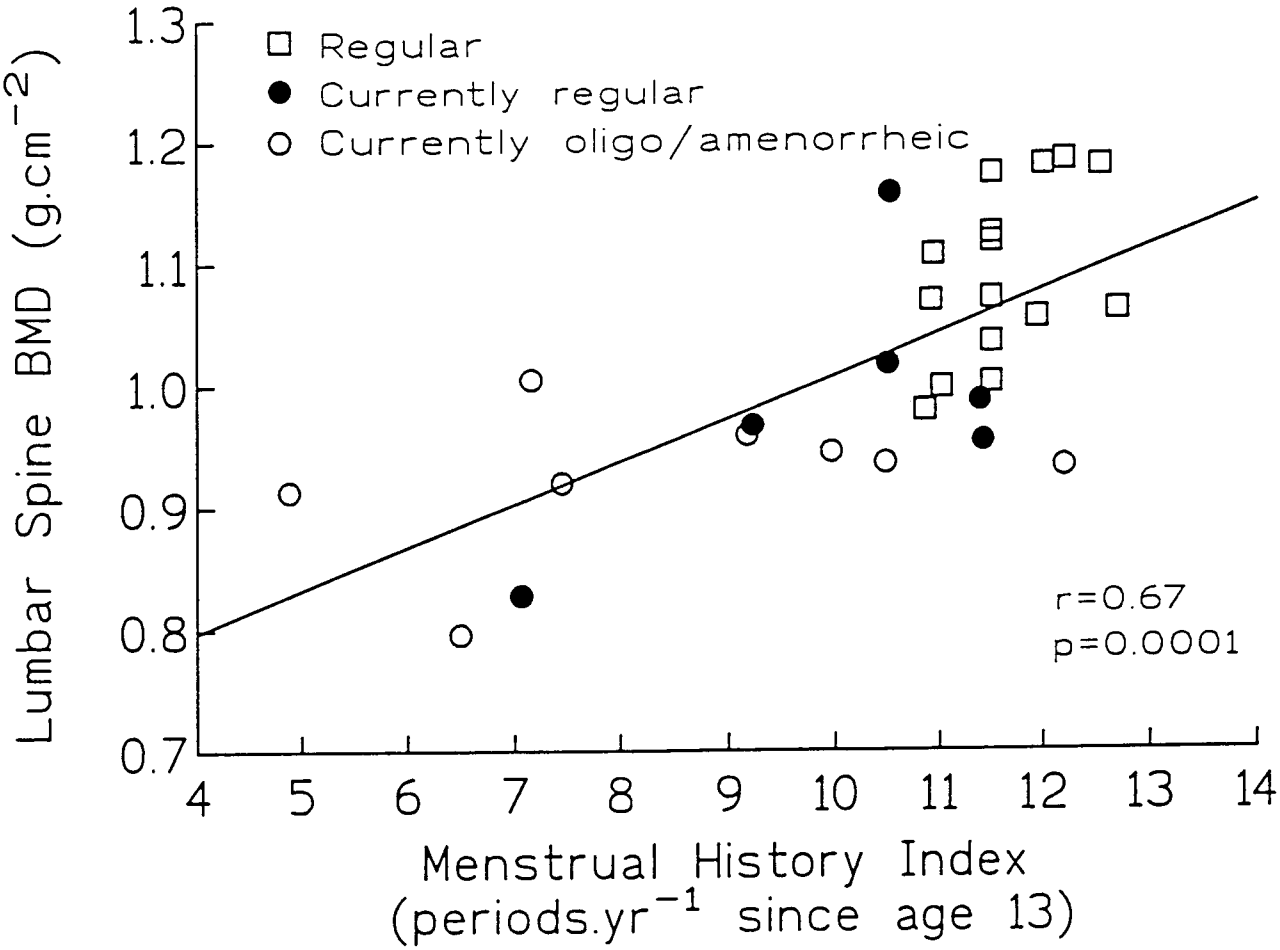

Fig 2.5: Lumbar spine bone mineral density (BMD) of premenopausal long distance runners relative to their Menstrual History Index. A Pearson-product moment correlation coefficient of r=0.67 was highly significant (p=0.0001).

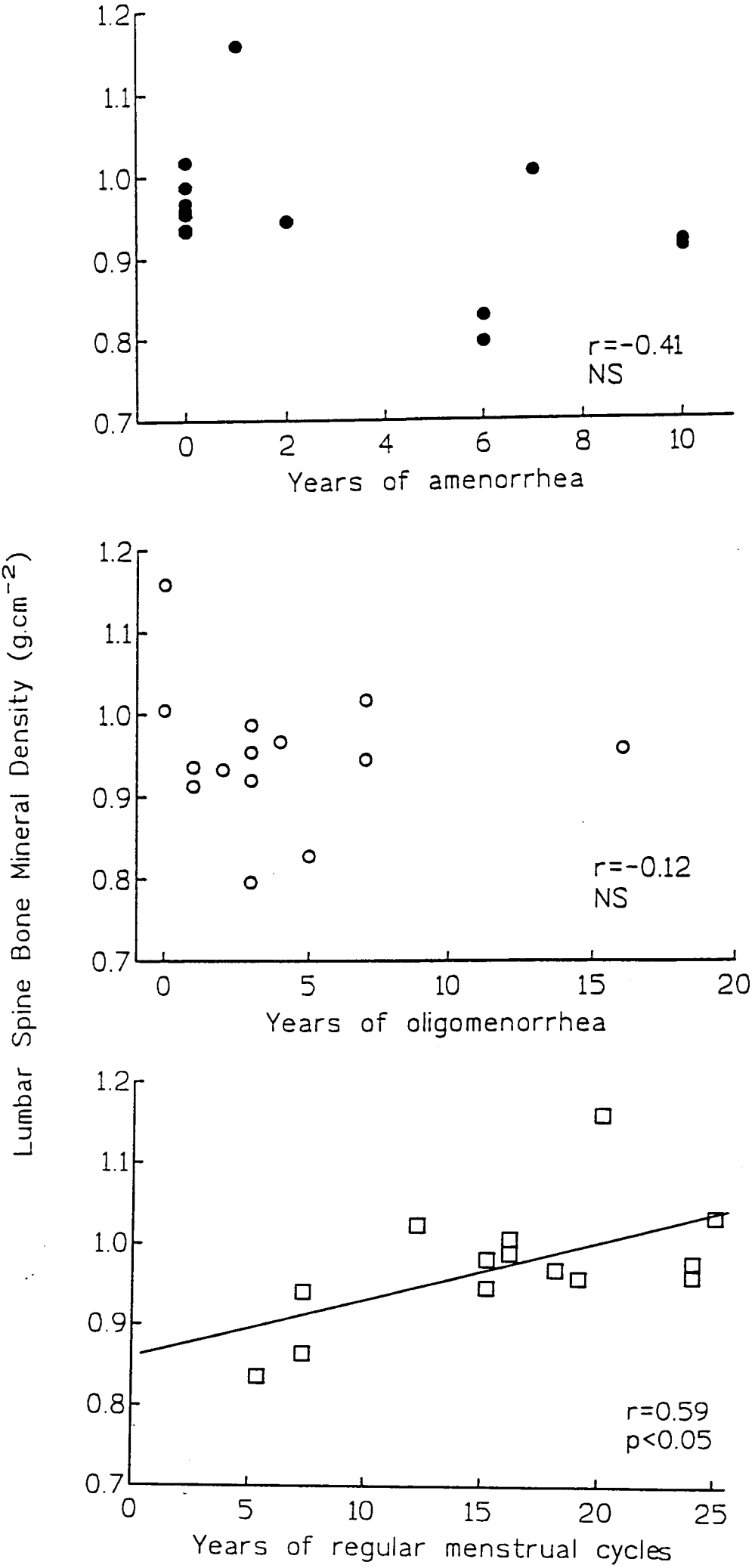

Fig 2.6: The lumbar spine BMD of those subjects with a history of oligo/amenorrhea (n=14) was not correlated with the reported years of regular menstrual cycles, oligomenorrhea or amenorrhea.

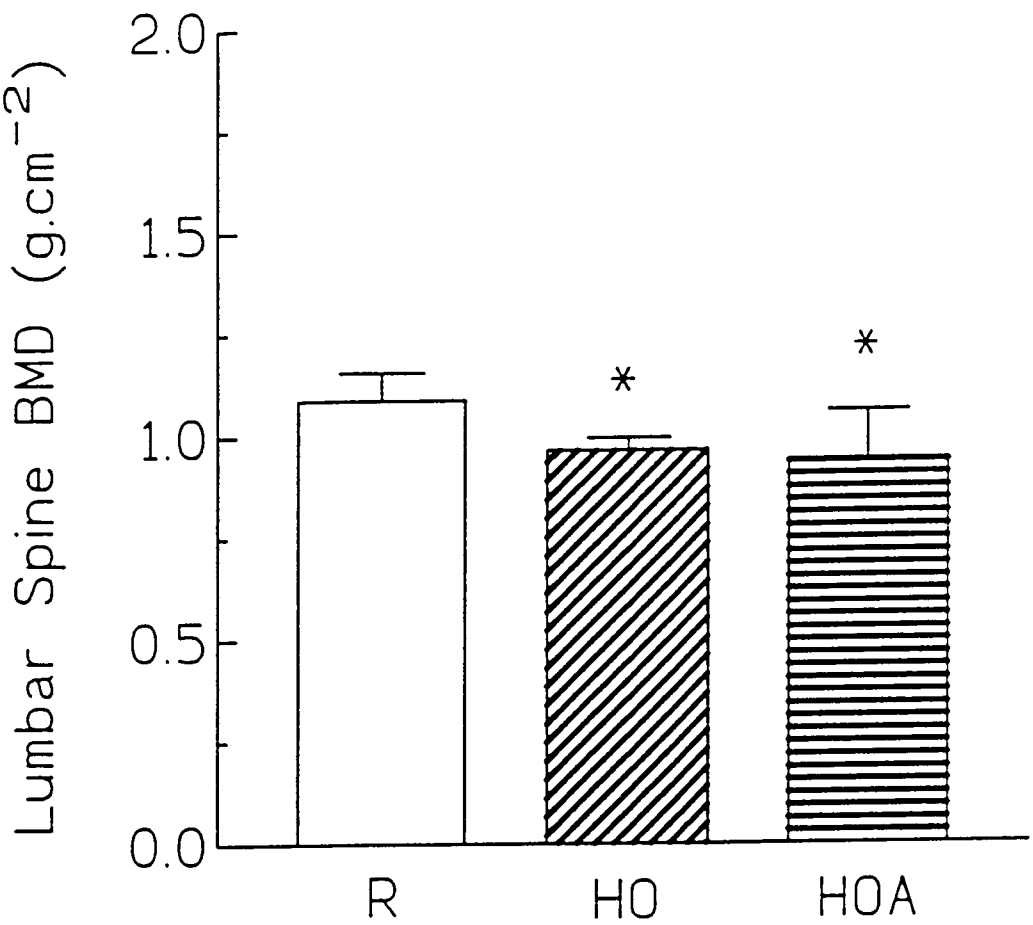

Fig 2.7: Lumbar spine bone mineral density (BMD) of premenopausal long distance runners who were divided into 3 groups according to previous menstrual status. Data presented as mean±SD. Statistical analysis by Kruskal-Wallis test. Subjects who had a history of oligomenorrhea but no history of amenorrhea (HO; n=7) were significantly (*$p<0.001$) lower than regular subjects (n=15). Subjects who had a history of either amenorrhea or oligomenorrhea (HOA; n=7) were also significantly (*$p<0.001$) lower than regular subjects, but not different from HO.

iii DISCUSSION

The results of the addendum are able to substantiate and solidify some of the major conclusion of study 1. The four additional subjects included provided a broader and more continuous spectrum of MHI values and any doubt that the findings of study 1 were a result of a small group of extremely low MHI values at the extreme of a wide distribution, can be extinguished. Similar subject numbers in the regularly menstruating group and the group with current and/or prior oligo/amenorrhea should prevent any bias that may have resulted from a larger number of subjects who had always had regular menstrual cycles in study 1. However, the possibility that the athletes with menstrual irregularity who responded may not reflect the overall population of athletes with menstrual dysfunction, but rather those who were concerned about their bone health, cannot been eliminated.

Once again overall MHI correlated better with lumbar spine BMD ($r=0.67$, $p=0.0001$) than did any of the other menstrual variables viz. years of regular menstrual cycles ($r=0.61$, $p<0.001$), years of amenorrhea ($r=-0.53$, $p<0.01$) and years of oligomenorrhea ($r=-0.44$, $p=0.01$). The only menstrual variable that was significantly correlated with lumbar spine BMD for those subjects with a history of oligo/amenorrhea ($n=14$) was years of regular menstrual cycles ($r=0.59$; $p<0.05$). This suggests once again that the number of periods experienced rather than the number of periods missed is important in the determination of bone mineral density. However, although lumbar spine BMD of the runners who had resumed regular menses (CR) was somewhat higher than the runners who were currently oligo/amenorrheic (COA), this was not significant and both groups had a significantly lower lumbar spine BMD than the regularly menstruating runners (R). These data suggest that, although resumption of menses is beneficial to the bone mineral density of the lumbar spine, any history of menstrual irregularity is still harmful to bone health.

Once again we found no difference in lumbar spine BMD between those subjects who had current or prior amenorrhea (HOA) and those who had current or prior oligomenorrhea but no

history of amenorrhea. However, both groups were significantly lower than those subjects who had never experienced menstrual irregularities, suggesting that a history of oligomenorrhea may be as detrimental to the lumbar spine as a history of amenorrhea, and that any history of menstrual irregularity will place one at risk of reduced bone mineral density.

iv CONCLUSIONS OF STUDY 1

The findings of the addendum support the significant findings of study 1 and show that in mature women distance runners low lumbar spine BMD is related to a history of oligo/amenorrhea and that a significant history of oligomenorrhea should not be considered to be less harmful to lumbar spine BMD than a history of amenorrhea. Menstrual regularity and the number of menstrual periods experienced may be more important than the number of periods missed in the determination of BMD. However, even those women who have resumed regular menses still have a distinctly lower lumbar spine BMD compared to women who have always had regular menstrual periods.

CHAPTER 3

Long term accretion of bone mineral density in premenopausal women with prior menstrual irregularity

STUDY 2

i. INTRODUCTION

We concluded from the previous study that a history of oligo/amenorrhea, regardless of the resumption of regular menstrual cycles, is associated with low lumbar spine BMD. Indeed, no studies have shown full restoration of lumbar spine BMD in previously oligo/amenorrheic subjects relative to controls. The large increases in lumbar spine BMD (6.3 % in 15.5 months; Drinkwater et al., 1986); (14.4 % over 2 years; Jonnavithula et al., 1993) have been shown in young groups of subjects (27.9 ± 2.0 yr; Drinkwater et al., 1986); (20.4 ± 5.7 yr; Jonnavithula et al., 1993). Therefore, the aim for study 2 is to determine whether (i) a history of oligo/amenorrhea, in relatively older women who have regained and *maintained* regular menses for a relatively long period of time since their episode/s of menstrual irregularity, will have a less pronounced influence on lumbar spine BMD, or (ii) the previously significant influence of oligo/amenorrhea on lumbar spine BMD would no longer exist when compared with women who have never had irregular menstrual periods.

Although it is accepted that large amounts of bone mass are attained during puberty (Bonjour et al., 1991), with an additional smaller gain in BMD into the late 20's (Recker et al., 1992a), in exercising women bone acquisition may continue for longer. There is controversy regarding the age at which bone loss begins (Buchanan et al., 1988; Lindsay

et al., 1993; Szejnfeld et al., 1993). There is evidence to show that sedentary women start to lose bone prior to menopause (Buchanan et al., 1988; Bonjour et al., 1991). Limited cross-sectional data seem to show that this may not apply to exercising women (Brewer et al., 1983), however no longitudinal data have confirmed this. There is also evidence to suggest that young women who have had amenorrhea but regain regular menses have a partial restitution of BMD (Drinkwater, 1989). However, it is not known whether exercising women with a history of menstrual irregularity will be able to regain BMD *throughout* their premenopausal years and eventually obtain a peak bone mass similar to their regularly menstruating peers.

ii HYPOTHESES OF STUDY 2

We hypothesise

i) that in more mature women distance runners with a history of menstrual irregularity (even if regular menstrual cycles have been regained for a longer period of time) there will still be a significantly lower lumbar spine BMD than in women who have had no history of menstrual irregularity,

ii) that the sedentary control group will have a lower lumbar spine BMD than the regularly menstruating runners, but a higher lumbar spine BMD than the runners with current menstrual irregularity and/or a history of oligo/amenorrhea, and

iii) that bone mass continues to be acquired during the fourth decade in exercising women., but that sedentary women may begin to lose bone mass during this time.

ii MATERIALS AND METHODS

Subjects

We attempted to contact all forty women who had been randomly selected for the first study three years previously. Some of the reasons for not participating in the second study included leaving the country (n=5), current pregnancy (n=2), and no longer taking part in regular running (n=5). We were unable to contact a further five women and four women were not willing to participate in another study. We therefore also contacted 15 women who had participated in a similar study five years previously, and from this sample 3 subjects were recruited and the rest of the questionnaires were returned to the sender due to change of address. A final sample of 22 runners agreed to participate in the study. Regular competitive running was not a requirement, but all the subjects were required to be running at least 3-4 times/week. Most subjects were also participating in some other form of exercise.

A control group of sedentary women were recruited by obtaining names and addresses of women who had undergone a bone density scan at Groote Schuur Hospital in the previous 3-5 years. The database contained only sixty women who were of similar age and had served as healthy controls for previous research. These women were all contacted, however exclusion criteria for the current study included: participating in regular exercise at present or in the last five years, presently menopausal or post-menopausal, and the experience of current or previous menstrual irregularities. A final sample of only 8 sedentary control (SC) women qualified and agreed to participate in the study. None of the sedentary control group had participated in formal exercise during adulthood.

Subject details including age, height, highest adult body weight, lowest adult body weight, training (where applicable), menstrual history and the use of oral contraceptive medications, were obtained from an initial questionnaire (see Appendix 1). Body Mass Index (BMI) and change in BMI (DBMI) were calculated as follows:

$$BMI = mass*height^{-2} \ (kg.m^{-2})$$

$$DBMI = \text{highest adult BMI - lowest adult BMI} \ (kg.m^{-2})$$

Protocol

An osteodensitometry scan was performed in the Department of Nuclear Medicine, Groote Schuur Hospital using a Hologic QDR-1000 (version 4.20) dual-energy x-ray bone densitometer (Hologic Inc., Waltham, MA). The machine is calibrated daily to assure proper operation of the system, by the scanning of an anthropomorphic spine phantom. There was a percentage coefficient of variation (CV) over the period during which the first scans were done of 0.44% (61 scans) and a CV of 0.36% (169 scans) during the period when the second scans were done. The difference in the mean value obtained for the phantom spine from time period 1 to time period 2 was 0.0006 $g.cm^{-2}$. An in-house study done at the Department of Nuclear Medicine, Groote Schuur Hospital, investigated the in-vivo precision of an Hologic QDR-1000 Bone Densitometer and the accuracy of the operator. Test-retest reproducibility was 0.99 for 20 women. Rate of change was calculated using the computers COMPARE function. Scans were made of the lumbar spine and the left proximal femur. Average bone mineral densities ($g.cm^{-2}$) were determined for lumbar vertebrae 1 through 4 and the total proximal femur, Ward's triangle, femoral neck, greater trochanter, and the intertrochanteric space. For 10 of the 29 scans of the proximal femur, an accurate value of the Ward's triangle could not be found, so this particular area was excluded from further investigation.

Values for bone mineral content (BMC, g) and projected area (A_p, cm^2) of the lumbar spine obtained from the bone scan were used to calculate bone mineral apparent density (BMAD, $g.cm^{-3}$) of L_2-L_4 (Carter et al., 1992). This is a more accurate method of comparing the density of bones of different sizes than the more traditional method of measuring BMD based on area only. $BMAD = BMC*(A_p^{-1.5})$.

Each subject was then interviewed and required to complete a detailed questionnaire in order to obtain more detailed information about their current menstrual status and previous menstrual history (see Appendix 2). Information included age at menarche, the estimated number of periods per year since menarche, the use of oral contraceptives, fertility drugs or hormone injections, as well as whether they had undergone procedures such as a hysterectomy or ovariectomy. Information on reproductive history, such as parity and total months of breastfeeding, was also obtained. This information was used to calculate the following:

(i) Menstrual History Index (modified from Grimston et al., 1990). This was calculated to determine the estimated number of periods per year since age 13.

Menstrual History Index = $(11.5*R + 7*O + 1.5*A)/(C-13)$ where:

R = number of years of regular menstrual cycles (defined as 10-13 menstrual periods per year and assuming an average of 11.5 $periods.yr^{-1}$);

O = number of years of oligomenorrhea (defined as 4-9 menstrual periods per year and assuming an average of 7 $periods.yr^{-1}$);

A = number of years of amenorrhea (defined as 0-3 menstrual periods per year and assuming an average of 1.5 $periods.yr^{-1}$);

C = current age.

(ii) The Menstrual History Index was then divided into the estimated number of periods per year for the following three time periods:

13-20 years of age

21-30 years of age (or 21 to present age)

31-40 years of age (or 31 to present age)

41 to present age

Information about previous dairy product intake was obtained from the questionnaire and was reported as the estimated number of portions consumed per week during junior school, high school, on leaving school and over the past year. One portion was equivalent to 1 cup of milk, 175 ml of yoghurt, a rounded scoop of ice-cream, a 250 g tub of cottage cheese, and a cheese meal or sandwich.

All subjects were also required to complete a 3 day dietary record in order to determine total energy intake (MJ) per day, as well as daily intake of calcium (mg), phosphorus (mg), protein (g), fat (g), carbohydrate (g), and fibre (g). Dietary intake was recorded on 2 week days and 1 weekend day and analysed using the food quantities tables of the National Research Institute for Nutritional Diseases (Parow, South African Medical Research Council Publications Unit, 1986) and a computerised dietary analysis programme (Foodfinder, Medical Research Council, Parow, South Africa).

Daily energy expenditure ($MJ.d^{-1}$) was estimated from a seven day activity diary. Subjects were required to divide the day into number of hours spent sleeping (Level 1), somewhat active, including activities such as leisurely walking, standing, driving and

reading (Level 1.5), active, including activities such as brisk walking, sweeping and mopping (Level 4), very active, including activities such as brisk uphill walking and climbing stairs (Level 6), and extremely active, including running (Level 9). This method was modified from the interview method of estimating daily energy expenditure used by Blair et al. (1985).

Each subject then visited the laboratory where body composition was measured and calculated according to the method of Durnin and Wormesley (1974). Skinfold measurements were taken by the same investigator, in all cases, on the right side at the triceps, biceps, subscapular, and suprailiac sites. Fat mass, % body fat, and fat-free mass were calculated. Somatotype was assessed using the Heath-Carter method (1967).

Venous blood samples were then drawn between 9.30 and 9.45 am from subjects who had not exercised that morning, in order to determine osteocalcin concentration by means of a specific radioimmunoassay performed on serum samples (OSTK-PR Kit, CIS bio international, ORIS Group, France). The principle is based on competition between osteocalcin radiolabelled with iodine-125 and osteocalcin contained in the standards or samples to be assayed for a given limited number of anti-osteocalcin antibody sites. At the end of the incubation period, the amount of radiolabelled osteocalcin bound to the antibody is inversely proportional to the amount of non-radiolabelled osteocalcin originally present in the assay.

Twenty-four hour urine samples were obtained from all the subjects and an aliquot was immediately frozen and stored in the dark. The concentration of free deoxypyridinoline cross-links (DPD), corrected for urinary concentration of creatinine, was determined by the Pyrilinks-D assay (Metra Biosystems, Inc., USA). The accuracy of this method has been confirmed due to a strong correlation of Pyrilinks-D values with total DPD

measured by HPLC (r=0.93), and with collagen crosslinks measured by Pyrilinks^{TM} (Metra Biosystems, Inc.)(r=0.96).

Data analysis

One-way ANOVA's were performed to compare data from runners who had always had regular menstrual cycles (R, n=12), runners with current or previous menstrual irregularity (OA, n=9), and sedentary controls who had always had regular menstrual cycles (SC, n=8). One-way ANOVA's were also performed to compare changes in mass, MHI and the different bone parameters in the three groups since the last study. ANCOVA's co-varying for age and age at menarche, together and separately, were performed when comparing years of regular menstruation and total number of periods of the three groups. ANCOVA's co-varying for age and body mass, together and separately, were performed when comparing the bone parameters of the three groups. ANCOVA's co-varying for age, current body mass, and change in body mass, together and separately, were performed when comparing the annualised changes in the bone parameters of the three groups. Kruskal-Wallis tests were performed to compare the BMD data of three groups of runners after dividing the subjects into those runners who had always had regular menstrual function (R), those who had oligo/amenorrhea in the past but who had now resumed regular menstrual cycles (CR, n=7), and those runners who were currently oligo/amenorrheic (COA, n=2). Kruskal-Wallis tests were also performed to compare the BMD data of three groups of runners after dividing the runners into those women who had always had regular menstrual cycles (R), those who had a history of oligomenorrhea with no history of amenorrhea (HO, n=5), and those runners who had a history of either amenorrhea or both amenorrhea and oligomenorrhea (HOA, n=4). Pearson-product moment correlation coefficients were calculated to determine which variables were significantly correlated with BMD, and change in BMD, for the whole sample of women,

as well as between selected variables to determine if they had an indirect effect on BMD. Pearson-product moment correlation coefficients were also calculated to determine which menstrual variables were significantly correlated with lumbar spine BMD in the sub-group of runners with current and/or previous menstrual irregularity (OA). Several physical characteristics (mass, BMI, DBMI, % body fat) which showed the largest simple correlations with BMD were entered into a stepwise selection in order to determine the most significant predictor/s of lumbar spine BMD from this category. A similar procedure was followed for the menstrual history variables in which total estimated periods per year from 21-30 years of age, total number of years of oligomenorrhea, total number of years of amenorrhea, and MHI were entered into the stepwise selection. Lumbar spine BMAD was calculated for all the women and ANOVA's were performed to compare the data of the three groups. Pearson-product moment correlation coefficients were calculated between lumbar spine BMAD and several physical characteristic variables (current body mass, height, BMI and DBMI), and the same variables were entered into a stepwise variable selection in order to determine the most significant predictor/s of lumbar spine BMAD.

iii RESULTS

Comparison of groups

Physical characteristics (Table 3.1)

Twelve runners (R) and eight sedentary controls (SC) had always been regular (10-13 periods.yr^{-1}), and nine runners had a history of oligo/amenorrhea, (OA, 0-9 periods.yr^{-1}). Subject no. 12 was an outlier and was excluded from all statistical analyses due to a change in lumbar spine BMD larger than 2 standard deviations above that for the group, as well as a DBMI value above 2 standard deviations above that of the group (see Table 3.1). Subjects ranged in age from 29 to 46 yr of age. The oligo/amenorrheic (OA) runners were significantly younger than the sedentary control group (OA: 35.9 ± 4.4 vs SC: 41.6 ± 3.1 yr; $p=0.01$) but did not differ in age from the regular group (R: 39.0 ± 3.8 yr). Body mass of the sedentary controls was significantly higher than the regularly menstruating runners (SC: 63.3 ± 8.6 vs R: 56.3 ± 7.9 kg; $p<0.05$) and the oligo/amenorrheic runners (SC: 63.3 ± 8.6 vs OA: 54.4 ± 3.8 kg; $p<0.05$). The BMI of the sedentary controls was significantly higher than the regular group (SC: 23.9 ± 3.4 vs R: 20.4 ± 1.8 kg.m^{-2}; $p<0.05$) and the oligo/amenorrheic group (SC: 23.9 ± 3.4 vs OA: 20.2 ± 1.5 kg.m^{-2}; $p<0.05$). The DBMI (highest - lowest BMI) was not significantly different among the groups. The anthropometric measurement of endomorphy was significantly higher in the sedentary controls than the other two groups. The mesomorphic component was similar between the three groups. The sedentary control group had a significantly lower measure of ectomorphy than the other two groups. Calculated % body fat was significantly lower in the oligo/amenorrheic group than the regularly menstruating runners and the sedentary controls (OA: 25.0 ± 3.8 vs R: 28.1 ± 3.6; or SC: 34.2 ± 2.8 %; $p<0.001$). The sedentary control group had a significantly

higher fat mass than the other two groups (SC: 21.8 ± 4.8 vs R: 15.8 ± 3.2; or OA: 13.7 ± 2.7 kg; $p<0.001$). There was no difference in fat free mass (FFM) among the three groups.

TABLE 3.1 Physical characteristics of ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Age (yr)	35.9 ± 4.4	39.0 ± 3.8	41.6 ± 3.1	p<0.01 OA vs SC
Mass (kg)	54.4 ± 3.8	56.3 ± 7.9	63.3 ± 8.6	p<0.05 OA vs SC p<0.05 R vs SC
Height (cm)	164.3 ± 4.1	165.8 ± 7.3	162.9 ± 5.9	NS
BMI ($kg.m^{-2}$)	20.2 ± 1.5	20.4 ± 1.8	23.9 ± 3.4	p<0.05 OA vs SC p<0.05 R vs SC
DBMI ($kg.m^{-2}$)	5.2 ± 2.2	3.0 ± 1.7	4.9 ± 2.6	NS
Endomorphy	3.5 ± 1.1	4.0 ± 0.9	6.3 ± 1.3	p<0.001 OA vs SC p<0.001 R vs SC
Mesomorphy	3.8 ± 1.3	3.7 ± 0.8	4.7 ± 1.0	NS
Ectomorphy	3.2 ± 0.9	3.2 ± 0.9	1.7 ± 1.2	p<0.01 OA vs SC p<0.01 R vs SC
Body fat (%)	25.0 ± 3.8	28.1 ± 3.6	34.2 ± 2.8	p<0.001 OA vs R vs SC
Lean body mass (kg)	40.8 ± 2.5	40.3 ± 5.6	41.5 ± 4.1	NS

Data are presented as means ± SD. Statistical analysis: one-way ANOVA.

Current menstrual status and history (Table 3.2)

Of the 9 subjects with a history of oligo/amenorrhea, 7 had regained regular menses, while one woman was currently amenorrheic and another was currently oligomenorrheic. The remaining seven OA subjects had been consecutively regular for between 1 and 25 years (11.7 ± 7.9 yr). There was no difference in age at menarche among the three groups (see Table 3.2). Years of regular menstruation, after co-varying for age, was significantly different among all the groups (OA: 15.2 ± 7.9 vs R: 25.9 ± 4.4 vs SC: 29.4 ± 2.8 yrs; $p<0.001$), however after co-varying for age at menarche as well as age, there was no

longer a significant difference in years of regular menstruation between the regularly menstruating runners and the sedentary controls. The oligo/amenorrheic group had a total of 4.9 ± 4.2 years of oligomenorrhea, and the mean years of amenorrhea was 2.4 ± 3.6 years. The oligo/amenorrheic group had a total of 217.7 ± 78.8 periods since menarche, which was significantly ($p<0.01$) less than the regularly menstruating runners (298.0 ± 50.6) after co-varying for age. Both groups had significantly ($p<0.01$) less total periods than the sedentary control group (337.8 ± 31.9) after co-varying for age. After co-varying for age and age at menarche, the three groups were still significantly different ($p<0.01$). The MHI was significantly lower in OA than R and SC (OA: 9.1 ± 2.0 vs R: 11.3 ± 0.5; or SC: 11.8 ± 0.4; $p<0.001$). The MHI of OA subjects was significantly different from the other two groups between age 13 and 20 ($p<0.01$), age 21 and 30 ($p=0.001$), and between age 31 and 40 ($p<0.01$). Only 2 women from the oligo/amenorrheic group were over the age of 40, and both of them were currently regular.

Reproductive status (Table 3.2)

Parity (number of pregnancies) and months of breastfeeding were similar in all three groups (OA: 1.0 ± 1.7 vs R: 1.3 ± 1.2 vs SC: 1.5 ± 1.1 children) and (OA: 7.2 ± 11.6 vs R: 6.0 ± 12.6 vs C: 9.3 ± 9.2 months). Four runners were currently taking oral contraceptives, 2 in the oligo/amenorrheic group, and 2 in the group that had always had regular menstrual cycles. None of the women in the sedentary control group were currently taking an oral contraceptive. There was no difference among the groups for total years on oral contraception (OA: 5.9 ± 5.4 vs R: 6.0 ± 4.4 vs SC: 8.5 ± 4.4 yr). One sedentary control subject had had a hysterectomy 2 years previously, but not an ovariectomy. She still experienced the symptoms associated with ovulation and the premenstrual period every month. Five women had undergone tubuligation.

TABLE 3.2 Variables related to the past and present menstrual and reproductive status of ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Menarcheal age (yr)	13.3 ± 1.7	13.3 ± 1.2	12.4 ± 1.1	NS
Menstrual History Index (periods.yr^{-1} since 13 yr)	9.1 ± 2.0	11.3 ± 0.5	11.8 ± 0.4	p<0.001 OA vs R p<0.001 OA vs SC
Estimated no. Periods.yr^{-1}:				
13-20 yr of age	8 .4 ± 3.6	10.9 ± 1.9	12.5 ± 1.7	p<0.01 OA vs R p<0.01 OA vs SC
21-30 yr of age	9.3 ± 3.1	11.5 ± 0	11.5 ± 0	p=0.01 OA vs R p=0.01 OA vs SC
31-40 yr of age	9.6 ± 2.4 (n=8)	11.5 ± 0	11.5 ± 0	p<0.01 OA vs R p<0.01 OA vs SC
41-46 yr of age	11.5 ± 0 (n=2)	11.5 ± 0 (n=5)	11.5 ± 0 (n=4)	NS
Total no. Periods #	217.7 ± 78.8	298.0 ± 51.0	337.8 ± 31.9	p<0.01 OA vs R vs SC
Years regular #	15.2 ± 7.9	25.9 ± 4.4	29.4 ± 2.8	p<0.001 OA vs R vs SC
Years amenorrheic	2.4 ± 3.6	0	0	p=0.01 OA vs R p=0.01 OA vs SC
Years oligomenorrheic	4.9 ± 4.2	0	0	p<0.001 OA vs R p<0.001 OA vs SC
Months of breastfeeding	7.2 ± 11.6	6.0 ± 12.6	9.3 ± 9.2	NS
Number of children	1 ± 1.7	1.3 ± 1.2	1.5 ± 1.1	NS
Total years on oral contraception	5.9 ± 5.4	6 ± 4.4	8.5 ± 4.4	NS

Data are presented as means ± SD. Statistical analysis: one-way ANOVA.
One-way ANCOVA, co-varying for age

Exercise status (Table 3.3)

Maximum weekly training distance (km.wk^{-1}) during any competitive season (ie. not necessarily the year of the study) was significantly higher in the oligo/amenorrheic runners than the regularly menstruating runners (OA: 109 ± 37 vs R: 72 ± 25 km.wk^{-1};

p=0.01). More than 70% of the runners participated in other activities such as cycling, swimming and walking, but running was their primary exercise activity. There was no difference between the two groups of runners for total daily estimated energy expenditure (OA: 10.8 ± 1.9 vs R: 11.8 ± 2.6 $MJ.d^{-1}$), and neither group was significantly different from the sedentary control group (SC:11.1 ± 3.1 $MJ.d^{-1}$). However, $hours.wk^{-1}$ at level 9 (extremely active) was significantly lower in the sedentary group than the two running groups (OA: 6.6 ± 2.5, R: 5.5 ± 3.4 vs SC: 0.3 ± 0.7 $hours.wk^{-1}$; p=0.0001). There was no significant difference among the three groups at the other levels of activity. The two groups of runners did not have significantly different personal best race times for the Two Oceans 56 km-marathon (OA: 301.4 ± 46.1 vs R: 326.6 ± 20.5 min) or a standard half marathon (21.1 km) (OA: 93.6 ± 13.2 vs R: 99.4 ± 10.3 min), and years of running training (OA: 7.1 ± 3.9 vs R: 7.3 ± 3.0 yr). None of the subjects in the sedentary control group had taken part in any formal exercise program during adulthood.

TABLE 3.3 Exercise histories and total estimated energy expenditure of ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Energy expenditure($MJ.d^{-1}$)	10.8 ± 1.9	11.8 ± 2.6	11.1 ± 3.1	NS
$Hrs.wk^{-1}$: level 1.5	79.0 ± 27.7	75.8 ± 19.2	89.1 ± 16.8	NS
level 4	20.4 ± 20.3	18.9 ± 12.1	19.6 ± 21.3	NS
level 6	5.3 ± 5.9	8.1 ± 9.1	3.7 ± 4.4	NS
level 9	6.6 ± 2.5	5.5 ± 3.4	0.3 ± 0.7	p<0.001 OA vs SC R vs SC
Maximum $km.wk^{-1}$	108.9 ± 36.8	72.1 ± 25.2	0	p=0.01 OA vs R
Personal best race time (min)				
56 km	301.4 ± 46.1 (n=8)	326.6 ± 20.5 (n=10)	0	NS
21.1 km	93.6 ± 13.2 (n=9)	99.4 ± 10.3 (n=11)	0	NS
Marathon training (yr)	7.1 ± 3.9	7.3 ± 3.0	0	NS

Data are presented as means ± SD. Statistical analysis: one-way ANOVA.

Bone mineral density (Table 3.4)

After co-varying for age and mass, lumbar spine BMD of the oligo/amenorrheic runners was significantly lower than the lumbar spine BMD of the regularly menstruating runners and the sedentary controls, who were not different (OA: 0.948 ± 0.070 vs R: 1.043 ± 0.100; or SC: 1.094 ± 0.077 $g.cm^{-2}$; $p<0.05$). There was a significant difference between OA and SC for BMAD (OA: 0.125 ± 0.009 vs SC: 0.146 ± 0.010 $g.cm^{-3}$;$p<0.01$) but neither were significantly different from R (0.135 ± 0.010 $g.cm^{-3}$). BMAD of those women who had oligo/amenorrhea in the past but who had now resumed regular menstrual cycles (0.123 ± 0.008 $g.cm^{-3}$) was significantly different from BMAD for the sedentary control group. The three groups did not differ for BMD of the proximal femur (total, neck, trochanter, and intertrochanter).

TABLE 3.4 Bone parameters of ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Lumbar Spine BMD ($g.cm^{-2}$) #	0.948 ± 0.071	1.043 ± 0.103	1.094 ± 0.077	p<0.05 OA vs R p<0.05 OA vs SC
Lumbar Spine BMAD ($g.cm^{-3}$)	0.125 ± 0.010	0.135 ± 0.010	0.146 ± 0.010	p<0.01 OA vs SC
Femur:				
Neck ($g.cm^{-2}$)	0.837 ± 0.066	0.843 ± 0.106	0.773 ± 0.075	NS
Trochanter ($g.cm^{-2}$)	0.669 ± 0.063	0.712 ± 0.127	0.683 ± 0.050	NS
Intertrochanter ($g.cm^{-2}$)	1.065 ± 0.092	1.093 ± 0.179	1.039 ± 0.076	NS
Total hip ($g.cm^{-2}$)	0.913 ± 0.069	0.934 ± 0.149	0.893 ± 0.057	NS

Data are presented as means ± SD. Statistical analysis: one-way ANOVA.
One-way ANCOVA, co-varying for age and mass

Dietary intake (Table 3.5)

There were no significant differences among groups for current reported daily energy intake (OA: 6.6 ± 2.1 vs R: 7.7 ± 2.2 vs SC: 6.3 ± 1.2 $MJ.d^{-1}$) and calcium intake (OA: 772.6 ± 293.9 vs R: 936.4 ± 371.4 vs SC: 765.7 ± 159.6 $mg.d^{-1}$) or for any other measured component of the diet. However subjects from all three groups exhibited extremely low levels of total energy intake and calcium intake. A total energy intake of less than 6.5 $MJ.d^{-1}$ was present in 4 of the 9 subjects in the OA group, 5 of the 12 subjects in the R group, and 5 of the 8 subjects in the SC group. Similarly, a calcium intake of less than the RDA of 800 $mg.d^{-1}$ was present in 5 of the 9 subjects in the OA group, 5 of the 12 subjects in the R group, and 3 of the 8 subjects in the SC group. A calcium intake of less than 500 $mg.d^{-1}$ was present in 2 of the subjects in the OA group, 2 of the subjects in the R group, and 1 of the subjects in the SC group. There was no difference in dairy product intake (portions of $dairy.wk^{-1}$) among the three groups.

TABLE 3.5 Dietary intake and history of dairy product intake of ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Total energy ($MJ.d^{-1}$)	6.6 ± 2.1	7.7 ± 2.2	6.3 ± 1.2	NS
Calcium ($mg.d^{-1}$)	773 ± 294	936 ± 371	766 ± 160	NS
Phosphorus ($g.d^{-1}$)	1078 ± 256	1290 ± 378	1146 ± 206	NS
Protein ($g.d^{-1}$)	60.4 ± 13.53	69.8 ± 17.0	66.4 ± 14.6	NS
Fat ($g.d^{-1}$)	54.5 ± 23.9	65.9 ± 27.2	55.5 ± 16.6	NS
Carbohydrate ($g.d^{-1}$)	188 ± 76	222 ± 57	174 ± 41.2	NS
Fibre ($g.d^{-1}$)	16 ± 3.3	19.2 ± 5.5	17 ± 8	NS
Portions of dairy products.wk^{-1}				
Past year	15.6 ± 6.4	13.8 ± 5.0	17.8 ± 10.5	NS
On leaving school	15.3 ± 5.7	11.9 ± 5.8	12.9 ± 6.1	NS
During high school	15.1 ± 6.7	10.7 ± 5.4	11.6 ± 7.5	NS
During junior school	17.7 ± 10.6	12.9 ± 6.7	13.4 ± 8.7	NS
Total portions/4	15.9 ± 6.4	12.3 ± 4.9	13.9 ± 6.1	NS

Data are presented as means ± SD. Statistical analysis: one-way ANOVA.

Biochemical markers (Table 3.6)

Osteocalcin and DPD/Creatinine were similar in the three groups (OA: 8.3 ± 1.4 vs R: 8.4 ± 1.6 vs SC: 8.1 ± 2.9 $ng.ml^{-1}$) and (OA: 3.9 ± 1.4 vs R: 3.5 ± 1.1 vs SC: 3.8 ± 2.0 nM Dpd.mM $Creat^{-1}$). Osteocalcin values generally vary between 2 and 12 $ng.ml^{-1}$ (Gundberg, 1990). Values obtained in this study ranged from 3.5 to 12.6 $ng.ml^{-1}$. Preliminary Pyrilinks-D reference ranges established by Metra Biosystems for normal premenopausal females (n=55) over 25 years of age were 2.0-6.0 nM Dpd.mM $Creat^{-1}$. The range of values in this study was 1.7-8.4 nM Dpd.mM $Creat^{-1}$.

TABLE 3.6 Biochemical markers of bone turnover of ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Osteocalcin ($ng.ml^{-1}$)	8.3 ± 1.4	8.4 ± 1.6	8.1 ± 2.9	NS
Volume of urine (ml) per 24 hours	1661.8 ± 948.4	1443.2 ± 730.8	1908.8 ± 855.6	NS
DPD/Creatinine (nM Dpd.mM $Creat^{-1}$)	3.9 ± 1.4	3.5 ± 1.1	3.8 ± 2.0	NS

Data are presented as means ± SD. Statistical analysis: one-way ANOVA.

Analysis by correlation

Lumbar Spine BMD for the whole sample of women correlated significantly with body mass ($p<0.05$, $r=0.39$), and BMI ($p=0.01$, $r=0.46$). Lumbar Spine BMD correlated significantly with the total number of years of oligomenorrhea ($p<0.05$, $r=-0.38$), the total number of years of amenorrhea ($p=0.01$, $r=-0.45$), and the overall MHI ($p=0.01$, $r=0.45$)(Figure 3.1). Lumbar Spine BMD for the whole sample of women also correlated significantly with the estimated number of periods per year from 21-30 years of age ($p=0.01$, $r=0.46$), but not for ages 13-20 yrs or 31-39 years.

Lumbar spine BMD for the sub-group of runners with current and/or prior oligo/amenorrhea (n=9) was not significantly correlated with any of the menstrual variables. Lumbar spine BMD for groups OA and R together, correlated significantly with total number of years of amenorrhea ($p<0.05$, $r=-0.44$) and estimated number of

periods per year from 21-30 years ($p<0.05$, $r=0.44$). Neither proximal femur or neck of the femur were significantly correlated with any of the menstrual variables for the sub-group of runners with current and/or prior oligo/amenorrhea, or for the whole sample of runners.

As BMD was significantly correlated with lumbar spine BMD, we determined which factors may be correlated with BMD and thus have an indirect influence on lumbar spine BMD. BMI was positively correlated with total daily estimated energy expenditure ($p<0.001$, $r=0.73$), as well as % body fat ($p<0.001$, $r=0.73$), and DBMI ($p<0.001$, $r=0.60$).

Although there was not a significant correlation between months of breastfeeding in the women who had had children ($n=16$) and lumbar spine BMD, an r-value of -0.40 was obtained. No dietary or training variables correlated with lumbar spine BMD for the whole sample of women. No other bone parameters, including the total proximal femur, neck of the femur, greater trochanter, and the intertrochanteric space, correlated with any body composition, menstrual, training or dietary variables. BMAD for the whole sample of women correlated significantly with BMI ($p<0.05$, $r=0.41$), but not with any other physical characteristics.

Change in lumbar spine BMD since the last study correlated significantly with change in mass since the last study ($p=0.05$, $r=0.36$)(Figure 3.2), but was not significantly correlated with change in MHI, estimated portions of dairy products per week over the past year, current calcium intake, or total energy intake.

Stepwise variable selection

Two physical characteristic variables emerged from stepwise variable selection as significant predictors of lumbar spine BMD (r^2=0.36, p=0.01). The prediction equation for BMD of the lumbar spine using physical characteristic variables was:

$BMD_{lumbar\ spine}$ = (BMI x 0.03) + (DBMI x -0.02) + 0.52 (r^2=0.36)

No physical characteristic variables emerged as significant predictors of the total proximal femur, the femoral neck, the greater trochanter, or the intertrochanteric space.

One menstrual variable emerged as the most significant predictor of lumbar spine BMD (r^2=0.20, p<0.05). The prediction equation for BMD of the lumbar spine using menstrual variables was:

$BMD_{lumbar\ spine}$ = (MHI x 0.03) + 0.72 (r^2=0.20)

The two most significant predictors of BMAD were BMI and DBMI (r^2=0.30, p<0.01). The formula which best predicted BMAD was:

BMAD = (BMI x 0.004) + (DBMI x -0.003) + 0.07 (r^2=0.30)

Total number of years of amenorrhea emerged as the most significant predictor of lumbar spine BMD for the groups OA and R together (r^2=0.20, p<0.05). The formula which best predicted BMD of the lumbar spine in the sample of runners only was:

$BMD_{lumbar\ spine}$ = (Total years of amenorrhea x -0.02) + 1.02 (r^2=0.20)

Changes since previous study (Table 3.7)

There was a significantly longer period of time between the baseline and follow-up bone density scans of the sedentary control group than the other two groups, although the range for all three groups was similar: 3-5.4 years for the OA group, 3-5.5 years for the R group and 3.7-5.7 years for the SC group. OA runners lost on average 2.5 kg of body mass while the SC group gained an average of 3.6 kg of body mass, however the variation within the two groups was large and neither were significantly different from the R group. Change in MHI was significantly greater in the oligo/amenorrheic runners than the other two regularly menstruating groups, R and SC (OA: 0.74 ± 0.9 vs R: 0 ± 0.04; SC: -0.13 ± 0.26; $p<0.01$). There was no significant difference in % change per year of the bone parameters between the three groups, with or without co-varying for age, current body mass and change in mass. Although the bone parameters tended to increase in the OA group and decrease in the SC group, these differences were not significant (see dashed line, Fig 3.3) It is also apparent from Fig 3.3 that for almost all subjects the absolute change in lumbar spine BMD per year was small and essentially insignificant. Only one subject in the whole sample of women showed a >1% decrease in BMD of the lumbar spine between the baseline and follow-up scans, however three subjects showed an increase of >1% in BMD of the lumbar spine. The greatest range of % change in BMD was seen in the trochanter.

TABLE 3.7 Changes in body mass, MHI and bone mineral densities since the previous study in ultramarathon runners who had current and/or prior oligo/amenorrhea, and ultramarathon runners and sedentary controls who had always had regular menstrual cycles.

	Current/prior oligo/amenorrhea (OA, n=9)	Always Regular (R, n=12)	Sedentary controls (SC, n=8)	Significance
Change in age (yrs)	3.7 ± 0.9	3.6 ± 0.8	4.7 ± 0.7	p<0.05 OA vs SC p<0.05 R vs SC
Range	(3 to 5.4)	(3 to 5.5)	(3.7 to 5.7)	
Change in mass (kg) *	-2.53 ± 4.10	-0.61 ± 2.51	3.63 ± 7.22	p<0.05 OA vs SC
Range	(-12.5 to +1.0)	(-5 to +3)	(-6.5 to +15.0)	
Change in MHI *	0.74 ± 0.9	0 ± 0.04	-0.13 ± 0.26	p<0.01 OA vs R p<0.01 OA vs SC
Range	(-0.34 to +2.5)	(-0.1 to +0.1)	(-0.75 to +0.03)	
Change in lumbar spine (%) #	0.10 ± 0.68	-0.17 ± 0.65	-0.11 ± 0.64	NS
Range	(-0.81 to +1.08)	(-1.04 to +1.31)	(-0.83 to +1.04)	
Change in neck (%) #	0.30 ± 1.34	-0.25 ± 0.85	-0.35 ± 0.71	NS
Range	(-1.67 to +2.09)	(-1.61 to +0.93)	(-1.05 to +1.07)	
Change in trochanter (%) #	0.28 ± 1.29	0.08 ± 0.78	-0.52 ± 1.24	NS
Range	(-0.99 to +2.83)	(-1.07 to +1.14)	(-3.09 to +0.87)	
Change in intertrochanter (%) #	0.24 ± 1.21	0.52 ± 0.69	-0.18 ± 1.2	NS
Range	(-1.31 to +1.86)	(-0.42 to +1.69)	(-1.97 to +2.09)	
Change in Total Hip (%) #	0.31 ± 1.01	0.36 ± 0.58	-0.28 ± 0.90	NS
Range	(-0.98 to +1.81)	(-0.39 to +1.23)	(-1.58 to +1.39)	

* Absolute change (post test - pre test)

\# Annualised change (post test- pre test) / (no.of years since pre test)

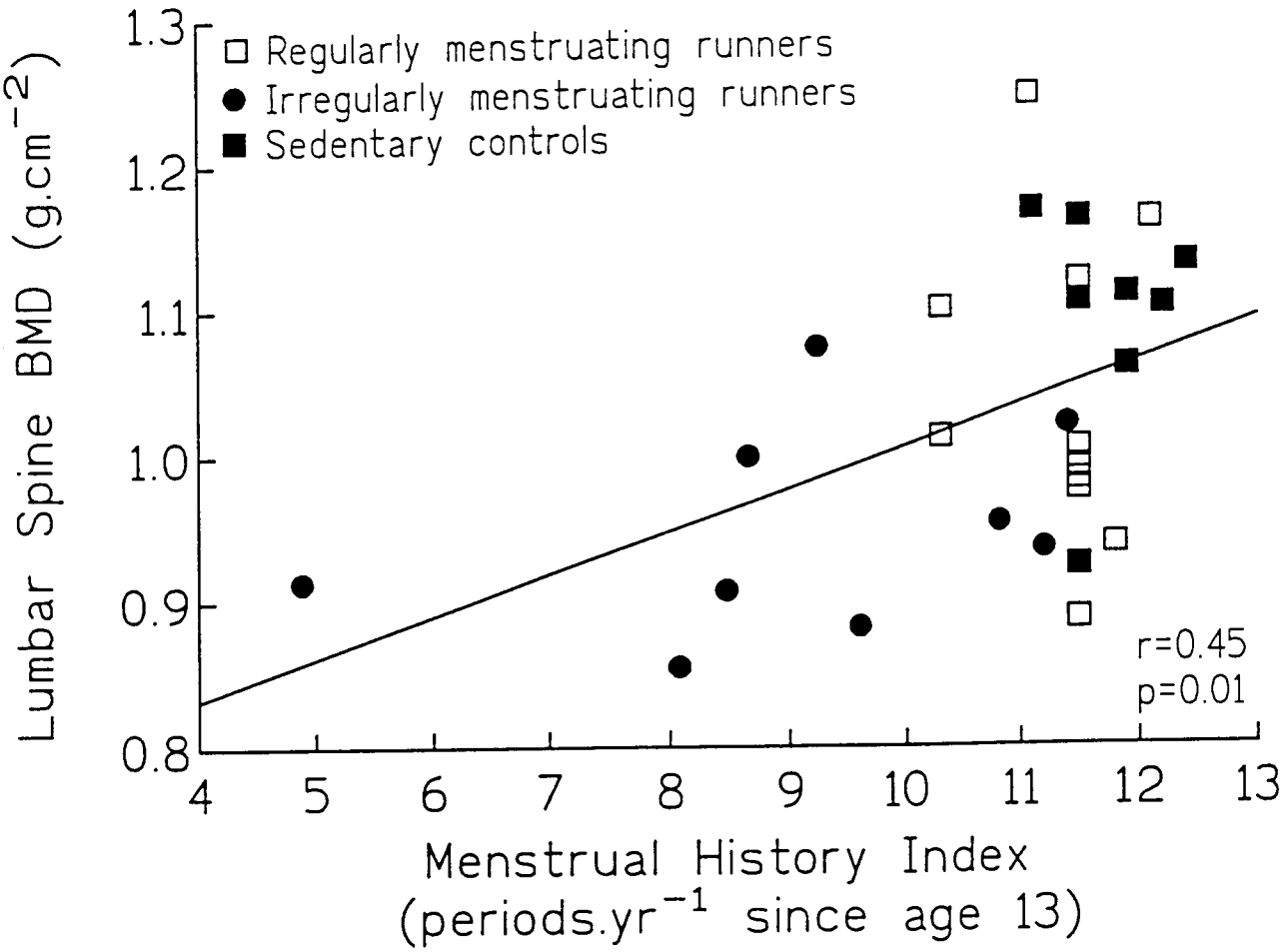

Fig 3.1: Lumbar spine bone mineral density (BMD) of premenopausal long distance runners, and sedentary controls, relative to their Menstrual History Index. A Pearson-product moment correlation coefficient of r=0.45 was significant (p=0.01).

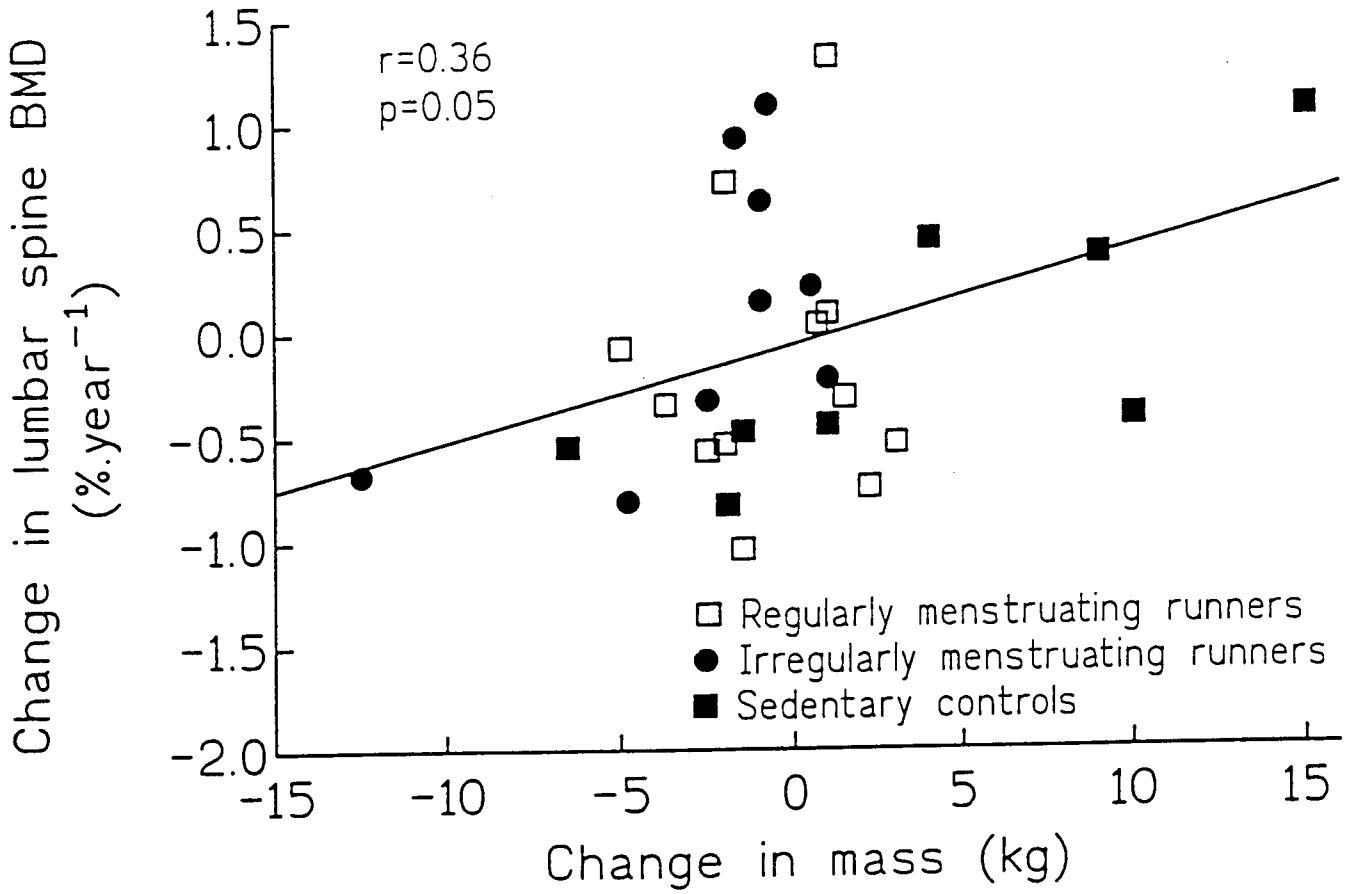

Fig 3.2: **Change in lumbar spine bone mineral density (BMD) of premenopausal long distance runners, and sedentary controls, relative to their change in mass since baseline. A Pearson-product moment correlation coefficient of r=0.36 was significant (p=0.05).**

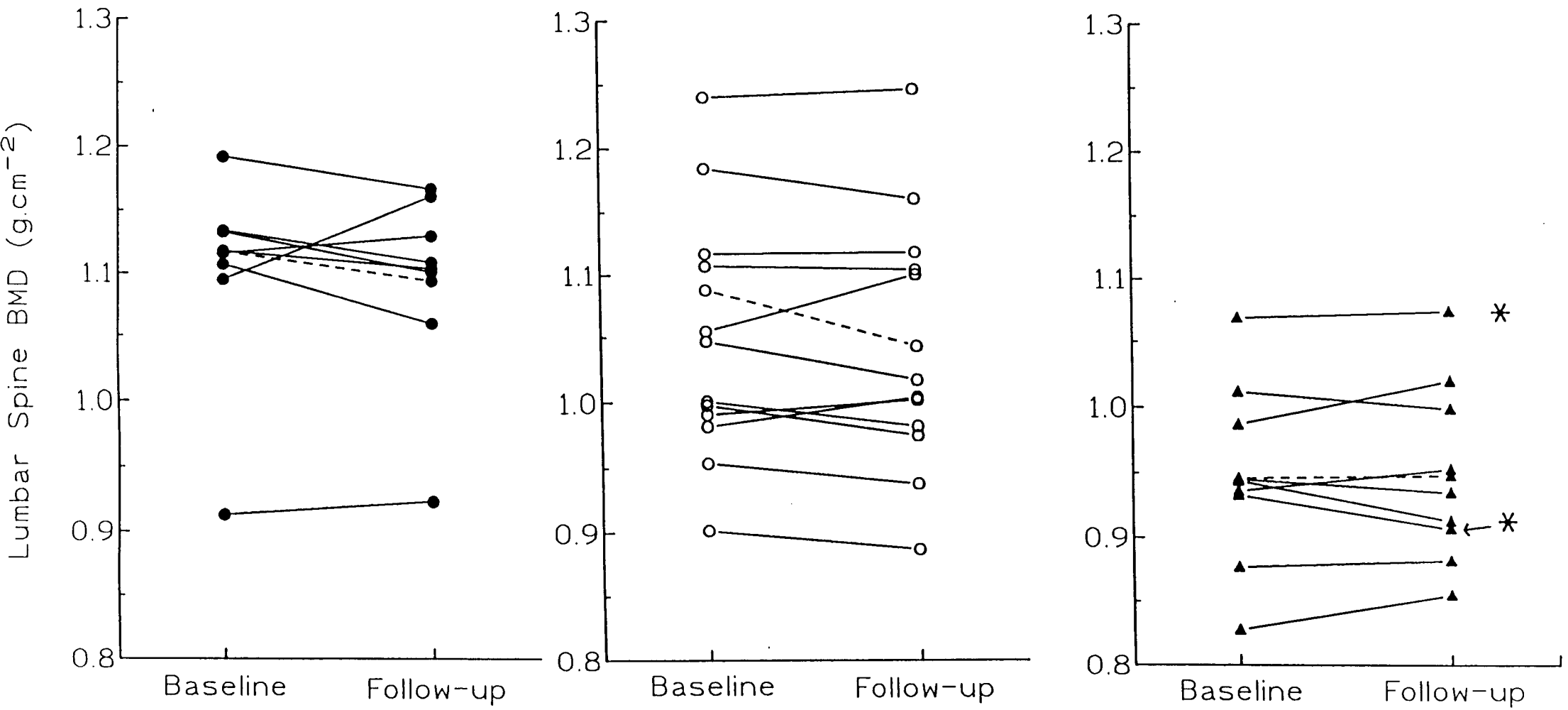

Fig 3.3: Lumbar spine bone mineral density (BMD), at baseline and follow-up, in sedentary controls, regularly menstruating runners and runners with a history of oligo/amenorrhea. Dashed lines represent the mean of all values. Closed circles = sedentray controls; open circles = regularly menstruating runners; closed triangles = oligo/amenorrheic runners. * currently oligo/amenorrheic runners.

iv DISCUSSION

This study in mature premenopausal women shows that despite an average of 9.0 ± 8.7 years of consecutively regular menses, women with a history of menstrual irregularity have reduced lumbar spine bone mineral density (BMD) relative to that of their athletic peers who had always had regular menstrual periods and sedentary controls who had also always menstruated regularly. Therefore, the main finding of this study is that lack of regular exercise is less detrimental to lumbar spine BMD in women in their fourth and fifth decades (mean age 38.8 ± 4.3 yr) than a history of menstrual irregularities associated with marathon running. In addition, this study found that lumbar spine BMD is relatively stable in women of this age.

Although the sample of subjects may not be entirely representative of the general population, this study is not supposed to be a record of the prevalence of osteoporosis among runners and sedentary controls, but rather to determine the effect of a previous history of menstrual irregularity on BMD in runners. Although it may have only been those runners and controls that were concerned about their bone health who responded to the letter, we are still able to look at the relationships between the different variables within the groups.

(a) Cross-sectional data

It is well known that low lumbar spine BMD can be associated with menstrual irregularity in athletes (Cann et al., 1984; Drinkwater et al., 1984; Lindberg et al., 1984; Marcus et al., 1985; Nelson et al., 1986; Cook et al., 1987; Drinkwater et al., 1990; Warren et al., 1991; Myburgh et al., 1993; Jonnavithula et al., 1993; Rutherford et al., 1993; Micklesfield et al., 1995). Many studies comparing BMD of groups of athletes have only considered the current menstrual status of the athletes when measuring bone mineral density (Cook et al., 1987; Warren et al., 1991). However, our data supports findings from other studies (Lloyd et al., 1988; Drinkwater et al., 1990; Grimston et al., 1990; Myburgh et al., 1993) that show that

menstrual history is more important than current menstrual status for the prediction of low bone mass. We also show that there is a graded relationship, with the lowest BMD seen in those subjects with the greatest cumulative history of menstrual irregularity.

The present study also confirms the data of Drinkwater et al. (1990) and Lindberg et al. (1987) who showed that even if previously irregular women athletes regained regular menses, lumbar spine BMD remained significantly lower than in women who had always had regular menses. There was still a significant difference in lumbar spine BMD between the regularly menstruating runners and controls, and the runners with a history of oligo/amenorrhea ($p<0.05$), despite only two women in the oligo/amenorrheic group still experiencing irregular menstrual periods. Even with the exclusion of these two currently irregular subjects, lumbar spine BMD was still significantly higher in the regularly menstruating group, as well as the sedentary control group, compared to those runners who were currently regular but had a history of oligo/amenorrhea. Contrary to the previous two studies in which subjects were re-tested after only 15 months, subjects in this study were re-tested after 3-6 years, but those who had regained regular menses, had had consecutively regular periods for 11.7 ± 7.9 years (range 1-25 yr) prior to the second scan. Therefore, our study adds to existing data, by suggesting that even though the resumption of regular menses has a positive effect on bone status, women who have a history of menstrual irregularity will still be at greater risk of reduced bone density than their regularly menstruating peers for many years.

The MHI is influenced by quite a few variables namely age at menarche, number of years of regular menstruation, oligomenorrhea and amenorrhea. In study 1 (Micklesfield et al., 1995) two of these variables correlated with lumbar spine BMD in the subgroup of subjects with a history of oligo/amenorrhea (years of regular menstruation and years of oligomenorrhea). With the addition of four more subjects to the sub-group of runners with current and/or prior oligo/amenorrhea, only years of regular menstruation was significantly related to lumbar spine BMD (addendum to study 1), while no relationship was found between lumbar spine BMD and the other two menstrual variables. However, the variable which was the most significant

predictor of lumbar spine BMD in the regularly and irregularly menstruating running groups was the total number of years of amenorrhea. With the inclusion of the sedentary controls all of whom were, and always had been, menstruating regularly, total number of years of oligomenorrhea was significantly related to lumbar spine BMD (r=-0.38). But MHI, a measure of the average number of periods per year from 13 to current age, emerged as the most significant predictor of BMD for all subjects. Despite these inconsistencies, both studies seem to suggest that a history of oligomenorrhea should be considered as a significant contributor to the MHI and to low lumbar spine BMD.

Our data also suggest that the age at which this menstrual irregularity occurred may also be important in the determination of peak bone mass. Findings of a study by Lloyd et al. (1988) concluded that regular menstruation and a normal age at menarche (implying adequate levels of circulating estrogen during adolescence) play a fundamental role in determining bone density in young women. Although the number of periods per year for the ages spanning 13-20 years, 21-30 years and 31-40 years were all significantly lower in the irregularly menstruating group in our study, only the age range from 21-30 years was significantly correlated with lumbar spine BMD for the whole sample of women, including the sedentary controls, thus conflicting with the findings of Lloyd et al. (1988). However, their subjects averaged 19.0 ± 0.6 years of age and therefore the relative influence of the second vs the third decade on lumbar spine BMD could not be determined. We suggest that (i) a significant amount of adult bone mass is still gained after adolescence, and (ii) that bone mass is lost more easily after than during adolescence. These issues will be discussed in more detail in section (b).

The significant correlation between lumbar spine BMD and body mass supports the concept that mechanical loading may be beneficial to the maintenance of bone density (Aloia et al., 1988). However, this association may be indirect as it has been suggested that there may be an association between low body weight and menstrual dysfunction (Lindberg et al., 1987). In comparison to controls who did not exercise regularly, the lumbar spine BMD of

oligo/amenorrheic athletes was still compromised, suggesting that regular exercise may not be sufficient to protect trabecular bone from the impact of hormonal imbalances that result from menstrual irregularities. These data also suggest that mechanical loading during daily tasks may be sufficient to load the spine of sedentary subjects.

Cortical bone mass did not appear to be affected by the history of menstrual irregularity. Although this has been a common finding of many studies (Cann et al., 1984; Drinkwater et al., 1984, Marcus et al., 1985; Nelson et al., 1986; Rutherford, 1993), all of these studies have used the mid-radius as their reference for cortical bone. A study by Myburgh et al. (1993), which also found no difference at the mid-radius between amenorrheic and eumenorrheic athletes, did find that BMD of the proximal femur and the femoral mid-shaft was significantly lower in amenorrheic athletes compared to eumenorrheic athletes. Exercise may also however play more of a protective role in the maintenance of cortical bone than trabecular bone, as the weight bearing areas measured consist of a higher proportion of cortical bone mass than trabecular bone mass. Although there were no significant differences between the three groups in any of the regions of the proximal femur in our study, the BMD of the total hip, intertrochanteric region and the neck of the femur did tend to be lower in the sedentary group suggesting that exercise is important in the maintenance of BMD in these predominantly cortical, weight bearing areas. A study by Heinrich et al., (1990) found BMC of female body builders was greater than the BMC of inactive females at all sites measured on the axial and appendicular skeleton, however similar to our results, no significant differences were observed between endurance trained and inactive subjects.

A study done in our laboratory by van Gend and Noakes (1987) concluded that short-term menstrual irregularity appears to be a direct result of the stresses of running training and racing. Their study showed that 41% of the runners experienced short-term menstrual irregularity during periods of intensive training and competition. Many studies have shown a direct relationship between menstrual dysfunction and training load (Feicht et al., 1978; Schwartz et al., 1981; Sanborn et al., 1982; Drinkwater et al., 1984; Lindberg et al., 1984;

Drinkwater et al., 1986; Cook et al., 1987; Drinkwater et al., 1990). Our data showing the significant difference in maximum weekly mileage ever undertaken by the subjects in the 2 running groups, appears to confirm their findings. However, it is not known whether the menstrual irregularity in this sample of women coincided with the increased training load, and other studies have not shown an association between training load and menstrual irregularity (Speroff and Redwine, 1980; Nelson et al., 1986; Myburgh et al., 1993). Therefore training load may be a contributing factor which becomes significant only if other risk factors are also present.

Analysis of our data revealed that several anthropometric variables were either different between the 3 groups, or correlated with lumbar spine BMD. In particular, we will discuss issues related to i) body fat content, ii) body size and shape (weight relative to height) and iii) changes in the latter.

i) Comparison between the 3 groups showed that the sedentary controls were heavier, more endomorphic, less ectomorphic, and had a higher BMI, estimated % body fat and fat mass than the other two groups. This may be largely a result of the differences in activity between the running groups and the sedentary group, but may also be due to a genetic predisposition to higher % body fat (Stunkard et al., 1986), which indirectly may have discouraged sports participation. The fact that the ectomorphic component ($p<0.01$), a measure of leanness and linearity, is more significantly different between the groups than BMI ($p<0.05$) suggests that the runners may have been originally more suited to running. However, there was no difference in mesomorphy, a measure on a scale of 1-7 of the degree of muscularity of the individual. Neither was fat free mass different between the three groups. Muscle mass has previously been related to bone mass (Aloia et al., 1995), and some cross-sectional studies have found that weight-training may be more closely associated with improved bone density than endurance training (Nilson and Westlin, 1971; Heinrich et al., 1990; Davee et al., 1990; Snow-Harter et al., 1992). However, our result is not surprising considering that endurance

running increases muscle oxidative capacity rather than muscle size (Holloszy and Coyle, 1984).

Estimated % body fat was also significantly lower in the OA group than the regularly menstruating runners. Since only 2 of the women in the OA group were currently experiencing menstrual irregularities, % body fat is not likely to be a direct mechanism responsible for menstrual irregularity, thus supporting data by Loucks et al. (1984) and Sanborn et al. (1987). Rather, those women who have a lower % body fat may be at a higher risk of menstrual irregularity as a result of other factors associated with low body fat such as higher training load, or inadequate energy intake, or both.

ii) Since conventional densitometry only takes into account cross-sectional area but not bone width in the third dimension, the effect of the 3-dimensional bone size is not entirely corrected for, resulting in a potential source of error when measuring bones of different sizes. Therefore, Carter et al.(1992) described a new method of assessing bone density. Bone mineral apparent density (BMAD) is a more accurate method of comparing the density of bones of different sizes since it is height and weight independent (Carter et al., 1992). In our study, the significant difference in BMAD between the OA group and the SC group provides further evidence that the difference in bone status between the two groups is the result of menstrual disruptions of the OA group, rather than a difference in body size or shape (range for height in this study is 152 cm - 175 cm). BMI was significantly correlated with BMAD ($r=0.41$, $p<0.05$) and, together with a negative influence by DBMI, was a significant predictor of BMAD ($r^2=0.30$, $p<0.01$). Therefore, the relationship of these same two variables with lumbar spine BMD was not likely a result of inadequate correction for bone size, but a true influence on these variables on osteopenia. Analysis of longitudinal data supplies some additional insights, particularly in individual subjects, (see section (b)).

iii) Of all the physical characteristics, DBMI and BMI were the most significant predictors of lumbar spine BMD ($r^2=0.36$; $p=0.01$). Disordered or restricted eating, which may be the

cause of large fluctuations in body weight (measured by DBMI, which had a negative influence on lumbar spine BMD) and the disruption of energy balance, is associated with amenorrhea, and consequently related to bone mineral loss (Wilmore et al., 1991). BMI as a significant predictor of lumbar spine BMD suggests that not only weight, but also height is important in the determination of bone mass. The significant correlation between BMI and DBMI ($r=0.60$, $p<0.001$) suggests that currently larger women may have experienced larger fluctuations in body weight in the past. Also, the relationship between BMI, % body fat ($r=0.73$, $p<0.001$), and estimated energy expenditure ($r=0.73$, $p<0.001$), suggests that these women are also currently more physically active, possibly in an attempt to decrease body fat. Therefore, the influence of body size, as well as the change in body size, on lumbar spine BMD must not be ignored.

(b) Longitudinal data

A longitudinal study by Jonnavithula et al. (1993) on a sample of young women (13-29 years) noted significant increases in spine bone mineral density of young (mean age 20.4 ± 5.7 yr - 23.0 ± 5.8 yr) amenorrheic exercising women over a 2 year period (14.3%), regardless of lack of menstrual periods. In our sample of much older women, lumbar spine BMD did not change significantly ($-0.07\%.yr^{-1}$) with an improvement in MHI. In contrast Lindberg et al., (1987) and Drinkwater et al., (1986) showed 6.6% and 6.2% increases per annum respectively. However, their subjects were younger than ours and had all been amenorrheic at baseline whereas our subjects had a prior history of oligo/amenorrhea and only one of our subjects was amenorrheic at baseline. Our study was designed to determine the potential long term accretion of bone mass in women with a history of menstrual irregularity. Our data suggests that peak bone mass may already have been reached at the time of the baseline measurements and that BMD may not increase substantially after ± 35 years of age, regardless of menstrual history. Although this conclusion is correct for all our subjects with a history of regular menses and 90% of our subjects with a history of menstrual irregularities, one subject did not follow this trend and will be presented as a special case report (see Chapter 4). Bone

mass also did not decrease in our subjects. Our data therefore concurs with Madsen (1977) who showed that BMC did not decrease until 50-60 years of age, as well as Recker et al. (1992b) who concluded that bone gain occurs in healthy young women during the third decade of life. Another study of healthy premenopausal women (aged 19-51 years) showed 2% increases per annum in peak bone mass up to age 34 years followed immediately by an average decline in lumbar spine BMD of 2.2% per annum (Krolner and Nielsen, 1982). Our data do not support this magnitude of decrease at this age. Buchanan et al., (1988) concluded that 0.7% of vertebral trabecular bone is lost per annum in the fourth and fifth decade of life. Data from some of our subjects concur, but there was no significant change in the mean.

Another study by Prior et al. (1990) in apparently regularly menstruating premenopausal women (n=66; 21-42 years of age) reported an average decrease of 2% per annum in spinal bone density. But a more substantial decrease (± 4%) was shown in two groups with either more than one menstrual cycle with a short luteal phase or anovulation. We did not measure either circulating estrogen or progesterone concentrations, but our insignificant change in lumbar spine BMD seems to indicate that the disturbances noted by Prior et al., (1990) were not present in the subjects in our study. The subject (37 years old) with the largest increase in MHI (7.1 to 9.6) since the previous test showed minimal improvement in lumbar spine BMD (+ 0.2 $\%.yr^{-1}$). However, during the age span of the subjects on this study the extent of change in lumbar spine BMD is variable, as well as the direction of that change, both of which appear to be independent of age and current menstrual status. For example, the subject who showed the largest increase in BMD (1.3 $\%.yr^{-1}$) had always had regular menses, was 42 years of age and had only gained 1 kg of body mass since the first scan.

Lindberg et al. (1987) associated their changes in BMD with increases in body mass. For the whole group we also found a significant positive correlation between the annualised change in lumbar spine BMD and the absolute change in body mass ($r=0.36$, $p=0.05$). The only subject who showed a negative annual change larger than 1% in lumbar spine BMD was 32 years old and had always had regular menses. She only lost 1.5 kg since her first scan three years

previously, but no other factors relating to diet or training had changed significantly. Several studies have stressed the importance of genetic factors in the determination of peak bone mass (Pocock et al., 1987; Matkovic et al., 1990; Seeman et al., 1990; Armamento-Villareal et al., 1992). Unfortunately family history of osteoporosis was not recorded in this study, and as all environmental factors have been well controlled for it can be hypothesised that there may be a genetic influence on rate of bone loss, classifying this subject as an "early loser". Three subjects showed a positive annual change of 1% or more in lumbar spine BMD. Although this does not represent a significant change and it may not be distinguishable from measurement error, it has been used as a level of rate of change from which to discuss particular individuals. One of the subjects (31 years old) had a history of menstrual irregularity but had regained and maintained regular menses for ten years prior to the second scan. Although there was no significant change in her body mass (-0.80 kg), she had dramatically reduced her training mileage which still included exercising 7 days a week. However, even with an increase in her lumbar spine BMD, this subject still had significant osteopenia. Another subject (43 years old) who exhibited a relatively large increase (>1 $\%.yr^{-1}$) in lumbar spine BMD was a sedentary control who had gained 15 kg since the initial study. The other subject (42 years old) who showed a >1$\%.yr^{-1}$ positive change in lumbar spine BMD did not show a significant change in body mass (+1 kg), training mileage, or menstrual status (she had always had regular menses). Therefore it is difficult to associate this increase with a change in lifestyle. These results are evidence that a wide variety of variables, some of which may not be related to lifestyle, influence lumbar spine BMD and even large gains in body mass may not always result in a large accretion of bone mass.

Two subjects were experiencing menstrual irregularities at the time of the second scan. One of the subjects (36 years old) was experiencing oligomenorrhea and had an MHI of 9.23, a score -0.34 lower than at the first scan. In the time period between the two scans she had decreased her training mileage substantially and showed a decrease of 1 kg of body weight. The annualised change in her lumbar spine BMD was +0.14$\%.yr^{-1}$ which is not clearly not significant. This subject does not associate the oligomenorrhea, which she has experienced

for a total of 4 consecutive years, as well as between menarche and the age of 18 years, with a change in training, emotional distress, weight loss or food restriction. The other subject with menstrual irregularity at the time of the second scan was currently amenorrheic and had an MHI of 4.88, a slight increase since the first scan (+0.80). This 34 year-old subject had a history of 10 years of amenorrhea and 1 year of oligomenorrhea. Her training varies between 40 $km.wk^{-1}$ when she is not training for competition, and 130 $km.wk^{-1}$ when she is training for competition. Lumbar spine (L_1-L_4) BMD of this subject had decreased (-0.68%.yr^{-1}) since her first scan five years previously and was 87% of age and sex matched normals.

Lindberg et al. (1987) re-evaluated runners after a 15 month period and found a substantial increase in vertebral bone density in response to reduced exercise in previously amenorrheic runners, however these runners were not compared to age-matched eumenorrheic controls. Other studies (Lloyd et al., 1988, Drinkwater et al., 1986) that have shown similar large increases in vertebral bone mineral content over time have been done in a much younger sample of women. We hypothesise that younger women have a better ability to improve bone mass than women in their fourth decade. Also, the first 1-2 years after resumption of menses may represent a period of rapid increase in lumbar spine BMD, which subsequently plateau's. A possible limitation of this study is that more regular follow-ups, such as once a year, would have provided more information on the rate of change in bone mass.

v CONCLUSIONS OF STUDY 2

From study 2 we have provided further evidence that despite resumption of regular menses, previously irregularly menstruating runners still have reduced lumbar spine BMD compared to regularly menstruating runners. In addition they are also lower than sedentary controls. This suggests that lack of regular exercise is less detrimental to lumbar spine BMD than a history of menstrual irregularity. A history of menstrual irregularity has a significant negative effect on trabecular bone mass, however cortical bone mass remains unchanged.

Although adolescence is an important period in the attainment of high bone mass, it is the period immediately following adolescence, the third decade, that may be more significant in ensuring the maintenance of a *high* peak bone mass. This study suggests that BMD may not increase substantially during the fourth and fifth decades, regardless of current or previous menstrual status. We propose that interventions to improve bone mass in athletes with a history of menstrual irregularity should be a priority in the third decade.

CHAPTER 4

CASE REPORT

i Introduction

Many studies have confirmed the significant direct relationship between current menstrual status and bone mineral density (Drinkwater et al., 1984; Lindberg et al., 1984; Marcus et al., 1985; Nelson et al., 1986; Cook et al., 1987; Drinkwater et al., 1990; Warren et al., 1991; Jonnavithula et al., 1993; Myburgh et al., 1993; Rutherford, 1993; Micklesfield et al., 1995), and more literature is emerging regarding the influence of overall history of menstrual dysfunction (Drinkwater et al., 1990; Grimston et al., 1990; Myburgh et al., 1993; Micklesfield et al., 1995) on bone status. These two variables, along with others such as body mass, functional loading, energy intake and calcium balance may determine peak bone mass. Complex interrelationships between these variables may also exist. It has been suggested that physical activity, especially if it is weight-bearing, may act to increase BMD (Nilson and Westlin 1971; Aloia, 1981; Marcus et al., 1985). However, large training volumes and inadequate energy intake are also associated with oligo/amenorrhea (Rippon et al., 1988), low endogenous estrogen concentrations (Drinkwater et al., 1986), low calcium intake (Nelson et al., 1986) and consequently, osteopenia. Few longitudinal studies exist which report significant changes in bone mass with resumption of menses (Drinkwater et al., 1986; Lindberg et al., 1987; Jonnavithula et al., 1993). Although dramatic improvements in lumbar spine BMD of up to 6.6% in the first year have been reported (Lindberg et al., 1987), mean lumbar spine bone mass was still significantly lower than in athletic controls who had always been regular. It is still unknown whether lumbar spine BMD can, with time, improve enough to equal age-matched normals. In this report, we present a 3 year

follow-up of a distance runner who was amenorrheic at baseline and menstruating regularly 3 years later.

ii Methods

The subject was originally recruited as part of a cross-sectional study of BMD in mature distance runners (Micklesfield et al., 1995) (T_1). Three years later she agreed to a follow-up study (T_2). She was also part of a larger study at T_2, but was a significant outlier compared with all the other subjects (n=29) and is therefore reported separately.

The subject had an osteodensitometry scan of the lumbar spine and the left proximal femur (Hologic QDR-1000, version 4.20).

A detailed questionnaire was completed in order to obtain more detailed information about her current menstrual status and previous menstrual history. This information was used to calculate the Menstrual History Index (modified from Grimston et al., 1990), as described in Micklesfield et al., (1995). Information about previous dairy product intake was also obtained from the questionnaire and was reported as the estimated number of portions consumed per week during junior school, high school, on leaving school and over the past year. For details see Micklesfield et al., (1995). A 3 day dietary record was obtained in order to determine total energy intake (MJ) per day, as well as daily intake of calcium (mg), phosphorus (mg), protein (g), fat (g), carbohydrate (g), and fibre (g). The record was analysed by a computerised dietary analysis programme (Foodfinder, Medical Research Council. Parow, South Africa). Daily energy expenditure ($MJ.d^{-1}$) was estimated from a seven day activity diary (modified Blair et al., 1985).

Body composition was measured and calculated according to the method of Durnin and Wormesley (1974). Somatotype was assessed using the Heath-Carter method (1967).
A venous blood sample were drawn in the morning in order to determine osteocalcin concentration by means of a specific radioimmunoassay performed on serum (OSTK-PR Kit, CIS bio international, ORIS Group, France).
A twenty-four hour urine sample was obtained from S12 in order to determine the concentration of free deoxypyridinoline cross-links (DPD), corrected for urinary concentration by creatinine (Metra Biosystems, Inc).

iii Report

Subject no 12 (S12) was a 29 year old ultramarathon runner at the time of the test 1 and was tested again 3 years later (test 2) at age 32 years. She started training for marathons at the age of 28 years. She participated in a 56 km ultramarathon three times in the period between the two tests, her best time was 4 hours and 42 minutes. Her height at test 1 and 2 was 170 cm, however her body mass increased from 58 kg to 60 kg from test 1 to test 2.

Her exercise schedule consisted of running five times a week for approximately an hour at a time. When training for marathon competition her weekly running distance was 75-95 km. She also participated in aerobic dance exercise four times a week and circuit weight training three times a week. Other regular, though not routine, aerobic activities included cycling and walking. She did not change her training significantly between test 1 and 2, and her competition times had stayed more or less similar.

At the time of her first bone density scan S12 was amenorrheic and had experienced on average 2 periods.yr^{-1} for the three years preceding her first scan. Before that she had

been either oligomenorrheic or amenorrheic since the age of 18 years. The reduction in the frequency of menses coincided with a rapid loss of weight, viz. 38 kg in 21 weeks, achieved through dieting. At the time of her first scan she weighed 58 kg (her lowest weight as an adult). At the time of her second scan she weighed 60 kg. Her highest weight as an adult was 96 kg, a year and a half prior to test 1. At the time of the second scan she had a BMI of 21 $kg.m^{-2}$, however due to the large fluctuation in weight her DBMI was 15.4 $kg.m^{-2}$. This value placed her more than 2 standard deviations above the average of the rest of the subject sample (4.21 $kg.m^{-2} \pm 2.2$; n=29). At the time of her first scan, her % body fat was 31.8%, however it had dropped to 25.9% at the time of her second scan. Lean body mass had increased from 68.2% to 74.1% in this time, which may be as a result of the inclusion of weight training in her exercise routine.

S12 started menstruating at the age of 14 years. At the time of the first scan S12 had an MHI of 6.50 which increased to 9.25 at the time of the second scan three years later due to the regulation of her menstrual cycle. She had experienced 6 years of amenorrhea and 3 years of oligomenorrhea in total prior to test 2.

S12 has two children aged 12 years and 8 years whom she breastfed for a total of 5 years. She had taken the oral contraceptive pill for a total of two years after giving birth to her second child. At the time of the first and second scan she was not taking any oral contraceptive.

Her estimated daily calcium intake at test 1 was 708 $mg.d^{-1}$ and at test 2, 548 $mg.d^{-1}$. It was derived mainly from the milk she had in her tea and coffee and some cheese in a salad. As a child she was deprived of dairy products and her dairy intake consisted of condensed milk and canned milk diluted with water. Estimated portions of $dairy.wk^{-1}$ during her junior school years was estimated to be 10, during high school 21 portions.wk-

^{1}, and since leaving school between 2 and 4 portions.wk^{-1}. Total energy intake at test 1 was 4492 kJ, and test 2 was 3940 kJ, which was 48% and 43% of the RDA for adult women (The Recommended Daily Allowance, 10th edition. © National Academy of Sciences, 1989).

S12 took no supplements but was treated for insomnia with a flunitrazepam (rohypnol, Roche).

Lumbar spine BMD at the time of the first bone density scan was 0.796 g.cm^{-2} and increased to 0.893 g.cm^{-2} at the time of the second scan, an annual increase of 4.0%, but a total of 12% in 3 years. The average BMD of the lumbar spine for the second scan was within the normal range when compared to age matched and young normals (reference curve for American females, Hologic QDR-1000, October 1984), but still only 86% of the mean. However the BMD of L1 was just below the fracture threshold of 0.827 g.cm^{-2} and L4 showed significant osteopenia which is worse than expected for her age and gender.

The BMD of the left hip was normal at the time of the first and second scan. Since the first scan, all areas in the left hip had increased in BMD. The annualised changes were: neck of the femur: +1.9% per annum, greater trochanter: +3.2% per annum, intertrochanteric space: +2.3% per annum, the total proximal femur: +2.6% per annum, Wards triangle: +0.8% per annum. All areas except Ward's triangle were above the T-score. Ward's triangle was equal to the T-score.

Table 4.1: Bone mineral density of the lumbar spine and the proximal femur at test 1 (T_1) and test 2 (T_2).

	BMD($g.cm^{-2}$)		% age matched		T-score	
Site	T_1	T_2	T_1	T_2	T_1	T_2
L_1	0.736	0.825	80%	89%	-1.72	-0.91
L_2	0.791	0.935	77%	91%	-2.16	-0.84
L_3	0.878	0.961	81%	89%	-1.87	-1.12
L_4	0.769	0.847	69%	76%	-3.16	-2.45
L_1-L_4	0.796	0.893	76%	86%	-2.28	-1.40
Femoral neck	0.978	1.034	110%	118%	+0.83	+1.40
Trochanter	0.751	0.826	104%	115%	+0.32	+1.15
Intertrochanter	1.202	1.285	105%	112%	+0.39	+0.99
Total hip	1.029	1.110	106%	114%	+0.53	+1.13
Wards triangle	0.771	0.789	102%	107%	-0.23	-0.06

Urinary calcium was measured in the first test at 0.210 $mmol.2hr^{-1}$, however for test 2 DPD/Creatinine was measured and a value of 5.3 nM Dpd.mM $Creat^{-1}$ was obtained. Similarly different blood tests were performed for the different tests. Estradiol concentration for the first test was 80 pMol/l, while osteocalcin had a value of 7.2 $ng.ml^{-1}$ for the second test. Osteocalcin values generally vary between 2 and 12 ng.ml-1 (Gundberg, 1990), and reference values for Pyrilinks-D range from 2.0-6.0 nM Dpd.mM $Creat^{-1}$.

iv Discussion

The annualised changes in BMD of S12 were significant and provide further evidence of the positive effect of the resumption of regular menses on BMD. However, lumbar spine BMD was still significantly lower than that of age matched normals (86%).

In S12 low lumbar spine BMD had a mulitifactorial origin including lifelong lower than recommended calcium intake, extreme weight loss (though no clinical treatment for an eating disorder) and a history of amenorrhea and oligomenorrhea. The menstrual irregularity was associated with a long period of breastfeeding and both the extreme weight loss and training for an ultramarathon, both of which occurred late in life. Following test 1 the subject was informed of the status of her bone health and advised of the lifestyle factors which contributed to this. S12 desisted from further weight loss, decreased running training at times, however not when training for an ultramarathon (± 6 months.yr^{-1}), but did not consciously alter calcium intake. Menses regularised spontaneously within one year after test 1 and coincided with the removal of an ovarian cyst. Regular menses probably had the largest influence on the gain in BMD in the subsequent three years. However regular weight training may also have contributed. Heinrich et al., (1990) and others (Nilson and Westlin, 1971; Davee et al., 1990, Snow-Harter et al., 1992) have suggested that weight training may be a better stimulus for improving bone status than running and swimming.

S12 was 32 years of age at test 2. Although several studies would predict that by this age peak bone mass would already be achieved (Lloyd et al., 1988; Bonjour et al., 1991; Armamento-Villareal et al., 1992), other studies infer that S12 could still gain bone mass up to the age of 34 years (Krolner and Nielsen, 1982), and perhaps right up to 50-60 years of age (Madsen, 1977). If lumbar spine BMD continued to increase at the present rate ie.

4%.yr^{-1}, S12 would reach the mean level of "young-normals" in 4 years. However, our other longitudinal data indicates <1%.yr^{-1} increase in previously irregularly menstruating women with a mean age of 35.9 ± 4.4 yr. These subjects had already menstruated regularly for an average of 11.7 ± 7.9 years, and were on average older than S12. It is unknown whether the better rate of improvement of BMD in S12 is related to age, severity of previous irregularity, or weight training and whether it will continue at the same rate or slow down.

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APPENDICES

APPENDIX 1

BONE DENSITY QUESTIONNAIRE

Name: _____
Address: _____ (h) _____ (w)
_____ _____
_____ _____
Tel No: _____ (h) _____ (w)

Date today: _____

PHYSICAL DETAILS

Age:_____ yrs (Birth date: _____)
Height: _____ m
Mass: Current weight: _____ kg
Lowest adult weight: _____ kg (When?_____)
Highest adult weight: _____ kg (excluding pregnancy)
(When?_____)

TRAINING

1. How many years have you been training for marathons?
_____ years
2. What is your minimum and maximum weekly mileage when training for marathon competition?
Minimum: _____ km/wk Maximum: _____ km/wk
3. What is your minimum and maximum weekly mileage when not training for marathon competition?
Minimum: _____ km/wk Maximum: _____ km/wk
4. Have you altered your training in the past five years?
If so,how? _____
_____
_____
_____
_____

5. Please record your personal best times for the respective distances for each year 1991-1994:

	10 km	1/2 marathon	Two Oceans	Comrades
1991				
1992				
1993				
1994				

6. Do you participate in any other forms of exercise, besides running? YES/NO
How many times per week do you participate in exercise, including running, at present?_____ times/wk.

7. Please complete the table below:

EXERCISE	FREQUENCY/ WEEK	DURATION/ WEEK	DISTANCE/ WEEK	MONTHS/YEARS PARTICIPATION
Running				
Aerobics				
Swimming				
Cycling				
Gym training				
Other				
Other				

MEDICAL HISTORY

1. Age at onset of menstruation? _____ years of age.

2. Are you presently
(i) pregnant YES/NO
(ii) on the pill YES/NO
(iii) menopausal YES/NO
(iv) post menopausal YES/NO

3. Have you had a hysterectomy or ovarectomy? YES/NO
If yes, please state which one: _____

4. Have you ever experienced any menstrual irregularity?
YES/NO

5. If the answer to no.4 was yes, did you experience the irregularity:

5. If the answer to no.4 was yes, did you experience the irregularity:
   (i) between the onset of menstruation and the age of 18 YES/NO
   (ii) with an increase in exercise YES/NO
   (iii) anxiety related YES/NO
   (iv) with food restriction YES/NO
   (v) unknown reason YES/NO
   (vi) within the past 5 years YES/NO

BONE DENSITY STUDY

1. Would you be prepared to do another:
   (i) bone density test YES/NO
   (ii) dietary record YES/NO
   (iii) blood test YES/NO

APPENDIX 2

DETAILED BONE DENSITY QUESTIONNAIRE (Initial consultation)

Name: _____ Date: _____

Tel No: _____ (h) _____ (w)

PHYSICAL DETAILS
Age:_____ yrs
Height: _____ m
Current mass (hospital scale)_____ kg

TRAINING

Comments_____
_____
_____
_____
_____

MEDICAL AND MENSTRUAL HISTORY

1a. Age at menarche: _____
1b. Fill in the following table of questions:
(i) Please mark in one of the columns the approximate number of periods you had per year at each age?
(ii) Were you using oral contraceptives at any of these ages?
(iii) Please indicate if you were pregnant.
(iv) Other: please indicate if you underwent any of the following at any age: breastfeeding, fertility drugs (type), hormone injections (type), hysterectomy, hysterectomy and ovarectomy, miscarriage (no. of weeks).
(iv) Any comments.

	(i)					(ii)	(iii)	(iv)	(v)
Age: 12	0	1-3	4-6	7-9	10-13	O/C	Pregnant	Other	Comments
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									

Age	0	1-3	4-6	7-9	10-13	O/C	Pregnant	Other	Comments
27									
28									
29									
30									
31									
32									
33									
34									
35									
36									
37									
38									
39									
40									

2a. How many children have you had?_____
b. How old are they?_____
c. How many months have you breastfed in total?_____
d. Did you take a calcium supplement at that time? Y/N

3a. Are you on any regular medication? Y/N
b. If "yes", please specify what medication and dose and for how long you have been taking this medication?

_____

4a. What was the date of your last period? __/__/__
b. How many days is your cycle (eg. 28) _____

DIET

1. Does your intake of dairy products vary a lot? Y/N
2. How many cups of milk do you think you drank per day?
   i) Over the past year? _____
   ii) In general since leaving school? _____
   iii) At high school? _____
   iv) At junior school? _____

3. How many portions of yoghurt did you drink per WEEK?
   (1 portion = a 175ml container as sold in shop)
   i) Over the past year? _____
   ii) In general since leaving school? _____
   iii) At high school? _____
   iv) At junior school? _____

4. How many portions of ice-cream did you eat per WEEK?
(1 portion = a scoop)
i) Over the past year? _____
ii) In general since leaving school? _____
iii) At high school? _____
iv) At junior school? _____

5. How many tubs of cottage cheese did you eat per WEEK?
i) Over the past year? _____
ii) In general since leaving school? _____
iii) At high school? _____
iv) At junior school? _____

6. How many times do you have a meal with cheese per WEEK?
(eg. cheese sandwich, macaroni cheese etc.)
i) Over the past year? _____
ii) In general since leaving school? _____
iii) At high school? _____
iv) At junior school? _____

Additional comments:

_____
_____
_____
_____